CLEANROOM MANAGEMENT

IN PHARMACEUTICALS AND HEALTHCARE

Edited by Tim Sandle and Madhu Raju Saghee

Cleanroom management in pharmaceuticals and healthcare

Edited by
Tim Sandle
Madhu Raju Saghee

Copyright © 2013 Euromed Communications Ltd

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means: electronic, electrostatic, magnetic tape, mechanical, photocopying, recording or otherwise, without permission in writing from the publishers.

ISBN 978-1-899015-83-2

Published by Euromed Communications Ltd, Passfield, England.
www.euromedcommunications.com

Designed by Dave Johnson
Printed in England

DEDICATION

We would like to dedicate this book to our wives, Jenny Sandle and Divya Saghee for their endless love and support and to our children, Jake Sandle and Ananya Saghee for they are the source of our energy and happiness

Tim Sandle and Madhu Raju Saghee

دیاگو شکو

CONTENTS

Foreword . . . 2

Preface . . . 4

About the editors . . . 7

Chapter 1
Introduction . . . 15
Tim Sandle and Madhu Raju Saghee

Chapter 2
History and development of cleanrooms
Tim Sandle

2.1 Introduction . . . 21
2.2 Early history: surgical endeavours . . . 21
2.3 Post-war: industrial growth . . . 24
2.4 1960: the space race and the cleanroom's first standards . . . 25
2.5 1970: pharmaceuticals . . . 29
2.6 1980: GMP standards . . . 29
2.7 1990s: towards a global standard . . . 30
2.8 Cleanrooms in the 21st century . . . 33
2.9 Conclusion . . . 33
2.10 References . . . 33
2.11 Appendix 1 . . . 37

Chapter 3
Cleanroom standards and GMP requirements
Mark Hallworth and Madhu Raju Saghee

3.1 Introduction . . . 39
3.2 History of cleanroom standards . . . 39
3.2.1 USAF (T.O.) 00-25-203 (1961) . . . 39
3.2.2 Federal Standard 209 . . . 39
3.3 ISO 14644 Clean Standard . . . 41
3.3.1 ISO 14644-1 . . . 42
3.3.2 ISO 14644-2: Specifications for testing and monitoring to prove continued compliance . . . 46
3.3.3 ISO 14644-3: Test methods . . . 46
3.3.4 ISO 14644-4: Design, construction and start-up . . . 46
3.3.5 ISO 14644-5: Operations . . . 48
3.3.6 ISO 14644-6: Vocabulary . . . 48
3.3.7 ISO 14644-7: Separative devices . . . 49
3.3.8 ISO 14644-8: Classification of airborne molecular contamination . . . 50
3.4 ISO 14698 Cleanroom biocontamination standards . . . 51
3.4.1 ISO 14698-1: Cleanroom and associated controlled environments – Biocontamination control – Part 1: General principles and methods . . . 51
3.4.2 ISO 14698-2: Cleanrooms and associated controlled environments – Biocontamination control – Part 2: Evaluation and interpretation of data . . . 52
3.5 Global GMP requirements for pharmaceutical cleanrooms . . . 52
3.5.1 EU GMP Annex 1 . . . 52

3.5.2 FDA Guidance on sterile manufacture . . . 54
3.5.3 The Pharmaceutical Inspection Co-operation Scheme (PIC/S) . . . 56
3.5.4 World Health Organization . . . 56
3.5.5 Comparing international standards . . . 56
3.6 Conclusions . . . 56
3.7 References . . . 57

Chapter 4
Design and construction of pharmaceutical cleanrooms
Alexander E Fedotov
4.1 Introduction . . . 59
4.2 What is a design? . . . 59
4.3 Design specification . . . 61
4.4 Concept of design . . . 62
4.5 Basic design . . . 63
4.6 Working and other drawings . . . 67
4.7 Construction of cleanrooms . . . 68
4.8 Modular and flexible cleanrooms . . . 69
4.9 Design qualification . . . 72
4.10 Examples of typical design mistakes . . . 72
4.11 Summary . . . 74
4.12 References . . . 74

Chapter 5
Air handling systems for the protection of pharmaceutical manufacturing processes
Hans H Schicht
5.1 Introduction . . . 75
5.2 Objectives of pharmaceutical air handling systems . . . 75
5.3 Sources of airborne particles . . . 76
5.4 Controlling airborne contamination: general remarks . . . 77
5.5 Key elements for ensuring air cleanliness in rooms . . . 77
5.5.1 Air filtration . . . 77
5.5.2 Airflow patterns in cleanrooms and how to establish them . . . 81
5.5.3 Area segregation concepts . . . 84
5.5.4 Infiltrations of false air through the building envelope . . . 85
5.6 Air handling concepts for GMP-compliance cleanrooms . . . 86
5.6.1 GMP stipulations . . . 86
5.6.2 Control parameters . . . 87
5.6.3 Decentralised units for local air cleanliness control . . . 87
5.6.4 Central air handling system concepts with turbulent airflow . . . 88
5.6.5 Protection configurations with unidirectional airflow . . . 92
5.7 HVAC system design . . . 93
5.7.1 Development of the basic HVAC system specification . . . 93
5.7.2 Determining the supply airflow rate . . . 93
5.7.3 Estimating the airborne micro-organism count . . . 94
5.8 Qualification, monitoring, verification for continued compliance . . . 95
5.8.1 Qualification checks and tests . . . 95
5.8.2 Design review and design qualification . . . 95
5.8.3 Installation and operational qualification . . . 96
5.8.4 Performance qualification . . . 97
5.8.5 Monitoring during production . . . 97
5.8.6 Operation and maintenance . . . 98
5.8.7 Verification for proving continued compliance . . . 98
5.9 The risk perspective . . . 99

5.9.1 HVAC system related risks . . . 99
5.9.2 Controlling the risks . . . 99
5.9.3 The FMEA risk analysis procedure . . . 100
5.10 Energy saving options . . . 101
5.11 Summary . . . 102
5.12 References . . . 103
Appendix 5.A . . . 106
Appendix 5.B . . . 109

Chapter 6
Cleanrooms in hospitals
Alexander E Fedotov
6.1 Introduction . . . 115
6.2 Hospital infections: history of protection . . . 115
6.3 Criteria for protection against infections . . . 116
6.4 Particles and micro-organisms in the air . . . 117
6.5 Sources of microbial contamination . . . 118
6.6 Protective measures . . . 119
6.7 Standards and norms for air cleanliness in hospitals . . . 120
6.8 Classification of hospital rooms . . . 122
6.9 References . . . 128

Chapter 7
Commissioning and qualification of cleanrooms
Kevin Beauchamp and Miroslav Tonovski
7.1 Introduction . . . 129
7.2 Planning . . . 129
7.3 The qualification . . . 130
7.4 Tests required for the commissioning and qualification of cleanrooms . . . 132
7.4.1 HVAC system component installation check . . . 132
7.4.2 Airlock test . . . 133
7.4.3 Fan commissioning test . . . 134
7.4.4 Ductwork leakage testing according to DW/143 . . . 134
7.4.5 Airflows and air-changes . . . 136
7.4.6 Differential pressures . . . 141
7.4.7 Temperature and humidity test . . . 146
7.4.8 Filter leakage test . . . 147
7.4.9 Airflow visualisation test . . . 158
7.4.10 Particle counting test . . . 159
7.4.11 Room recovery test . . . 165
7.5 Conclusion . . . 168
7.6 References . . . 168

Chapter 8
Cleanroom certification and ongoing compliance
Tim Sandle and Madhu Raju Saghee
8.1 Introduction . . . 169
8.2 Cleanrooms . . . 171
8.3 Cleanroom certification to ISO 14644 . . . 172
8.3.1 ISO 14644-1 (Part 1: Classification of air cleanliness) . . . 172
8.3.2 ISO 14644-2 (Part 2: Specifications for testing and monitoring to prove continued compliance with ISO 14644-1) . . . 176
8.3.3 Other parts of the ISO 14644 standard . . . 179

8.4 Reporting . . . 180
8.5 Risk-based approach in cleanroom commissioning and qualification . . . 180
8.6 Conclusion . . . 181
8.7 References . . . 182

Chapter 9
Fundamentals of pharmaceutical isolators
Brian Midcalf, John Neiger and Tim Sandle

9.1 Introduction . . . 185
9.2 Isolator basic concepts . . . 185
9.3 Why isolation technology is required . . . 187
9.4 Key application of isolators . . . 188
9.4.1 Non-pharmaceutical applications: biomedical research . . . 188
9.4.2 Pharmaceutical industry applications: primary manufacture . . . 188
9.4.3 Hospital pharmacy applications . . . 189
9.5 Isolator design . . . 190
9.5.1 Construction of main enclosure and transfer devices general . . . 191
9.5.2 Transfer devices . . . 192
9.5.3 Access devices . . . 195
9.5.4 Sanitisation systems . . . 201
9.5.5 Filtration and airflow . . . 202
9.5.6 Leakage and leaktightness . . . 205
9.5.7 Isolator controls . . . 207
9.5.8 Waste removal . . . 208
9.6 Isolator siting . . . 209
9.6.1 Siting . . . 209
9.6.2 Clothing regimes . . . 210
9.6.3 Cleanroom testing . . . 210
9.6.4 Isolator exhausts . . . 210
9.7 Performance, monitoring and testing . . . 211
9.8 Isolators used in pharmaceutical manufacturing . . . 213
9.9 Regulatory aspects on isolators – EU GMP, US FDA, PIC/S, Pharmacopoeia . . . 214
9.9.1 EU GMP . . . 214
9.9.2 FDA Aseptic Filling . . . 214
9.9.3 PIC/S . . . 215
9.9.4 World Health Organization . . . 217
9.9.5 Pharmacopoeia . . . 217
9.10 Overview on the ISO Standard for isolators . . . 217
9.11 Challenges with isolators . . . 218
9.12 Cleaning . . . 219
9.12.1 General considerations . . . 219
9.12.2 Cleaning schedule or timing . . . 220
9.12.3 Cleaning methods . . . 220
9.12.4 Cleaning agents . . . 221
9.12.5 Cleaning validation . . . 221
9.13 Staff training . . . 222
9.14 Glossary . . . 225
9.15 References . . . 225

Chapter 10
The choice of isolators: A risk-based decision
Didier Meyer

10.1 Introduction . . . 227
10.2 Selecting isolators . . . 229

10.3 Isolator sterilisation and decontamination . . . 236
10.4 A logical approach to isolator design . . . 237
10.5 Types of isolator application . . . 239
10.6 A logical approach to qualification validation . . . 244
10.7 Regulations recommendations to follow . . . 245
10.8 Financial matters . . . 245
10.9 Personnel management matters . . . 245
10.10 Pros and cons of isolators versus cleanrooms and RABS . . . 245
10.11 Conclustion . . . 246
10.12 References . . . 246

Chapter 11
Validation concepts in pharmaceutical aseptic application isolators
Rajesh Thempadiyil
11.1 Introduction . . . 249
11.2 History and key definitions . . . 249
11.3 Basis for positive pressure isolators . . . 250
11.4 Validation of isolators . . . 250
11.4.1 Validation life cycle approach . . . 251
11.5 Regulatory requirements for isolators for aseptic use . . . 252
11.6 Key features of isolator design . . . 252
11.6.1 Development of the user requirements specification and design specification . . . 252
11.6.2 Isolator – relevant GMP guidance, standards and guidelines: GMP guidance . . . 253
11.6.3 Design verification . . . 253
11.6.4 Environmental conditions . . . 254
11.6.5 Air handling system and air flow considerations . . . 254
11.6.6 Enclosure and construction material . . . 256
11.6.7 Ergonomics and access devices . . . 257
11.6.8 Transfer devices . . . 257
11.6.9 Glove integrity testing provisions . . . 258
11.6.10 Decontamination system . . . 258
11.6.11 Process monitoring and control system . . . 259
11.7 Qualification, monitoring and verification for continued compliance . . . 259
11.6.1 Qualification activities . . . 259
11.7.3 Monitoring during production . . . 259
11.7.4 Operation and maintenance . . . 261
11.7.5 Verification for proving continued compliance . . . 261
11.8 Risk-based approach for qualification of an isolator system . . . 262
11.9 Summary . . . 262
11.10 References . . . 262
Appendix 11.A . . . 266
Appendix 11.B . . . 278

Chapter 12
Risk-based product and occupational exposure control in multi-product facilities
Julian Wilkins
12.1 Introduction . . . 287
12.2 What is risk-MaPP® and why is it important? . . . 287
12.3 Routes of exposure . . . 291
12.4 Factors to consider in conducting a quality risk management lan . . . 292
12.5 What is the current regulatory position on cross-contamination and risk-MaPP? . . . 302
12.6 Conclusions . . . 304
12.7 References . . . 305

Chapter 13

Future of aseptic processing

James L Drinkwater

13.1 Introduction . . . 307
13.2 Changes in product profiles . . . 308
13.3 Development of risk-based initiatives such as quality risk management and quality by design (QbD) . . . 308
13.4 Understanding risks associated with microbiological bio-contamination . . . 309
13.5 A risk based methodology and key considerations for selecting a barrier technology (isolator or RABS) for aseptic processing . . . 310
13.5.1 More assured and possible ways to validate biological decontamination processes . 313
13.5.2 Comparison between vapour and atomised decontamination agent generation and distribution processes . . . 314
13.5.3 Hydrogen peroxide vapour cycle development – key requirements for cycle optimisation . . . 315
13.6 Process optimisation and improved efficiency with consideration to reduced process down times . . . 317
13.7 Development of monitoring technologies and interaction with biological decontamination processes . . . 317
13.7.1 Single use product bag and integrated delivery line systems . . . 318
13.8 The development of pre-sterilised disposable technologies . . . 318
13.9 Process and validation efficiency improvements together with considerations saving . . . 318
13.9.1 Considerations in energy saving in bio-contamination control . . . 319
13.10 Summary: the future of aseptic processing . . . 319
13.11 References . . . 320

Chapter 14

Aseptic process simulations/media fills

Marco Budini

14.1 Introduction . . . 321
14.2 Documentation . . . 322
14.3 Risk assessment . . . 324
14.4 Media selection . . . 324
14.5 Frequency and number of runs . . . 326
14.6 Size of runs . . . 326
14.7 Duration of runs . . . 327
14.8 Line speed . . . 327
14.9 Container size . . . 328
14.10 Closure type . . . 328
14.11 Processes/line configuration and set-up . . . 328
14.12 Fill volume . . . 330
14.13 Number of persons and activities . . . 330
14.14 Interventions . . . 330
14.15 Line clearance . . . 332
14.16 Environmental monitoring . . . 333
14.17 Personnel monitoring . . . 333
14.18 Other worst case scenarios . . . 333
14.19 Considerations regarding isolators . . . 334
14.20 Cleaning after process simulation . . . 334
14.21 Incubation of filled units . . . 334
14.22 Visual inspection after incubation . . . 335
14.23 Growth promotion test (GTP) . . . 336
14.24 Interpretation of results – acceptance criteria . . . 337
14.25 Invalidation and abortion of process simulation runs . . . 337

14.26 Investigation of process simulation contaminations – failures . . . 338
14.27 Conclusion . . . 340
14.28 References . . . 341

Chapter 15
Microbial risk management during aseptic manufacture
Tim Eaton
15.1 Introduction . . . 343
15.2 Risk management system stages . . . 343
15.3 Stage 1 – identification of contamination sources and routes of transfer . . . 343
15.3.1 Sources of contamination . . . 344
15.3.2 Routes of transfer to product . . . 344
15.3.3 Contamination risk diagrams . . . 345
15.4 Stage 2 – risk assessment method for microbial contamination . . . 346
15.4.1 Fundamental contamination transfer model . . . 346
15.4.2 Simplified risk assessment model . . . 347
15.4.3 Risk scoring method . . . 347
15.4.4 Allocation of risk factor scores . . . 348
15.4.4.1 Risk factor A – Concentration of microbial contamination on, or in, a source . . . 348
15.4.4.2 Risk factor B – Proportion of contamination transferred to product . . . 348
15.4.4.2.1 Airborne transfer . . . 348
15.4.4.2.2 Contact transfer . . . 349
15.4.4.3 Risk factor C – Time, or frequency, of contamination transfer . . . 349
15.4.4.3.1 Airborne contamination . . . 349
15.4.4.3.2 Surface contact . . . 349
15.5 Stage 2 – practical risk assessments for aseptic manufacturing operation . . . 349
15.5.1 Description of aseptic manufacturing operation . . . 349
15.5.2 Identification of contamination sources . . . 350
15.5.3 Examples of the overall risk assessment method . . . 351
15.5.3.1 Risk from supply air . . . 351
15.5.3.2 Risk from cleanroom surfaces . . . 352
15.5.3.3 Risk from operator's hands . . . 352
15.5.4 Calculation of risk ratings . . . 353
15.6 Stage 2 – (continued) – risk assessments for critical areas . . . 353
15.6.1 Airborne deposition . . . 353
15.6.2 Surface contact . . . 357
15.6.3 Examples of risk assessments for critical areas . . . 358
15.6.3.1 Calculation of contamination rate from airborne deposition . . . 358
15.6.3.2 Calculation of contamination rate from surface contact . . . 358
15.7 Stage 3 – Establishment of an effective monitoring programme . . . 359
15.7.1 Valid sampling methods . . . 359
15.7.2 Sampling locations and monitoring frequency . . . 359
15.7.3 Setting the monitoring limits . . . 360
15.7.4 Recording of the control and monitoring methods . . . 360
15.8 Stage 4 – verification of the risk control system . . . 360
15.8.1 Introduction . . . 360
15.8.2 Assessment and reassessment the overall effectiveness of the control system . . 361
15.8.3 Review of the risk assessment method . . . 362
15.8.4 Modification of environmental sampling and alert or action levels . . . 362
15.9 Stage 5 – establishment of appropriate documentation . . . 363
15.10 Stage 6 – training the personnel . . . 363
15.11 References . . . 363

Chapter 16
Airflow studies and airflow mapping
Tim Sandle, Marco Budini and Rajesh Thempadiyil
16.1 Introduction . . . 365
16.2 Regulatory requirement . . . 365
16.3 Operational state . . . 367
16.4 Smoke generating devices . . . 367
16.5 Video recording . . . 369
16.6 Undertaking an airflow visualisation study . . . 369
16.7 Key objective of airflow visualisation studies . . . 370
16.8 What to include in airflow studies . . . 372
16.9 Reviewing airflow studies . . . 374
16.10 Documenting airflow studies . . . 375
16.11 Reporting airflow studies . . . 376
16.12 Frequency of airflow studies . . . 376
16.13 Alternative to airflow studies . . . 377
16.14 Conclusion . . . 377
16.15 References . . . 377
Appendix 1 . . . 379

Chapter 17
Cleanroom containment sources and control measures
Eric Strauss
17.1 Introduction . . . 381
17.2 Materials . . . 382
17.2.1 Utilities . . . 382
17.2.2 Product materials . . . 383
17.2.3 Supplies . . . 384
17.3 Machine . . . 385
17.3.1 Component preparation equipment . . . 385
17.3.2 Component sterilisation equipment . . . 386
17.3.3 Pass-through devices . . . 386
17.3.4 Filling machines . . . 387
17.4 Method . . . 389
17.4.1 Work instructions . . . 389
17.4.2 Environmental monitoring . . . 389
17.5 Men (personnel) . . . 391
17.5.1 Gowning . . . 391
17.5.2 Training . . . 392
17.5.3 Aseptic training . . . 393
17.6 Summary . . . 393
17.7 References . . . 394

Chapter 18
Particle counters and particle counting
Tony Harrison
18.1 Introduction . . . 395
18.2 Air particle counters and the GMP cleanroom . . . 395
18.3 How particle counters work . . . 396
18.4 Performance and calibration of particle counters . . . 396
18.5 Practical operations . . . 400
18.5.1 Sample tubing . . . 400
18.5.2 Sampling in unidirectional airflow . . . 401
18.5.3 Sampling process gases . . . 401

18.5.4 Interference from cleanroom activities . . . 401
18.5.5 Monitoring in closed barrier systems . . . 402
18.5.6 Continuous particle monitoring and alarms . . . 402
18.6 Troubleshooting . . . 402
18.7 Summary . . . 404
18.8 References . . . 404

Chapter 19
Environmental monitoring in cleanrooms
Tim Sandle and Madhu Raju Saghee
19.1 Introduction . . . 405
19.2 Personnel and responsibilities . . . 406
19.2.1 Responsibilities . . . 406
19.2.2 Personnel . . . 407
19.3 Cleanrooms . . . 407
19.3.1 Design of cleanrooms . . . 408
19.3.2 Cleanrooms and contamination control . . . 409
19.3.3 Classification of cleanrooms . . . 409
19.3.4 Cleaning and sanitisation . . . 410
19.4 Environmental monitoring programme: Non-viable particle counts . . . 410
19.4.1 Particle counters . . . 412
19.4.2 Particle sizes . . . 412
19.4.3 Particle counting . . . 412
19.4.4 Isokinetic probes . . . 413
19.4.5 Sample volumes . . . 413
19.4.6 Particle count limits . . . 413
19.5 Environmental monitoring programme: Viable counts . . . 414
19.5.1 Microbiological environmental monitoring . . . 414
19.5.2 Sample types . . . 415
19.5.3 Limits for viable environmental monitoring . . . 419
19.5.4 Locations for monitoring . . . 422
19.5.5 Sampling responsibilities . . . 423
19.5.6 Frequency of monitoring . . . 424
19.5.7 Duration of monitoring . . . 424
19.5.8 Microbial indentification . . . 425
19.5.9 Devising an effective environmental monitoring programme . . . 427
19.5.10 Data examination . . . 428
19.5.11 Corrective and preventative actions . . . 429
19.6 Conclusion . . . 432
19.7 References . . . 433

Chapter 20
Cleaning and disinfection practices
Tim Sandle and Madhu Raju Saghee
20.1 Introduction . . . 435
20.2 Cleaning and disinfection concepts . . . 435
20.2.1 Cleaning . . . 435
20.2.2 Detergent . . . 436
20.2.3 Disinfectants . . . 436
20.2.4 Sanitiser . . . 436
20.2.5 Antiseptics . . . 437
20.3 Regulatory requirements . . . 437
20.4 Selection of detergents and disinfectants . . . 438
20.4.1 Criteria for the selection of detergents . . . 438

20.4.2 Types of detergents . . . 438
20.4.3 Criteria for the selection of disinfectants . . . 439
20.4.4 Types of disinfectants . . . 441
20.5 Disinfectant efficacy . . . 443
20.6 Cleaning and disinfection techniques . . . 447
20.6.1 Order of cleaning and disinfection . . . 447
20.6.2 Treatment of detergents and disinfectants . . . 447
20.6.3 Preparation of detergents and disinfectants . . . 447
20.6.4 Cleaning materials . . . 448
20.6.5 Cleaning steps . . . 448
20.6.6 Cleaning techniques . . . 449
20.6.7 Cleaning and disinfection frequencies . . . 451
20.6.8 Fogging . . . 451
20.6.9 Cleaning and disinfection protocols . . . 451
20.6.10 Other considerations . . . 452
20.7 Qualification of disinfectants . . . 452
20.8 Hand sanitisation . . . 457
20.8.1 Selecting hand sanitisers . . . 457
20.8.2 Hand sanitisation techniques . . . 458
20.8.3 Validating hand sanitisation . . . 458
20.9 Conclusion . . . 460
20.10 References . . . 460

Chapter 21
Cleanroom clothing
Matts Ramstorp
21.1 Introduction . . . 463
21.2 Particles generated by humans . . . 465
21.3 The human microbial flora . . . 466
21.4 Cleanroom garment demands . . . 466
21.4.1 The European GMP and cleanroom garment demands . . . 467
21.4.2 Recommended practices and cleanroom garments . . . 468
21.4.3 General requirements . . . 468
21.4.3.1 Disposable or reusable cleanroom garments . . . 468
21.4.3.2 Comfort . . . 470
21.4.3.3 Filtration efficiency . . . 472
21.4.3.4 Particle release from the garment material . . . 472
21.4.4 Private clothing worn underneath cleanroom garments . . . 473
21.5 Cleanroom clothing parts . . . 473
21.5.1 Body covering parts . . . 473
21.5.2 Shoe covers . . . 474
21.5.3 Face masks . . . 475
21.5.4 Beard cover . . . 475
21.5.5 Hair cover . . . 475
21.5.6 Gloves and finger cots . . . 476
21.6 Maintenance of cleanroom garments . . . 477
21.7 Testing cleanroom garments . . . 479
21.7.1 Test methods on textile fabrics as well as cleanroom garments . . . 480
21.7.1.1 The particle penetration test . . . 480
21.7.1.2 The bubble point method . . . 480
21.7.1.3 The releasable large particle test . . . 481
21.7.1.4 The helmke drum test . . . 481
21.7.1.5 The microbial penetration test . . . 482
21.8 Modern approach to testing cleanroom garments . . . 483

21.9 Use of cleanroom garment . . . 487
21.9.1 Bio-activities . . . 488
21.9.2 Non-bio-activities . . . 488
21.10 Changing into cleanroom garment . . . 489
21.11 Risk factors associated with cleanroom clothing . . . 490
21.12 Standards . . . 491
21.12.1 Gloves and finger cots . . . 491
21.12.1.1 ASTM . . . 491
21.12.1.2 ISO . . . 492
21.12.2 Cleanroom garments . . . 492
21.12.2.1 AATCC . . . 492
21.12.2.2 INDA . . . 493
21.12.2.3 ASTM . . . 493
21.12.2.4 ISO . . . 494
21.13 Acknowledgement . . . 494
21.14 References . . . 495

Chapter 22
Quality assurance in hospital pharmacies
Richard Bateman
22.1 Introduction . . . 499
22.1.1 Quality assurance . . . 500
22.1.2 Good manufacturing practice . . . 500
22.1.3 Quality control . . . 501
22.1.4 Registration and inspection of services . . . 501
22.2 Design, use and maintenance of cleanroom, clear air devices and isolators . . . 501
22.2.1 General requirements . . . 501
22.2.2 Production areas . . . 502
22.2.3 Storage areas . . . 502
22.2.4 Quality control areas . . . 502
22.2.5 Ancillary areas . . . 503
22.2.6 Equipment . . . 503
22.2.7 Special requirements for preparation of terminally sterilised products . . . 503
22.2.8 Special requirements for aseptic preparation activities . . . 503
22.3 Personnel and training . . . 503
22.4 Hygiene . . . 504
22.5 Personnel . . . 504
22.5.1 Special requirements for aseptic preparation activities . . . 505
22.6 Gowning and cleaning . . . 505
22.6.1 Clothing . . . 505
22.6.2 Cleaning . . . 505
22.7 Compounding of sterile products . . . 506
22.7.1 Principles . . . 506
22.7.2 General requirements . . . 506
22.7.3 Product risk assessment . . . 506
22.7.4 Processing operations . . . 507
22.7.5 Labelling . . . 507
22.7.6 Packaging operations . . . 507
22.7.7 Rejected and returned materials and products . . . 507
22.7.8 Sterile processing . . . 507
22.7.9 Preparation of terminally sterilised products . . . 508
22.7.10 Sterilisation by moist heat . . . 508
22.7.11 Aseptic processing . . . 508
22.8 Documentation and records . . . 509

22.8.1 Principles . . . 509
22.8.2 Principles of documentation control . . . 509
22.8.3 Types of documentation . . . 510
22.8.4 Specification . . . 510
22.8.5 Instructions . . . 511
222.8.6 Batch records/worksheets . . . 511
22.8.7 General procedures and addition documentation . . . 512
22.9 Starting materials, stability and storage . . . 513
22.9.1 Starting materials . . . 513
22.9.2 Stability and shelf life . . . 513
22.9.3 Storage . . . 513
22.10 Environmental monitoring and control . . . 514
22.10.1 Monitoring the process . . . 514
22.10.2 Monitoring the equipment and clean air devices . . . 514
22.10.3 Monitoring the operations . . . 514
22.10.4 Environmental monitoring . . . 514
22.10.5 Microbiological monitoring . . . 515
22.10.6 Physical environmental monitoring . . . 515
22.11 Product testing and approval (including sterility assurance) . . . 515
22.11.1 Preparation for individual patients . . . 515
22.11.2 Batch preparation . . . 516
22.11.2.1 General requirements . . . 516
22.11.2.2 Sampling . . . 516
22.11.2.3 Testing . . . 517
22.11.2.4 Release . . . 517
22.12 Internal audit in the hospital pharmacy . . . 517
22.13 Complaints and recalls . . . 518
22.13.1 Recalls . . . 518
22.14 Summary . . . 518
22.15 References . . . 519

Chapter 23
Building management systems for cleanroom process parameters monitoring and control
Sunil Chand Singhai and Rajesh Thempadiyil
23.1 Introduction . . . 521
23.2 Review of existing regulatory requirements . . . 522
23.3 Review of relevant regulatory citations . . . 524
23.4 Scope of BMS in pharmaceutical manufacturing and its criticality . . . 525
23.5 Components of a typical BMS . . . 525
23.6 Design and validation of BMS . . . 527
23.6.1 Risk assessment . . . 528
23.6.2 Design of building management system . . . 529
23.6.3 Development of the user requirements specification . . . 529
23.6.4 Qualification of building management system . . . 531
23.6.5 Approach for handling of alarms . . . 534
23.6.6 Performance qualification . . . 535
23.7 Operation and maintenance . . . 536
23.8 Summary . . . 536
23.9 References . . . 537

Chapter 24
Energy management and sustainable cleanrooms
Nigel Lenegan and Ulla Thomsen
24.1 Introduction . . . 539

24.2 HVAC in the pharmaceutical industry . . . 539
24.3 Primary sources of contamination . . . 540
24.3.1 Viable (microbial) particulate contamination . . . 540
24.3.2 The usefulness of ventilation as a contamination control measure . . . 541
24.3.3 Attitudes to cleanliness . . . 541
24.4 Classification and HVAC techniques . . . 542
24.5 HVAC compliance demands in the pharmaceutical industry . . . 542
24.6 Demand-side energy use . . . 544
24.6.1 Metering and sub-metering . . . 544
24.6.2 How HVAC design has an impact on energy consumption . . . 545
24.6.3 System description . . . 546
24.6.4 Component efficiencies . . . 546
24.6.5 Air conditioning parameters affecting energy consumption . . . 547
24.6.6 The energy consumption impact of humidity requirements . . . 547
24.6.7 Separating the flow for different purposes . . . 548
24.6.8 Energy saving and installation costs reduction hand-in-hand . . . 548
24.6.9 Theoretical “ideal” airflow requirements . . . 548
24.7 Conclusion . . . 550
24.8 References . . . 550

Chapter 25
Auditing cleanroom operations
Tim Sandle and Madhu Raju Saghee
25.1 Introduction . . . 551
25.2 Part A: GMP auditing . . . 552
25.2.1 Types of audit . . . 552
25.2.2 Auditors . . . 552
25.2.3 Undertaking audits . . . 553
25.2.4 Review of audits . . . 555
25.2.5 Regulatory trends . . . 555
25.3 Part B: Auditing cleanrooms . . . 555
25.3.1 General controls and facility operations . . . 555
25.3.2 Quality systems . . . 555
25.3.3 Personnel and staff training . . . 556
25.3.4 Auditing cleanrooms . . . 557
25.3.5 Space and design . . . 557
25.3.6 Process flow . . . 558
25.3.7 Certification . . . 558
25.3.8 Physical operations . . . 558
25.3.9 Equipment . . . 560
25.3.10 Personnel . . . 560
25.3.11 Aseptic processing . . . 560
25.3.12 Auditing cleaning and disinfection . . . 561
25.3.13 Auditing environmental monitoring . . . 562
25.3.14 Auditing cleanroom documentation . . . 564
25.4 Conclusion . . . 565
25.5 References . . . 565

Chapter 26
Developments in cleanroom technology
Tim Sandle and Madhu Raju Saghee
26.1 Introduction . . . 567
26.2 Cleanroom design . . . 567
26.2.1 Design principles . . . 567

26.2.2 Conventional and modular cleanrooms . . . 568
26.2.3 Isolators and RABS . . . 569
26.2.4 Cleanroom robotics . . . 569
26.2.5 Energy conservation . . . 569
26.2.6 Antibacterial materials . . . 570
26.2.7 Cleanroom decontamination . . . 570
26.3 Cleanroom equipment . . . 571
26.3.1 Air showers . . . 571
26.3.2 Pass through chambers . . . 571
26.3.3 Cleanroom ovens . . . 571
26.3.4 Cleanroom weighing stations . . . 572
26.3.5 Filling machines . . . 572
26.3.6 Environmental monitoring . . . 572
26.4 Cleanroom apparel . . . 573
26.4.1 Wipes . . . 573
26.4.2 Cleanroom mats and flooring . . . 573
26.4.3 Garments . . . 574
26.4.4 Cleanroom consumables . . . 574
26.4.5 Furniture . . . 574
26.4.6 Documentation . . . 574
26.5 Single-use technology . . . 575
26.5.1 Aseptic connections . . . 575
26.5.2 Disposable product holding systems . . . 576
26.5.3 Biocontainer sampling bags . . . 576
26.6 Risk management . . . 577
26.7 Summary . . . 579
26.8 References . . . 579

FOREWORD

Cleanrooms and all the other contamination control technologies and techniques play an essential part in the delivery of safe, efficacious and reliable products. Nowhere is this more important than in the life sciences and healthcare sector, where environmental cleanliness is mandated in our GMPs for various steps in the manufacture of sterile products. Interestingly, whilst the cleanliness levels haven't really changed in 35 years, the techniques we use to deliver the cleanliness, and the technology to monitor our success (or lack of it…failure) are continually evolving and developing and have changed a great deal over that time. What was cGMP last year won't be in 2020!

Keeping up to date is an essential responsibility of the industry professional. It is extremely valuable to address the historical context, and relate this to the evolution of the technology, standards and regulation, as well as all aspects of systems engineering and applications.

It's not all about aspiring to ever greater levels of cleanliness assurance. The concepts of appropriateness of the solution we choose, its practicality and energy and occupation health attributes are complex and essential things we have to address. Risk, its assessment, identification, mitigation where necessary and management have to be dealt with on several levels. At one level we have to choose the contamination control technique (e.g. isolators versus cleanrooms); and on another we are challenged about energy consumption in particular. It is the easiest thing in the world to over-engineer and add safety margins and factors. It is actually quite tough to engineer down to the point of failure. Who wants to take the risk? Various aspects on energy saving potential must be considered, from good low energy system design practice to efficient air mixing and maximising the effectiveness of the clean air system. More and more clean air isn't necessarily better or required. It is certainly essential to challenge old wives' tales of 20 air changes per hour.

Different facets of the life sciences need different solutions, so understanding the requirements of a hospital operating theatre and the characteristics of a blow-fill-seal machine will lead to very different solutions. We mustn't stand still and just trot out the same old solutions. Innovation and an open mind are likely to lead us to discover novel and cost effective solutions.

Demonstrating the performance of a cleanroom or clean air device through performance qualification and real time monitoring is an essential part of both regulatory compliance and good operational practice. Monitoring technologies are about to change radically as we get our hands on affordable rapid microbiological monitoring tools. If we add these to the capability of our particle monitoring systems we have the chance to move closer to the cleanroom practice seen in the micro-electronics world. Here, greater emphasis is placed on critical point performance monitoring, and much less on invasive and disruptive testing and certification. These formalised steps are still essential for procurement sign-off, and period confirmation that all is well, but real time information, attached to product quality attribute data is the way of the future.

So what about the future for the cleanroom and clean air systems? They will almost certainly be smaller and focused on critical points, more energy efficient, better monitored and smarter. There is no reason why we won't see common use of more robust filters, much less air, and smarter air distribution. Our design tools will move from a reliance on rule of thumb to placing confidence in predictive tools such as computational fluid dynamics and system modelling to enable smarter solutions. Where we do over-engineer to include margins of safety for the unknown, we should use the better performance knowledge to allow us the refine the performance for maximum efficiency. The future of effective environmental cleanliness is in our hands.

"Cleanroom Management in Pharmaceuticals and Healthcare" provides cleanroom practitioners ranging from concept designer through to user and maintenance engineer with a unique tool to help improve the breed and achieve regulatory compliance where that is needed. It achieves this by creating a foundation in history and established practice, and then moving forward to help us understand how state of the art technologies and engineering solutions can help deliver best practice and excellent reliable systems performance… an essential read for practitioners in cleanroom technology.

Gordon J Farquharson BSc (Hons), CEng

Chair BSI LBI/030; Chair CEN TC243; Convenor ISO TC209 WG1

Critical Systems Ltd, Surrey, UK

PREFACE

"Everything should be as simple as it is, but not simpler."
Albert Einstein (1879-1955)

The aim of this book is to be a self-contained treatise on the operation and management of cleanrooms. The book does this by balancing theoretical concepts with practical examples. Our aim was first, to introduce and explain concepts about cleanrooms, and second, to show how those concepts can be applied. The way in which the book will be judged as a success is the extent that it becomes well-thumbed and worn at the corners, for we want the book to be well-used by practitioners.

The book is intended to be accessible to experience cleanroom users, engineers, microbiologists, Quality Assurance personnel, graduate students, as well as general readers with an interest in the environments in which their medicines are prepared or in which their surgical procedures are conducted.

Whilst there are books available which look at certain aspects of cleanrooms, such as the way they are designed and the way they operate, there was no book available which, in a single volume, captured all aspects of cleanroom operations – from how to select disinfectants to conducting airflow studies, and from commissioning cleanrooms to building isolators. Neither was there a book available that embraced pharmaceutical cleanrooms, hospital pharmacies and laboratory clean air devices. With this book, we have captured all of these important aspects of cleanroom use and application in a single volume.

Thus the origins of the book were borne out of the frustration of the editors in not having a single source to turn to for cleanroom management. In the absence of such a source, we decided to put together our own. In doing so, we have taken the opportunity to examine what has changed with cleanrooms into the 21st century and, as part of that review, we have ensured that the book reflects the latest in clean air containment, single-use disposable technology, and rapid microbiological methods.

In order to produce a work that captured our aims, we drew upon the leading experts in their fields. Being foremost microbiologists by background, the editors realised that for the book to be of use and interest to people working within a range of disciplines,

including engineering and physical sciences, we needed to draw upon the expertise of leaders in cleanroom technology. As the biographical details attest and as the introduction to this book affirms, the authors in this book are amongst the 'leading lights' in the world of cleanrooms and have a combined experience of 400 years. We would like to express our gratitude to our authors for delivering a set of detailed, comprehensive and impressive chapters that serve to capture the pertinent aspects of cleanroom management. We would like to thank Rajesh Thempadiyil, who should be listed as one of the principal editors for helping us in envisaging this book. We would like to thank James Drinkwater for applying his specialist knowledge in reviewing some chapters of the book and the Pharmaceutical and Healthcare Sciences Society (PHSS) for providing technical reports used in shaping this book. We would like to thank Joe Ridge of Euromed Communications for his patience and diligence in the production of this work.

The book is structured in such a way that it first introduces cleanrooms, describing the socio-economic and scientific drivers that triggered their development. The book then goes on to to discuss cleanroom standards and the important elements of Good Manufacturing Practice that relate to cleanrooms. The book then considers how cleanrooms are designed and the engineering aspects of their construction, commissioning and operation. The book then proceeds to look at containment devices and isolators. This is followed by chapters that tie the developed cleanroom with processing. Here the chapters examine such aspects as operator qualification, risk assessments, media fills, cleaning and auditing cleanrooms.

We hope that we have produced a book of interest, which informs as well as challenges, and fosters a greater understanding of cleanroom operation and practice throughout the pharmaceutical, medical device and health care sectors.

Tim Sandle, St Albans, UK

Madhu Raju Saghee, Hyderabad, India

ABOUT THE EDITORS

Tim Sandle

Dr Tim Sandle is the Head of Microbiology at the UK Bio Products Laboratory. His role involves overseeing a range of microbiological tests, batch review, microbiological investigation and policy development. In addition, Tim is an honorary consultant with the School of Pharmacy and Pharmaceutical Sciences, University of Manchester and is a tutor for the university's pharmaceutical microbiology MSc course. Tim is a chartered biologist and holds a First Class Honours Degree in Applied Biology; a Masters degree in Education; and a PhD in the safety testing of blood products.

Tim serves on several national and international committees relating to pharmaceutical microbiology and cleanroom contamination control (including the ISO cleanroom standards), and has acted as a spokesperson for several microbiological societies. He is a committee member of the UK and Irish microbiology society, Pharmig and is editor of its newsletter. Tim has written over 150 book chapters, peer-reviewed papers and technical articles relating to microbiology; these include co-editing the book "Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices". In addition, Tim runs an on-line microbiology blog (www.pharmig.blogspot.com).

Madhu Raju Saghee

Madhu Raju Saghee is Assistant Manager – QA, at Novartis India. His areas of expertise include all aspects of quality and compliance for systems, processes, facilities and operations for drug products, particularly for sterile products. Madhu is also an honorary member of the Pharmaceutical & Healthcare Sciences Society (PHSS), acting as the Director for India. Madhu has written many articles pertaining to regulatory compliance, quality assurance, quality risk management, cleanroom contamination control and microbiology. He is the co-editor of the book *"Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices"*.

Madhu has a Master of Science Degree in Microbiology from Andhra University. He is an active member of various industry associations, including PDA, PHSS and ISPE. He can be reached at madhuraju.sagi@gmail.com

CONTRIBUTING AUTHORS

Richard Bateman

Qualified with a BPharm degree from the Welsh School of Pharmacy in 1989, and became a member of the Royal Pharmaceutical Society in 1990. He obtained the PTQA (Pharmaceutical Technology and Quality Assurance) MSc from the University of Leeds in 2000.

Having held a variety of posts in hospital pharmacies in London he took up the post of Pharmacy Production Manager at Ealing Hospital in 1993. He then moved to Great Ormond Street Hospital as Quality Assurance Manager in 1997.

In 2000 Richard moved to the regional quality assurance service (part of East and South East England Specialist Pharmacy Services) based at Guys Hospital.

Richard has completed two terms as Chairman of the NHS Pharmaceutical Quality Assurance Committee and one as secretary. He participates in many working groups of the committee and has contributed to numerous guidance documents.

As part of the regional QA service, he is responsible for external audit and provision of technical information, advice and training to approximately 80 Hospital Trusts in the UK

Richard is also a member of the Pharmaceutical Aseptic Services and the Technical Specialist Education and Training groups in the UK and is a module director and course management committee member for the Leeds University PTQA course.

Richard also holds Qualified Person status for Investigational Medicinal Products.

Kevin Beauchamp

Kevin has worked in the industry and been involved with the validation of safety cabinets, cleanroom and containment facilities for nearly 30 years, starting off in 1983 by joining Pharmaceutical Engineering and Design Ltd (Tanshire Group in Godalming) as Operations Manager of the Environmental validation department. This involved working throughout the world; Scandinavia, Spain, Canada, etc. undertaking validation project management and the practical validation tests of both large Porton Down projects and small projects for hospital pharmacy, including the production of protocols and reports for clients' Licence applications. In 1994 Kevin joined Crowthorne Hi-Tec Services Ltd in Farnborough as general manager with a service team of 18 engineers and in 2004 became co-owner as part of the management buy-out team. Kevin is a committee member of S2C2 and is their representative on LBI/30. He is also a member and sits on the CTCB-I (Cleanroom Training Certification Board – International) committee as a trainer and examiner. Kevin is now involved with many large new build projects throughout the world for both pharmaceutical and micro-electronics facilities.

Marco Budini

Marco obtained his Masters Degree in Chemistry from University of Florence in 1975. From 1977 to June 2003 he was with Eli Lilly, Italy, covering several assignments in QC, QA and Manufacturing as Chemical Laboratory Head, Injectable Products Manufacturing Head, QA Manager, Technical Service & Validation Advisor. From July 2003 up to retirement (June 2007) he was with Novartis Vaccines (formerly Chiron Vaccines) as Head of Quality Operations – Secondary Manufacturing – Italy, leading the Quality Operation Unit of the Fill & Finish site located in Rosia (Siena). Currently he works as freelance consultant covering pharmaceutical technology and compliance topics. He has served as Past Vice President of PDA-Italy Chapter and is past annual contractor with Florence University as lecturer on validation topics.

James Drinkwater

James is currently the Process Director for Bioquell UK – Bio-decontamination Solutions in Life Sciences together with being the Chairman of the Pharmaceutical and Healthcare Sciences Society (PHSS), and leader of the PHSS RABS special interest group.

Having worked 10 years in the pharmaceutical industry (Amersham International – now GE Healthcare) followed by positions of Technical Director at three Isolator Barrier technology UK companies, his current specialisation is in the area of contamination and infection control with special interest in aseptic processing. Bio-decontamination process integration involves work in many sectors including, Pharmaceutical, Biologics, Biomedical, Bio-safety and Healthcare.

Tim Eaton

Tim Eaton is a process chemist with over 20 years experience of steriles manufacture with Zeneca Pharmaceuticals and then AstraZeneca. During this time he has had extensive roles in technical support, production management and specialist activities for aseptically prepared products. He has had responsibilities for the design, construction, start up and validation of multimillion pound aseptic manufacturing facilities and has managed the introduction, technical transfer and scale-up activities for a number of sterile products. He has published a number of papers relating to cleanroom activities and has also presented at various industry forums in Europe and the US. In his current role of Sterile Manufacturing Specialist, he has responsibilities for the derivation, optimisation and implementation of best practices for aseptically prepared products.

Alexander E Fedotov

Dr Fedotov has 40 years' experience of working in research and industry. His primary specialty is automatic control systems. For the last 25 years he has worked in cleanrooms and more recently in GMP areas.

He is a managing director of a private company "Invar-project" that is located in Moscow, Russia. The company is active in cleanroom design and testing for different areas of application, especially for the pharmaceutical industry and hospitals.

In 1991 he became one of the founders of the Russian Association of Engineers for Contamination Control (ASENMCO). He is now President of ASENMCO, and editor of the Russian language magazine "Technology of Cleanliness". He has also edited the books "Cleanrooms" in 2003 and "Basics of GMP" in 2012, both in Russian.

He is a chairman of Russian Technical committees for standardisation TC 184 "Industrial cleanliness" and TC 458 "Manufacturing and quality control of medicinal products" and is the Russian delegate to Technical committee ISO/TC 209 "Cleanrooms and associated controlled environments".

Mark Hallworth

Mark Hallworth is the Pharmaceutical Manager for Particle Measuring Systems, Boulder, CO, USA. Mark has managed the design, installation and validation of over 200 environmental monitoring system projects worldwide. He has designed several products specific for pharmaceutical environmental monitoring, including particle counters for explosive and corrosive areas and 21 CFR part 11 compliant software for batch test and release. He currently lectures for the PDA, ISPE and other international pharmaceutical societies on environmental monitoring and GMP compliance design and validation. Mark recently was awarded the IEST James Mildon award for "significant contributions to the advancement and increase of knowledge in the field of contamination control".

He can be reached at mhallworth@pmeasuring.com or at Particle Measuring Systems, 5475 Airport Boulevard, Boulder, CO, USA.

Tony Harrison

Tony is the Convenor of the International ISO Technical Committee 209 Working Group 02 (ISO TC 209 WG02) who are tasked with re-writing ISO 14698-1 & -2 for Microbiological Control in Cleanrooms and is the UK subject matter expert to the International ISO TC209 Working Group 01 currently revising ISO 14644-1 & -2 for airborne particulate contamination in cleanrooms. Tony has spent the last 10 years in applied metrology for the pharmaceutical and healthcare manufacturing industries. Prior to that, he worked for companies providing process control solutions for manufacturing industries. Earning a Bachelor's Degree in Electrical & Electronic Engineer in 1975, Tony is currently employed by Pharmagraph (supplier of cleanroom monitoring systems) and currently holds the position of Vice Chairman of the UK Pharmaceutical & Healthcare Sciences Society (PHSS).

He can be reached at mhallworth@pmeasuring.com or at Particle Measuring Systems, 5475 Airport Boulevard, Boulder, CO, USA.

Nigel D Lenegan

Nigel has worked in the building services sector for nearly 25 years, starting off in 1987 as a 16 year-old apprentice building services engineer. He has a First Class Honours Degree in Building Services Engineering and is a Chartered Engineer and full member of the Chartered Institute of Building Services Engineers. He has held responsible positions across all parts of the building services industry in Design & Build contracting, M&E Consultancy, End User Global Technical Services and Major Capital Equipment sales and delivery.

In 2006, while at AstraZeneca, he realised the pharmaceutical sector was facing a number of challenges on energy use, carbon emissions and corporate social responsibility and in response

he co-founded the Global Sustainable Facilities CoP for the International Society of Pharmaceutical Engineers, now with over 1,500 members across the world. In 2008 he set up Energy & Carbon Reduction Solutions, a specialist demand and supply side energy use, HVAC technical support and validation support consultancy for complex facilities within the Pharmaceutical, Higher Education and Healthcare sectors.

His work with AstraZeneca and GSK on ground-breaking experiments on airflow velocity and air-change reduction in sterile manufacturing and involvement in other similar experiments around the world alongside experience with life science Universities and Healthcare Pharmacies has led to a new era of low energy cleanroom and laboratory operation and design.

In 2010, his developing reputation and contacts led him to become involved in the British Standards technical committee – LBI/030/0-/02 (BS8568) Energy management of cleanrooms, which is to be published early 2012. He is also part of a global team of subject matter experts for cleanroom HVAC design, validation, operation and energy reduction and regularly contributes to blogs and delivers lectures and training on the subject.

Didier Meyer

Didier Meyer holds a BSc in Biochemistry. He worked 7 years with Millipore Europe in various positions of sales, marketing and training. He has been working since 1983 with Getinge (former La Calhène) in their Sales & Marketing department. He is currently Marketing Manager for Isolation Technology at Getinge Life Sciences.

pharmaceutical technology and compliance topics. He has served as Past Vice President of PDA-Italy Chapter and is past annual contractor with Florence University as lecturer on validation topics.

Brian Midcalf

Brian Midcalf has spent most of his full time employment as a Regional Quality Assurance Pharmacist for the NHS, based in Leeds, UK. With over 30 years service to the NHS he has held a number of appointments, including membership of the Committee on Dental and Surgical Materials, Committee on Safety of Medicines Subcommittee Chemistry and Pharmacy Standards, British Pharmacopoeia Committee B, BSI/ISO LBI-30 and has been a member of and chaired the National Quality Assurance Committee.

As an original member of the Course Management Committee for Pharmaceutical Technology and Quality Assurance (PTQA), Brian has been a director for a number of modules and for the development of some new courses. Since retirement from the NHS, he has acted as Assistant PTQA Course Director based in the University of Leeds.

In support of the use of barrier technology in hospital pharmacies, Brian became convenor and chairman of the Working Party on Pharmaceutical Isolators and was joint editor of ‘Isolators for Pharmaceutical Applications’ and ‘Pharmaceutical Isolators’. He has chaired eight Pharmaceutical Isolator conferences and participated in numerous conferences and seminars. Brian was registered as a Qualified Person with RPSGB and is a Fellow of the Royal Pharmaceutical Society.

John Neiger

John Neiger was a founding director of Envair Limited in 1972, becoming Chairman from 1990 until 2004. He is currently retained there as a consultant. Envair specialises in the design and manufacture of a wide range of clean air and containment equipment including unidirectional airflow cabinets and enclosures, microbiological safety cabinets and pharmaceutical isolators. He is now pursuing a second career as a writer and as editor of *Clean Air and Containment Review*.

John is a member of various standards committees including BSI CH 101, which concerns itself with microbiological safety cabinets, and BSI LBI 30, which concerns itself with the ISO 14644 series of international cleanroom standards. He has also taken part in two international working groups: WG 6, which assembled ISO 14644-6: Terms and definitions, and WG 7, which

developed ISO 14644-7: Separative devices, the standard covering isolators. He was an active co-editor with Brian Midcalf of *Pharmaceutical Isolators*, which was published by The Pharmaceutical Press in May 2004.

John has presented papers in a number of countries on the subject of isolators.

Matts Ramstorp

Dr. Ramstorp has a Master Degree in Chemical Engineering from LTH, University of Lund, Sweden. He received his Doctoral Degree in Applied Biochemistry at the same university. During his PhD-studies he was heavily involved in the development of the area of biotechnology, both in Lund and also at the ETH in Zürich, Switzerland. Matts Ramstorp then left for industry and worked as senior adviser for Pall Filtration in the Nordic countries. In 1991 he started his own company BioTekPro AB together with his wife, a company with training and education of cleanroom personnel as well with pre-inspections and trouble shooting. During the 1990s Dr. Ramstorp was working intensely with R3-Nordic, the contamination control society of the Nordic countries. During this period he was the chief secretary and later general secretary of the association. He was the president of ICCCS, International Society of Contamination Control Societies, 1998 to 2000.

During the period 2002 and 2008 he was the first Professor in Cleanroom Technology in Sweden at the Design Centre, LTH, University of Lund. Dr Ramstorp has written more than ten different textbooks in the area of contamination control and cleanroom technology in Swedish, some of which have been translated into English, Chinese and Persian. He is a well known lecturer within the industrial as well as the academic world.

Madhu Raju Saghee

Madhu Raju Saghee is Assistant Manager – QA, at Novartis India. His areas of expertise include all aspects of quality and compliance for systems, processes, facilities and operations for drug products, particularly for sterile products. Madhu is also an honorary member of the Pharmaceutical & Healthcare Sciences Society (PHSS), acting as the Director for India. Madhu has written many articles pertaining to regulatory compliance, quality assurance, quality risk management, cleanroom contamination control and microbiology.

Madhu has written many articles pertaining to regulatory compliance, quality assurance, quality risk management, cleanroom contamination control and microbiology. Madhu is the co-editor of the book *“Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices”*.

Madhu has a Master of Science in Microbiology from Andhra University and is an active member of various industry associations, including PDA, PHSS and ISPE. He can be reached at madhuraju.sagi@gmail.com

Tim Sandle

Dr Tim Sandle is Head of Microbiology at the UK Bio Products Laboratory. His role involves overseeing a range of microbiological tests, batch review, microbiological investigation and policy development. In addition, Tim is an honorary consultant with the School of Pharmacy and Pharmaceutical Sciences, University of Manchester and is a tutor for the university’s pharmaceutical microbiology MSc course. Tim is a chartered biologist and holds a First Class Honours degree in Applied Biology; a Masters Degree in Education; and a PhD in the safety testing of blood products.

Tim serves on several national and international committees relating to pharmaceutical microbiology and cleanroom contamination control (including the ISO cleanroom standards), and has acted as a spokesperson for several microbiological societies. He is a committee member of the UK and Irish microbiology society, Pharmig, and is editor of its newsletter. Tim has written over 150 book chapters, peer-reviewed papers and technical articles relating to microbiology, He is co-editor of the book “Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices”. In addition, Tim runs an on-line microbiology blog (www.pharmig.blogspot.com).

Hans H Schicht

Dr Hans H Schicht, Dr sc techn, studied mechanical and chemical engineering at the Swiss Federal Institute of Technology in Zürich. After 20 years industrial activities in industrial air-conditioning in research and management positions (including 7 years in Brazil) he became an independent consultant specialising in cleanroom technology and contamination control. He participated in many international contamination control conferences as lecturer, organized symposia and seminars and is author of numerous technical publications. He is a past president of the *Swiss Contamination Control Society* (SRRT) and chaired the *International Confederation of Contamination Control Societies* (ICCCS) from 1988 to 1990. As the official Swiss representative, he was actively engaged for many years in the Technical Committees ISO/TC 209 and CEN/TC 243, both committed to cleanroom technology. He is honorary member of the Brazilian and Egyptian contamination control societies and a fellow of the Institute of Environmental Sciences and Technology (IEST) in the United States of America, and member of a number of professional societies serving cleanroom technology and the pharmaceutical industry.

Sunil Chand Singhai

Mr. Singhai has a Masters Degree in Pharmaceutics and is currently working as Vice President – Technology in Dr. Reddy's Laboratories Ltd., Hyderabad, India. His work involves technology absorption, evaluation and transfer from in-house and overseas locations to various sites, upgrading technology, trouble shooting and cost improvement programmes. He is involved in Global Capacity Planning which involves project design, execution and validation. He has been a speaker at various international conferences on new technologies and projects. He has created the first totally contained cytotoxic and hormonal, injectable and OSD facility with all international approvals in India. He is Chairman of the Generics and Contract Manufacturing Sub Committee for ISPE OSD International and Advisor to COP OSD International.

Earlier he was responsible for biotech business and has completed the Development Centre, Analytical & Process Research & Development facility and Aseptic Fill Finish facility and a pilot production facility for recombinant proteins from bacterial and cell culture expression systems.

In his previous assignment with Ranbaxy Laboratories, he headed Manufacturing. This facility was created in 1993 and obtained international regulatory approvals. Prior to this, he was with Warner Hindustan, a subsidiary of Warner Lambert Inc, USA as Production Head.

Eric Strauss

Eric Strauss is currently VP of Quality – Global Branded Products at Teva Pharmaceuticals Industries Ltd. He has over 25 years experience in pharmaceutical microbiology and quality assurance of aseptic processing. He started his career in the microbiology laboratory at Teva in Israel as an analyst and transitioned to developing and validating new methods as well as adopting new technologies in the lab. Eric moved to a start-up pharmaceutical company where he managed all microbiological services for manufacture and innovative R&D. He took on responsibilities in quality assurance and supporting regulatory filings and inspections.

Eric returned to Teva and worked in the local operations division managing microbiological compliance for sterile and non-sterile manufacturing sites throughout the division. He moved to a global position supporting all Teva's sterile manufacturing around the globe and currently focuses on the global branded product portfolio for Teva. Eric holds a Masters Degree in Biology from the City University of NY and is accredited as a Specialist Microbiologist in Consumer and Industrial Microbiology by the National Registry of Certified Microbiologists (ASM). Eric has served on the editorial board of Pharmaceutical Microbiology Forum (PMF) Newsletter and has been an active member of the local Israel chapter of the PDA. Eric can be reached at eric.strauss@teva.co.il

Rajesh Thempadiyil

Rajesh Thempadiyil is a science graduate currently working with Dr Reddy's Laboratories, India. Rajesh has 12 years of industry experience and has worked in pharmaceutical quality assurance,

pharmaceutical validation and computerised system validation during his career at USV, Alkem Laboratories, Strides Arcolab, Orchid Chemicals & Pharmaceuticals and Dr Reddy's Laboratories. His areas of expertise include validation and qualification, aseptic processing, sterilisation, isolation technology, cGMPs, technical training and computerised system validation. He is an active member of PDA and ISPE.

During his career he has been involved in design and validation of sterile & non-sterile contained and non-contained drug dosage form facility, including design and validation of positive-pressure isolator filling lines. He has successfully handled projects such as validation of enterprise management systems (mySAP), business process design and validation of manufacturing execution systems, etc. in computerised system validation.

Ulla Thomsen

Ulla Thomsen is currently working as an HVAC specialist in Novo Nordisk, Denmark. She has extensive experience in designing energy efficient and energy saving HVAC systems.

Miroslav Tonovski

Miroslav Tonovski graduated at the Mechanical Faculty, Department of Air-conditioning, at "Sts Cyril and Methodius University in Skopje. Later on he completed his Master studies in the area of Cleanroom Testing Methodology. Miroslav was engaged by several companies operating in the field of cleanroom design and installation.

His primary field of expertise is cleanroom validation, although he has been involved in different phases of cleanroom projects. In 2009, he was appointed Technical Director of KTM Proekt, a company specialised in Cleanroom Testing. In 2010, Miroslav became one of the founders and was elected President of the Cleanroom Association of South Eastern Europe "VAT Cleanrooms SEE".

Tim Triggs

Tim Triggs is Business Development Director of DOP Solutions Limited, a technical based instrument and training company focused on the testing of HEPA filter systems and cleanrooms. Tim's career includes 14 years with GlaxoSmithKline in the UK where he worked in the operational functions of QA/QC, engineering, production and business process (lean sigma). During his time with GSK Tim developed a strong understanding in the areas of GMP compliance for global supply and new products and was engaged in many FDA and MHRA audits. He holds qualifications in engineering and pharmaceutical sciences and is a member of the Institute of Healthcare Engineering and Estates Management, a contributor to BSI LBI/30 and a committee Member of the Pharmaceutical and Healthcare Sciences Society.

Julian Wilkins

Julian Wilkins is founder and vice president of PharmaConsult US, Inc. In 1991 he founded a UK-based isolator company for the emerging need for pharmaceutical containment. The company carried out many projects worldwide for aseptic and potent containment at all scales of pharmaceutical operation. He moved to the US in 1997 and set up PharmaConsult US in 1999. Since its formation, the company has provided independent advice, design and support for containment projects for companies including Bristol-Myers Squibb, Chiron, GSK, Merck & Co., Pfizer, Roche Colorado, Sanofi Sythelabo, Tyco/Mallinckrodt and Wyeth. He is also an adjunct professor at Steven's Institute in the Pharmaceutical Manufacturing Engineering Master's program.

Wilkins is a core team member of the Risk-MaPP Baseline® Guide team. He is a past recipient of the prestigious ISPE Member of the Year award and has spoken at many seminars worldwide on the subject of containment and has contributed articles and chapters to periodicals and books on containment.

Introduction

Tim Sandle, PhD, Head of Microbiology, Bio Products Laboratory, St Albans, UK
Madhu Raju Saghee, Assistant Manager – QA, Novartis India Ltd, Thane, India

This is a book about cleanrooms: their design, construction, qualification, operation, monitoring and assessment, as well as the mechanisms required to maintain control. In covering all of these aspects in one volume this is a unique book and one which will appeal to the engineer, the cleanroom manager, the microbiologist, quality assurance personnel, as well as to the general reader.

Any introductory chapter to a book should begin with a definition of its subject: what are cleanrooms? Here there are a surprisingly wide range of definitions. Here is ours: a cleanroom is a specially designed work area in which the air quality, temperature and humidity are controlled with the objective of minimising contamination. Once established, cleanrooms are classified by the cleanliness of the air (concentration of airborne particulates). More detailed and elaborate explanations emerge as the book progresses. The aim of this introductory chapter is to briefly outline the importance of cleanrooms and to discuss the book. With the latter we seek to do two things: explain why we have produced the book and commissioned the authors and to outline the chapter contents.

In dealing with the former, cleanrooms provide critical support for the production of life saving products and components (pharmaceutical preparations and medical devices), life saving operations (surgical units) and are part of the fabric of modern industrial production (electronics manufacture). Given this central role, the modern conception of the 'cleanroom', with specially filtered air, set pressure differentials, appropriate airflows, gowned staff and with the application of disinfectants, is a development of the 1950s (although aspects of 'clean air' date back much further as the second chapter of this book explains). From the 1960s conception, cleanrooms have evolved in terms of improvements to the control of the physical environment, to minimising contamination risks from operators through the use of barrier technology, and more recent use of single-use systems (biodisposable technologies).

Although technologies have become more advanced, design processes streamlined, and more focus placed on making cleanrooms 'greener' in terms of energy conservation, the fundamental design feature of cleanrooms remains: cleanrooms are designed to minimise and control contamination. Contamination, in this book's core focus, which is health care and pharmaceuticals, is micro-organisms and particles (some of which will be microbial). There are many sources of contamination. However most contamination can be traced to one or more of four "generic" sources: people, air, water and equipment. Even with the most advanced cleanrooms these sources cannot be completely eliminated, since they are often inherent features of the design or manufacturing process or arise through human activities. The atmosphere within pharmaceutical manufacturing environments will contain dust, micro-organisms, condensates, and gases. Manufacturing processes will also produce a range of contaminants. Wherever there is a process which grinds, corrodes, fumes, heats, sprays, turns, etc., particles and fumes are emitted and will contaminate the surroundings. Although they cannot be eliminated they can be controlled. Control is important because contamination can lead to expensive downtime, increased production costs and, most importantly, could result in patient harm.

The fundamental aspect of control is designed to minimise the concentration of airborne particles through minimising the introduction, generation and retention of such particles. The cleanliness of the air is controlled by an HVAC system (Heating, Ventilation and Air-Conditioning), pressure, air supply, and sometimes other parameters such as temperature and humidity. The design, construction and operation of cleanrooms are important in order to achieve environmental control, and thus contamination control. With contamination control, air flow is the answer to many contamination problems. There are four principles which apply to the control of air-borne micro-organisms in cleanrooms, which are fundamental design concepts. These are: air filtration, dilution, air movement and, for the cleanest activities in relation to aseptic processing, directional air flow.

Due to the contamination risks, health care and pharmaceutical cleanroom operations are highly regulated. These regulations offer some areas of guidance, but leave others only partially covered (such as how often to test something) or leave entire aspects of monitoring only loosely covered (the microbiological environmental monitoring programme being a prime example). Regulatory documents are supported by a host of guidelines from cleanroom societies and trade associations, other advice can be sought from manufacturers and suppliers. Here there is a mix of some very good advice, bad advice and often contradictory statements on offer.

The confusion arises partly because many aspects of cleanroom operations appears simple in themselves but how they fit together is not always straightforward. Cleanroom systems are made up of independent measures and controls which are for the most part directed towards particular sources of contamination. For these systems to be successful they must work in harmony. It is important therefore to understand not only what each control is intended to do and how it works, but also to design the process and the facility surrounding it and the movement of materials and personnel into an effective integrated system. This holistic understanding is not always readily obtained.

Despite the widespread use and undoubted importance of cleanrooms, there have been very few books written about cleanrooms. Within more general text books relating to pharmaceutical processing, health care and sometimes microbiology, there are occasional chapters which deal with cleanrooms. Within this comparative small array of literature there is none which deals with the entire scope of cleanroom activities: from design and build, to physical parameters; from day-to-day operations and aspects of personnel behaviour, to microbiology. It is in light of this shortfall that our motivation for this book came about. With this book we have attempted to capture all of these dimensions of cleanrooms (a totality that we describe as **"cleanroom management"**). Thus the book will appeal to both the specialist reader seeking information about one aspect of cleanrooms and the generalist wishing to understand cleanrooms holistically. With the specialist reader there are aspects of interest to engineers, cleanroom managers, quality assurance personnel, quality control staff, microbiologists, cleanroom operators; as well as each aspect being presented in such a way that the general reader too, wishing to understand more about cleanrooms, will find much of interest.

This book is of relevance to all who use or work within cleanrooms centred with the pharmaceutical and health care sectors. This includes pharmaceutical manufacturing, sterility testing, the preparation of medicines within a hospital setting, and bespoke cleanroom operations. Although the book does not directly address cleanrooms used within the electronics industry there will be aspects, not least in design of cleanrooms, which readers more familiar with this industry will also find of interest.

This explains why we felt a single volume of cleanrooms was necessary. With regards to the how, we contacted a range of international experts on cleanrooms from the health care and pharmaceutical sectors. We were pleased that the overwhelming majority shared our vision for the

book and agreed to contribute. As the introduction proceeds to briefly examine the book's contents, and as the later biographies of the chapter authors underlines, the book's contributors are well respected subject matter experts.

In order to allow the reader to navigate through the book and to find appropriate areas of interest, we have divided the book and its chapters into a series of sections. The main purpose of this introductory chapter is to present a short overview of the chapters and to explain the structure.

The first section of the book acts as an introduction to cleanrooms. With the main chapter in this part – Chapter 2 – Dr. Tim Sandle provides an introduction to the history of cleanrooms, dealing with the early concepts of 'clean spaces' through to the technological driver of the post-war era and the later application of cleanrooms to pharmaceutical processing. This chapter places the subsequent chapters in the book, which focus on current applications of cleanroom technology, within the socio-economic historical context.

The second part of the book is concerned with the design and building of cleanrooms. In Chapter 3, Mark Hallworth introduce cleanroom standards and GMP requirements. Here the focus is upon ISO 14644, an international standard for cleanroom design and operations. Reference is also made to the biocontamination control standard ISO 14698. With GMP standards, the key areas here are the FDA guidance documents for aseptic processing and to EU GMP.

With the key standards established, Chapter 4 by Alexander Fedotov presents the necessary steps involved with the design and selection of pharmaceutical cleanrooms, with the main focus being on cleanrooms used for pharmaceutical manufacturing. Mr. Fedotov's chapter looks at the cleanroom design process (including Design Qualification); the processes involved in building a cleanroom, including modular cleanrooms; for existing cleanrooms the chapter presents practical advice on adapting established facilities.

In relation to pharmaceutical operations, more detailed information is provided by Dr. Hans Schicht, who looks at air handling systems for the protection of pharmaceutical manufacturing processes in Chapter 5. This chapter, which balances the theoretical with the practical, considers how basic specifications for air handling systems are developed. The chapter then proceeds to explain the steps relating to the design, installation, operational and performance verification of air-systems. For in-place air-handling systems, the chapter concludes with the requirements for system monitoring and periodical verification for continued compliance.

With Chapters 4 and 5 dealing with pharmaceutical operations, Chapter 6 considers the operational issues relating to cleanrooms in hospitals. In this chapter, Alexander Fedotov considers how cleanrooms need to be designed and operated in such a way which safeguards patient safety.

With the above chapters dealing with design and construction, the third section of the book presents with the next phase: the commissioning and qualification of cleanrooms. Kevin Beauchamp and Miroslav Tonovski present an overview of the requirements for commissioning and qualifying cleanrooms in Chapter 7. Here the authors address necessary physical tests relating to cleanrooms including testing HEPA filters, pressure differentials, air velocities and so forth. Whilst the requirements for physical testing are unavoidable in order to show that a cleanroom is working satisfactorily, current industrial practices are increasingly orientated towards risk based approaches. The final chapter in this section (Chapter 8) is about cleanroom certification and ongoing compliance. Here Dr. Tim Sandle and Madhu Raju Saghee outline the requirements for certification of cleanrooms. The differences between initial validation and recertification are discussed.

The fourth part of the book is about more advanced aspects of cleanroom operations. As cleanrooms have evolved and greater assurance about cleanliness is required then there has been a tendency towards barrier devices. In Chapter 9, John Neiger, Brian Midcalf and Dr. Tim Sandle provide an introduction to pharmaceutical isolators. The following chapters provide more specific information relating to barrier systems. In Chapter 10, Didier Meyer iterates the design, construction and functional specifications of isolators from an engineering perspective. In Chapter 11, T. Rajesh provides a framework for validating isolators using a risk based approach before use within a pharmaceutical setting.

Chapter 12 addresses a more specialist matter. There is worldwide regulatory concern with cross contamination by high hazard compounds in aseptic processing and oral solid dosage manufacturing. To help the reader through this, Julian Wilkins provides the necessary information pertaining to a risk based product and occupational exposure control in multiproduct facilities.

The next four chapters address aseptic processing, arguably the most complex area of pharmaceutical operations and one subject to the greatest contamination risks which must be understood and minimised as part of sterility assurance. In Chapter 13, James Drinkwater discusses how risk based methodology can assist with product protection. In this chapter, Mr. Drinkwater examines how and why barrier technology should be selected for aseptic processing and how process optimisation and improved efficiency can be applied to reduce process downtimes.

An important area for the qualifying of aseptic filling is media simulation trials. In Chapter 14 Marco Budini provides a detailed explanation of this activity for both conventional cleanrooms and isolators. Media simulation trials are designed to assess microbial contamination at six-monthly intervals; contamination, however, is a risk during each filling run. In view of this, Tim Eaton discusses microbial risk management during aseptic manufacturing in Chapter 15. In the chapter, Mr. Eaton outlines risk assessment tools, techniques and considerations for their application.

In the last chapter in this section, airflow visualisation patterns and airflow mapping is discussed. This examination is an important and necessary tool for assessing contamination risk including whether cleanroom operations, such as personnel interventions, raise the level of risk. This chapter (Chapter 16) has been written by Dr. Tim Sandle, Marco Budini and T. Rajesh.

Contamination risks in the wider context of all cleanroom operations are the central point of the fifth part of the book (both microbial and particulate). As way of an introduction in Chapter 17, Eric Strauss explains cleanroom microbiology and sources of contamination within cleanrooms (air, people, equipment and water). An emphasis is placed upon risk assessment and mechanisms to reduce contamination within cleanrooms.

Focusing upon airborne particulates, Tony Harrison, in Chapter 18, looks at particle counters and the science of particle counting. In his chapter, Mr. Harrison explains how particle counters work; how they should be calibrated and provides some guidance on what to do when things go wrong. Viable contamination is assessed through environmental monitoring programmes. Environmental monitoring takes a holistic look at the operation of all of the environmental control systems by measuring their end product, i.e. the overall cleanliness of the cleanroom. From experience, environmental monitoring is a necessary and valuable means of disclosing lapses in control, which may not be signalled by any other means. Although most cleanrooms will be regularly assessed for microbial limits, not all environmental monitoring regimes do so according to regulatory expectations. To meet these aims rationales are required for sample selection and monitoring frequencies. The basis of a robust monitoring programme is set out in Chapter 19 by this book's editors: Dr. Tim Sandle and Madhu Raju Saghee.

Keeping with the contamination control theme in Chapter 20, Dr. Tim Sandle and Madhu Raju Saghee discuss cleanroom cleaning and disinfection programmes. Disinfection places a key part in keeping cleanrooms clean for although the aspects of physical operation described earlier keep cleanroom clean in the static state it is only through the removal and reduction of micro-organisms that cleanrooms with people present can be kept within a state of control. In relation to people, operators within cleanrooms must be gowned correctly and the clothing worn must be of an appropriate quality. This forms the subject of Professor Matts Ramstorp's chapter (Chapter 21) which examines gowns, masks and gloves, as well as the process and qualification of garment laundering.

Monitoring is an important part of contamination control programmes. However, monitoring, as a feature of quality control, is just one aspect of the quality assurance system. In aligning quality assurance to cleanrooms from the hospital perspective, Richard Bateman in Chapter 22 considers best practices in hospital and commercial compounding.

The sixth and final part of the book looks at cleanrooms from the operational perspective and this part will be of interest to cleanroom managers. In Chapter 23, T. Rajesh looks at Building Management Systems, which are the primary way by which cleanroom managers can assess the data pertaining to cleanroom control. In this chapter the validation of such systems is also discussed. In Chapter 24 Ulla Thomsen and Nigel Lenegan look at the energy management of cleanrooms, particularly in relation to EN16001.

For cleanrooms to remain in compliance they should be subjected to regular audits by quality personnel. Given the complexity of today's cleanrooms this can be daunting task. To assist quality staff in this activity, and to allow cleanroom managers to prepare for inspections, in Chapter 25, Dr. Tim Sandle and Madhu Raju Saghee provide a step-by-step guide to the auditing of cleanrooms.

The final chapter of the book (Chapter 26) stands in contrast to the book's first main chapter (Chapter 2, which retells the history of cleanrooms). In Chapter 26 Tim Sandle and Madhu Raju Saghee look at recent and proposed developments with cleanroom technologies. Here the authors see the further use of barrier systems and the wider application of single-use sterile disposable technologies as being the fastest growing and most likely developments in relation to cleanroom use.

It is the opinion of the editors that this book – "*Cleanroom Management in Pharmaceuticals and Health Care*" – presents a detailed and comprehensive account of cleanroom design and operations. It has been a great honour for us to be associated with the expert authors who have contributed to this book. We hope that readers find the book as useful, interesting and insightful as we have. We welcome any comments for future editions.

History and development of cleanrooms

Tim Sandle, Head of Microbiology, Bio Products Laboratory, UK

2.1 Introduction

Cleanrooms are the key technological factor in the overall contamination control strategy of pharmaceutical and healthcare facilities. Cleanrooms are designed to protect products, personnel and equipment from being adversely affected by contaminants, through creating clean and controlled areas. Cleanrooms are used in a variety of different sectors including microelectronics, semiconductor manufacture, industries undertaking micromedical work, optical applications, biotechnological applications, pharmaceutical manufacturing, certain food and beverage industries, operating theatres and other healthcare applications. Although this book is concerned with cleanrooms and clean air devices used for pharmaceutical and healthcare applications, the development and use of cleanrooms cannot be considered without reference to these other industrial sectors.

This chapter outlines the history of cleanrooms or 'clean rooms' (until the 1980s 'clean room' as two separate words was more common, whereas from the 1980s the portmanteau word 'cleanroom' became generally accepted and this is the term used throughout the international cleanroom standard ISO 14644). This chapter discusses the development and progress of cleanrooms and describes the significant historical milestones, taking the eighteenth century concept of a 'clean space' to the 21st century cleanroom (these key developments are summarised in **Appendix I**). The purpose of this chapter is to contextualise the subsequent chapters, particularly those which discuss technological applications and the use of cleanroom standards, as well outlining an historical narrative which will be of general interest to the reader.

The history of cleanrooms is intimately entwined with two strands of technological development: medicine and military. The medical origins can be traced back to the attempts to create a clean environment, first for field surgery and later for operating theatres; whilst the military applications stem from attempts to assemble precision engineered mechanisms in environments where dust particles posed a risk to the device mechanics. Further on, developments with the atomic power, spacecraft and electronics spurred technological advances in cleanrooms, followed by the later application of cleanrooms in the pharmaceutical industry, led to the modern cleanroom technology of the 21st century.

2.2 Early history: surgical endeavours

Fixing a point in time, when it was commonly accepted that the surrounding environment had an affect on the level of cleanliness within a defined space and recognising that this required practical steps to be taken in order to improve cleanliness, is not straightforward. Such an exercise will provide a different answer depending upon the industry or technology surveyed. This task is made more complicated by the need to follow two strands, at least up until the post-war era. One strand can be considered 'industrial', for which the military, atomic and space fields were of the greatest influence on clean area development, and the other 'medicinal', of which the development of operating theatres is the most significant.

One of the earliest recorded 'industrial' concerns about environmental cleanliness in an industrial setting was by a Swiss watchmaker, whose concerns with dust led him to cover his watches with

***Figure 1:** Operator undertaking environmental monitoring in a cleanroom (image courtesy of Tim Sandle).*

a glass jar when they were not being worked on. Thus, arguably, this use of a bell jar was one of the first design concepts of a clean space as a working environment. In addition to an enclosed space, the most important mechanism for keeping the space clean is by air filtration. The history of air filters is linked with fire fighters. Until the early nineteenth century it was common for fire fighters to enter burning buildings with a wet cloth tied around their faces as a crude protection from smoke inhalation. This precarious activity was somewhat improved by the development of an air filter by John and Charles Dean in 1823, who devised a smoke protection filter which afforded some protection to fire fighters from the acrid smoke and dangerous chemicals in the air when fighting fires[1]. The air filter device was later adapted for use by underwater divers and for the gas masks invented by Cluny Macpherson which were used in World War I[2], as well as providing the design concept for the HEPA (high efficiency particulate air) filter.

When the medical strand is examined there are various points in the development of surgery where medical staff expressed concerns about environmental contamination. There is, for example, documentary evidence indicating that surgeons in the American Civil War (1860-1865) acknowledged the environmental risks from field surgery, where, for example, army physicians speculated that the spread of pus-formation from one patient to another was probably airborne[3]. Furthermore, in 1864 the American Medical Association campaigned for ventilation of the hospitals to be improved as a means to creating cleaner air[4].

Although contamination from air, equipment, surfaces and people was generally acknowledged as an important risk during surgical procedures, little improvement was made to the cleanliness until the work of Lord Joseph Lister came to the fore. One of Lister's concerns was with the spread of post-operative infections and in response he pioneered the use of aseptic methods in surgery.

Figure 2: *Surgery, at the time of the American Civil War (image: Creative Commons).*

Inspired by the work of Louis Pasteur on ways to destroy micro-organisms, Lister experimented with surgical dressings soaked with carbolic acid (phenol) to cover the wound; and with hand-washing, sterilising instruments and spraying carbolic in the operating theatre whilst surgery was performed, in order to limit infection[5]. In 1867, Lister described the first antiseptic:

> *"Carbolic or phenic acid, a volatile organic compound which appears to exercise a peculiarly destructive influence upon low forms of life, and hence is the most powerful antiseptic with which we are at present acquainted".*[6]

From Lister's success in reducing post-operative infections, other medical developments in surgery followed. In 1878 Robert Koch discovered that bacteria in the blood caused septicaemia, a finding which led to greater support for the use of antiseptics[7]. The growing demand for antiseptics led to the American entrepreneurs James Wood Johnson and Edward Mead Johnson founding Johnson and Johnson for the first large scale production of anti-bacterial chemicals[8].

Scientists, following on from the work of Lister and Koch, reasoned that reducing bacteria present within the hospital environment would similarly reduce the dispersion of infectious diseases and promote patient recovery. For example in the late nineteenth century, Professor Neuber, and later, Ernst Bergmann, focused on the cleanliness of surgeons[9]. Improved hygiene was gradually adopted part of the operating theatre routine. Bandages, instruments and clothes were all steam-sterilised to remove dirt and micro-organisms, which reduced the use of chemical disinfection.

Greater discipline was also introduced to the practises of theatre staff by minimising infections via surgeons' hands and nails. Here the work of William Halsted, who introduced the use of rubber gloves, caps masks and gowns for surgery, was important[10]. Arguably it was improved post-

operative survival rates which helped to advance surgical techniques in the early years of the twentieth century.

2.3 Post-war: industrial growth

Although the cleanliness of medical techniques continued to move forwards in the first part of the twentieth century, notably as a result of the field medicine of World War I, there was little advance with the concept of the 'clean air space' in the first decades of the century. Consideration was given to controlled areas within factories which manufactured small bearings and gears used in aircraft, where attempts were made to eliminate the gross contamination associated with manufacturing areas (such as heavy dust-laden air which could cause seizure of gears mechanisms). Contamination control was attempted through good housekeeping practices and by segregating the work area from other manufacturing operations.[11]

Whilst methods of minimising air cleanliness were attempted (most notably with ultraviolet radiation in the 1930s[12]), the next significant step in the idea of a clean space was that century's second war and driven by industrial rather than medical applications. With World War II there was an escalation of military technological developments. The need to assemble small-sized critical components, from gun mechanisms to aircraft gyroscopes, in areas free from contamination led to the development of 'white rooms'. These were areas where the level of ventilation was increased and the air was filtered through air conditioning systems. These filters, part of the air handling unit, were initially called super impingement or super interception filters and were later referred to as absolute filters. Attempts were also made with room pressurization[13]. A further development took place with filtration, where the gas masks were designed as something akin to the HEPA filter whereby esparto grass and asbestos was used as the filtration medium[14].

The development during the war of atomic weapons was part of the driver for the design of more sophisticated air filters for creating clean working environments. This process led to the construction of the first 'HEPA filters' as instructed by the Atomic Energy Commission (AEC),

Figure 3: *Production of gas filters for the Swiss Army (image courtesy of Schicht Hans H.: 40 years of cleanroom technology – some historical remarks. In: Proceedings of the 17th International Symposium on Contamination Control, Bonn, Germany, 6-9 Sept. 2004, p. 1-10. Edited by: Verein Deutscher Ingenieure (Association of German Engineers), Düsseldorf, Germany 2004.*

USA (the filters continued to be termed ‘absolute filters’, the acronym HEPA was not used until the 1950s). The absolute filters were developed, as part of the atomic weapons Manhattan Project, in order to remove and to capture radioactive dust and particles from the air inside the laboratories. The development was undertaken by the research and development firm Arthur D. Little. The Little firm designed the first absolute filters to capture particles of a size of 0.3 μm and larger based in the design specification of the filter to effectively capture condensed radioactive iodine. These first air filters were very large compared to the HEPA air filters produced today[15].

Absolute filter technology was declassified after World War II and became available for industrial use in the early 1950s, providing the primary part of heating, ventilation and air-conditioning systems (HVAC). The first filter mechanisms, based on grass, were replaced with glass fibres intertwined with paper. The use of HEPA filters formed part of the adoption in industry of many aspects of wartime technology. Many aspects of post-war industrial developments required clean working environments and the maintenance of cleanrooms increased in importance. Such industries includes the preparation of mechanical instruments, electronics (especially the use of semiconductors) and the nuclear and space industries. In the era of mass production, these sectors required larger cleanrooms with larger volumes of filtered air. This was part of the greater recognition of the need to control particulate levels, such as from dust, fibres and skin cells, particularly as dust could cause post-manufacture operational problems to precision instruments if sufficient quantities settled onto gears or bearings. The construction of these turbulent flow cleanroom areas was helped by the development of ventilation distributors which allowed greater volumes of air to be supplied in a more uniform manner[16].

As well as within industry, post-war developments in cleanroom applications continued within hospitals. In the 1950s there was extensive research undertaken into examining patterns of hospital acquired infections and the microbiological risks posed to patients during surgical operations[17]. This led to further attempts to improve the ventilation of operating areas, on the basis that addition of filtered air, in sufficient quantities, would considerably increase the level of air cleanliness (this was sometimes referred to as ‘positive ventilation’[18]). The most important technological development was the production of artificial ventilation whereby air speed and flow were subject to mechanical control. As this technology advanced, artificially generated clean air became 'cleaner' and was delivered through more reliable systems. This led to the USA, towards the late 1950s, to stipulate that the requirements for air circulatory systems employed in surgical suites were to be of a minimum of 12 changes per hour in existing facilities and a minimum of 25 changes per hour in new facilities[19].

2.4 1960s: the space race and the cleanroom’s first standards

A very significant innovation occurred in the nuclear industry which had become the receptacle of huge financial investment following the creation of the atomic bomb. This was the application of the HEPA filters to create laminar airflow. The concept of laminar airflow (now described today as ‘unidirectional airflow’) is that air is introduced into the cleanroom at a high velocity which causes the air to travel along a unidirectional path over a required distance. In doing so, contamination is swept away from the critical area unlike the more random distribution and transition of contaminants in turbulent flow cleanrooms. The concept of laminar airflow led to the development of rooms and specialised airflow cabinets whereby greater levels of cleanliness could be achieved[20].

The development of laminar flow technology was completed in 1961 by a team led by Willis Whitfield at the Sandia Corporation (later the Sandia National Laboratories) based at Albuquerque, New Mexico, USA, in partnership with the US Atomic Energy Commission. The objective of Whitfield’s work had been to devise an air control system which protected personnel from nuclear particles. The

Figure 4: *Cleanroom, circa 1960s. National Climatic Data Center, USA (image: Creative Commons).*

starting basis of the research was that current cleanroom designs were inefficient when measured against the pace of industrial developments, such as the problems of dust particles contaminating electronic components[21]. The primary way to create very clean environments was as equally reliant upon intensive manual cleaning as it was to ventilated air. Whitfield was able to achieve higher cleanliness standards by using fans to both push outside air through filters into a room whilst simultaneously using fans to remove the air from the room at an equal measure through exhaust pipes located in the cleanroom walls. A later development provided air from the ceiling and removed air ('air exhaustion') through grills in the floor, based on the principle that particles deposited into the air stream would, through gravitational affects, be more easily removed from the room by the airflow[22].

The applications of HEPA filtration which followed on from the Sandia developments were adopted by a number of other industries[23]. In some industries the technology was orientated towards protecting people from contamination against a range of hazards such as radiation and chemicals. Whereas in other sectors such as car production, the company General Motors was one of the first car manufacturers to use clean spaces to protect circuits from dust[24]. In the 1960s a series of different patents pertaining to cleanrooms were issued; for example a patent for a vertical laminar flow room that was issued by Charles Moll and William Andersen, based on work undertaken at the Westinghouse Electric Corporation. It was known as an 'ultra clean room'.[25]

The widespread use of cleanrooms across a range of industries and the improvements to building design technologies led to a requirement for a means by which cleanliness could be measured, standardised and compared. The standardisation of cleanroom cleanliness was first marked by the US Air Force Technical Order 00-25-203[26]. This was the first standard for a controlled area. The Technical Order adopted the classification system Class I to Class IV, with Class I established as the cleanest standard. As with modern standards, the classification was based on, the maximum quantity of particulate matter of a specific size that pertained to a particular room class. In addition to a classification standard, the Technical Order also described the process for entering a clean area, clothing requirements and some rudimentary room design issues[27]. The Technical Order became the basis of the stricter requirements of the development of rockets and later of the US Federal Standard 209.

A powerful driver for technologies designed to produce cleaner manufacturing areas was the space race[28]. The US National Aeronautics and Space Administration (NASA) centre utilized cleanrooms and laminar airflows to advance rocket technology[29]. NASA initially adopted the Air Force Technical

Order and classification system, which was only based on particulate assessment, and added to this a requirement for microbiological monitoring due to the need to minimise microbial levels on critical components required for the assembly of spacecraft (through a series of handbooks such as NASA SP-5076 which was concerned with contamination control)[30]. For microbiological testing, the NASA guidelines recommended the use of Reyniers slit air-samplers and RODAC plates for surface sampling; For the monitoring of the hands of personnel, it required them to touch sterile stainless steel strips and then subsequently washing the strips with peptone water, with the peptone water. That was then subject to further dilution and transfer onto microbiological culture media. The culture medium used for all tests was Trypticase Soy Agar (with an incubation of 32°C for 72 hours). As an indicator of the level of contamination control of this time, research reports conducted in the mid-1960s indicated that where particle control was generally achieved microbiological control was often lacking.[31,32]

The work on cleanroom standards by the US Army and NASA paved the way for the US government to issue the first federal standard for cleanrooms, through approval by the General Services Administration, on 16th December 1963. This was Federal Standard 209[33]. The FS 209 standard established classes for air cleanliness based on specified concentrations of airborne particles (of a size 0.5 µm of larger) suspended in one cubic foot of air. The classes were applicable to the types of industries in which cleanrooms were being used. For example, Class 1 was applicable to microelectronics, Class 10 to semiconductors, Class 100 to aseptic manufacturing, Class 1,000 to optics, Class 10,000 to support areas for aseptic filling, and so on (albeit the pharmaceutical industry only utilised cleanroom concepts with any great seriousness in the 1970s). When the particles in a room were measured, the class limit was set on the basis of which ever particle limit was not exceeded. In addition, the standard outlined the method and monitoring plan required in order to determine particle concentrations. The standard contained both mandatory and non-mandatory (guidance) sections. The application of the standard was made easier by particle counting technology becoming more accurate and the use of particle counters became more widespread.

The influence of this standard was not confined to the USA and it became a *de facto* global standard for cleanrooms. The first draft of Good Manufacturing Practice standards (first issued by

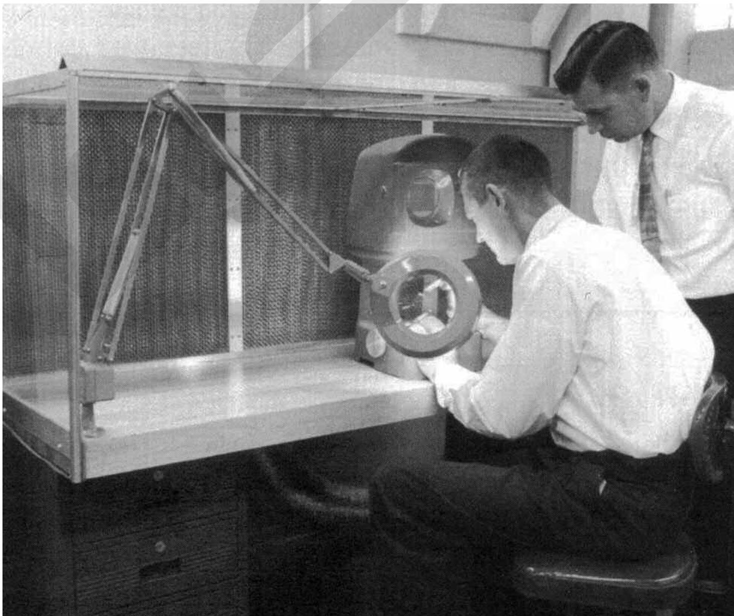


Figure 5: *Whitfield working under a laminar airflow at Sandia Corporation, circa 1961 (image courtesy of Schicht Hans H.: 40 years of cleanroom technology - some historical remarks. In: Proceedings of the 17th International Symposium on Contamination Control, Bonn, Germany, 6-9 Sept. 2004, p. 1-10. Edited by: Verein Deutscher Ingenieure (Association of German Engineers), Düsseldorf, Germany 2004.*

the FDA in 1962, WHO 1969, and Europe in the 1970s), despite often deploying different notational systems to classify or to grade cleanrooms, referred to the FS 209 standard for cleanroom particulate levels. The FS 209 standard was subsequently revised as 209a (1966), 209b (1973, with an additional amendment issued in 1976), 209c (1987), 209d (1988) and 209e (1992). The primary changes with each edition related to revisions of the air cleanliness classes in terms of classification by particulates, driven in part by improvements to particle measuring technology. The final revision, the 209e standard, adopted metric measurements.

As with industrial developments, the 1960s also saw cleanroom improvements in the medical field. These were advancements of the ventilated room concept. The most significant work was undertaken in 1961 by Professor Sir John Charnley, a U.K based orthopaedic surgeon, and Hugh Howorth, who used unidirectional airflow to create a downward flow of air from a much smaller area of the ceiling directly over the operating table[34]. This 'clean air enclosure' recirculated a continuous flow of filtered air, which was under positive pressure, over the zone under which operations took place and also functioned to remove airborne contamination generated during surgery[35]. The innovation became known as the Charnley-Howorth system[36] and was adopted by many hospitals for operations where high-risk surgery (such as transplant surgery) was undertaken[37]. The role that personnel played in cleanroom, as sources of contamination, or as impediments to the airflow was given increased attention. For example the studies of Blowers and Crew on developing turbulent airflow examined how people disrupted the airflow over operating tables[38]. Such studies further promoted the requirement for laminar airflow for critical activities[39].

Aside from cleanroom design technology, another cleanroom development of the 1960s was with the use of specialist clothing in cleanrooms[40]. It had long been recognised that clothing served as

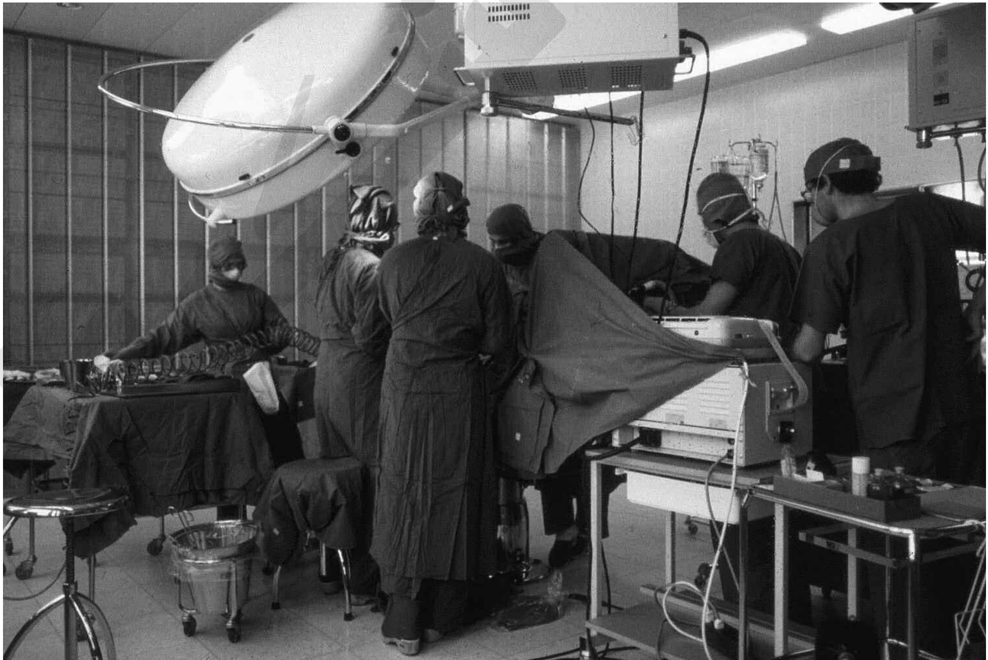


Figure 6: *Operating theatre for orthopaedic surgery with horizontal unidirectional airflow, circa 1973 (image courtesy of Schicht Hans H.: 40 years of cleanroom technology – some historical remarks. In: Proceedings of the 17th International Symposium on Contamination Control, Bonn, Germany, 6-9 Sept. 2004, p. 1-10. Edited by: Verein Deutscher Ingenieure (Association of German Engineers), Düsseldorf, Germany 2004.*

an important particulate barrier. However, it was not until August 1968 that the first standard relating to the requirements of cleanroom clothing was issued (ASTM F51-68[41]). The standard described the particle shedding limits in relation to different types of fabrics and described a check on the effectiveness of garment decontamination which involved examining an area of fabric using an optical microscope. Quite surprisingly, given the pace of other cleanroom developments, the standard was not revised until 2000 (around the same time as ISO 14644 Part 5 for Cleanroom Operations was issued). Nonetheless, the standard reflected the improvements taken in minimising the amount of contamination generated from cleanroom personnel.

2.5 1970s: pharmaceuticals

During the 1970s the cleanroom technology began to be used to a greater extent by the pharmaceutical industry. Although a sterility requirement for the preparation of aseptically produced injectable medicines was established in the 1920s and the requirement for biological preparations to be produced under relatively ‘clean’ conditions had became generally accepted post-World War II[42], the increased application of laminar airflow technology in the 1970s allowed medicines to be produced under far cleaner and more controlled conditions. This development was driven by both technology and by regulatory developments (which had begun with the FDA Good Manufacturing Guidelines of 1963[43]). For example, the UK published its first GMP standards (colloquially termed the ‘Orange Guide’) in 1971, where the use of cleanrooms was specified. However, the initial coverage given to cleanrooms was limited and referred to only very broad environmental cleanliness requirements. There was no inclusion, for example, of air supply or air filtration requirements.

There were other developments with standardisation during the 1970s, in terms of international collaboration and the formulation of cleanroom standards. Although several national societies for contamination control had been formed during the 1960s, the first major international society was founded in 1972: the International Confederation of Contamination Control Societies (ICCCS). With standards, a significant advancement was made with the British Standard for cleanrooms and clean air devices: BS 5295, which was issued in 1976 (later revised in 1989). The British Standard was more extensive in its scope and purpose than FS 209 (the Federal Standard, for instance, did not have a specification for HEPA filters). The BS 5295 covered cleanroom operational aspects like air flow, filters, temperature, humidity, lighting and noise levels. Nevertheless, the standard took no account of microbiological control. Despite the limitation given to microbiological testing, the wider use of cleanrooms and clean zones within the pharmaceutical industry and the parallel evolution of GMP standards, the importance of particulate and microbiological environmental monitoring programmes for cleanrooms generally advanced during the 1970s, and became established during the 1980s[44].

Other developments which took place were with the development of microbiological safety cabinets in the early 1970s. This centred on research undertaken within the US National Institute of Sanitation and by the UK Medical Research Council[45]. This research led to the development of safety cabinets with different safety features depending upon the nature of the biological hazard (typically three different classes of cabinet used appropriate for one of four different biohazard categories[46]).

2.6 1980s: GMP standards

The main development with cleanrooms during the 1980s was no so much with technology but instead with an increase in GMP standards relating to pharmaceutical processing (although improvements with cleanroom design continued throughout the decade[47]). There was little co-ordination between the different bodies responsible for standards, regulations and guidelines, which created considerable drift between the national standards for cleanrooms and the GMP requirements for pharmaceutical processing in terms of the operational and testing requirements.[48]

The first significant GMP 'guidance' was with the publication by the Parenteral Drug Association of an Aseptic Validation Technical Report in 1981[49], which pre-dated the regulatory guideline on this subject, from the FDA, by six years. The FDA issued its first guideline on aseptic manufacturing in 1987: the 'Guideline on Sterile Drug Products Produced by Aseptic Processing'[50]. The guideline made reference to Federal Standard 209 for the purposes of particle classification, and contained the most detailed regulatory information to date relating to sterility assurance, cleanroom control and to microbiological testing. In relation to cleanrooms, the document defined a 'critical area', where the sterilized dosage form, containers, and closures are exposed to the environment, as well as support areas and areas for component preparation[51]. Despite advances with cleanroom technology, most notably the wider use of isolators, the standard was not updated again until 2004.

The microbiological limits in the FDA Guidelines, as with those in the European GMPs, appeared similar to the NASA standards for microbiological testing. A further difference emerged with the United States Pharmacopeia (USP) general Chapter <1116> for cleanrooms. Although the status of the chapter was non-mandatory, it generally became regarded as the best practice guide expected by regulators[52]. The chapter not only set out an alternative particle classification scheme and microbiological monitoring limits, it also established monitoring frequencies[53]. There was also a slight shift in cleanroom philosophy whereby the class of a cleanroom was less tied to the result it achieved when re-qualified, instead the classification of cleanroom became more closely tied to the specific activity being performed[54].

2.7 1990s: towards a global standard

The 1990s saw major changes to cleanroom standards and considerable technological changes to cleanrooms with the advent of isolation technology.

In examining cleanroom standards first, the decade initially saw a further increase, in terms of numbers and stringency, of cleanrooms standards. This was driven, primarily, by the demands of the microelectronics industry[55]. A revised edition of the Federal Standard was published in 1992 as FS 209E[56]. The major change with the standard was that the airborne concentrations of particles in a cleanroom were given in metric units, (particles per m^3) for the first time, and the classifications of the room were defined as the logarithm of the airborne concentration of particles (in relation to the 0.5μm size)[57]. This was to be the last edition of this standard as several nations began the process of constructing a global cleanroom standard.

The genesis of a single international cleanroom standard, covering all industries which used cleanrooms, began in 1991 at a meeting of the International Confederation of Contamination Control Societies. The objective of the meeting was to replace the competing national and supranational standards for cleanrooms with an international standard for the technical and performance specification of cleanrooms. Quite why the direction was not taken towards international acceptance of the Federal Standard 209 is unclear, although some speculate that this related to a desire by some European nations for a degree of independence from the USA[58].

A year later, in 1992, the International Standards Organisation (ISO) Technical Secretariat established Technical Committee ISO/TC 209 'Cleanrooms and associated environments', with a view to progressing a new cleanroom standard. The lead participant was Europe, through its CEN/TC 243 body for 'Cleanroom Technology'. The USA entered the process a year later. The first draft of the new 'international' standard was issued in 1993[59]. During this embryonic stage the possibility of one standard combining both particulate levels and microbial limits was considered[60]. This was quickly abandoned, and the development of a biocontamination standard was later developed (what was to become ISO 14698: Cleanrooms and Associated Controlled Environments – Biocontamination Control). ISO 14698, in its current form, is generally regarded as secondary to GMP guidance.

Figure 7: *CEN/TC 243 meeting at AFNOR, Paris, 8 Oct. 1993 (image courtesy of Schicht Hans H.: 40 years of cleanroom technology – some historical remarks. In: Proceedings of the 17th International Symposium on Contamination Control, Bonn, Germany, 6-9 Sept. 2004, p. 1-10. Edited by: Verein Deutscher Ingenieure (Association of German Engineers), Düsseldorf, Germany 2004.*

The international cleanroom standard – ISO14644 – was issued in 1999[61]. The standard introduced a new classification system (ISO Class *n*, with cleanrooms classified on scale of decreasing cleanliness from class 1 to class 9, across three different room occupancy states: as built, at rest and in operation). There were noteworthy similarities and differences to the Federal Standard 209. What was most similar was that the class name indicated the maximum allowed number of particles of a given diameter. What was different was that the number of classification states was greater. Standard 209 contained six classes, whilst the ISO 14644-1 classification system added two cleaner standards and one dirtier standard (so that ISO class 3 was approximately equal to FS209E class 1, and ISO class 8 approximately equaled FS209E class 100,000). With the ISO classification the reference particle diameter was across a scale of 0.1µm and larger, which was different from the Federal Standard which used the given class number as the maximum allowable concentration of particles, with a reference particle diameter of 0.5 micron. The standard was adopted by most nations during 1999, although the USA did not adopt the standard until 2001[62]. The adoption of ISO 14644 led to various other national standards being superseded, including: UK: BS 5295, Australia: AS 1386, France: AFNOR X44101, Germany: VD Guideline 2083 Sheet 3 – Messtechnik in der Reinraumluft I.2083 and Japan: JIS B 9920. These major national standards had each referenced FS 209 for particulate levels.

During the 1990s other changes took place with regard to cleanrooms standards. This included the first significant revisions to the standards for cleanroom garment testing. The key change was the standardisation of examining garments for contamination. For this the Helmke Drum test became the most widely used test method to examine the decontamination process (a process whereby garments are placed in a rotating drum and tumbled to release particulate matter, which is counted by an optical particle counter)[63]. In relation to cleanroom design, a European-wide standard for HEPA filters was introduced in 1998, which replaced several national standards. This was the EN 1822 and was driven by the need to make trade across Europe easier between member nations[64]. EN 1822 was later revised, and expanded to five parts, including ULPA (Ultra Low Penetration Air) filters.

Figure 8: *Isolator protected ampoule filling machine (Copyright: Cilag AG, CH-8201 Schaffhausen, image courtesy of Schicht Hans H.: 40 years of cleanroom technology – some historical remarks. In: Proceedings of the 17th International Symposium on Contamination Control, Bonn, Germany, 6-9 Sept. 2004, p. 1-10. Edited by: Verein Deutscher Ingenieure (Association of German Engineers), Düsseldorf, Germany 2004.*

In terms of technical innovations, the major shift in cleanroom operations during the 1990s was the more widespread use of isolation technology. Isolators, like conventional cleanrooms, are primarily a post-World War II technology. The origin of isolators was as glove-boxes manufactured to be containment devices. Barrier devices, as rigid wall constructs, began to be used in the 1940s for the holding of animals undergoing medical testing or to be reared 'pathogen free'[65], as well as in the emerging nuclear industry[66]. In both instances the object was containment: of pathogens or radiation[67]. These glove-box isolators were relatively small and did not contain air filtration systems. The pace of isolation technology was relatively slow with a flexible isolator (an actual closed environment rather than a barrier device) developed in the late 1950s[68] and an isolator with an attached air-handling system manufactured in the 1960s. It was during this decade that the concept of an isolator being used to minimise contamination inside, as an addition to preventing contamination from getting outside, began to be progressed.

One of the reasons for the wider use of isolators was the drive to reduce cleanroom operating costs in the electronics industry. This led to increased investment in 'mini-environments' or 'contained environments' which utilised barrier technology so that the product was kept relatively cleaner, whilst also allowing for a lower cleanroom class for the surrounding environment[69].

As with many other cleanroom innovations, pharmaceutical organisations followed behind the electronics industry with the incorporation of isolators. The use of isolators, for both pharmaceutical manufacturing and for sterility testing, began slowly during the 1980s[70] and accelerated in the late 1990s (between 1998 and 2000 the number of isolators operational in the pharmaceutical industry reportedly doubled[71]). The adoption of isolators increased much faster

during the 1990s, although it was not until 1997 that section on isolators was added to European GMPs (EU GMP Guide Annex 1), followed by an FDA draft concept paper in 2002. The primary drive for the use of isolators for aseptic filling was the increased assurance that isolators provided in relation to reducing contamination risks[72] (with a requirement, in some circumstances, to protect operators as with the case of cytotoxic drug manufacture). A similar trend was seen with hospitals, where isolators began to be used in hospital pharmacies from the mid-1980s, and became increasingly used for small scale drug manufacture during the 1990s.

2.8 Cleanrooms in the 21st century

This book, as a practical guide to the use and management of cleanrooms, describes modern cleanroom technology and it is thus appropriate that this chapter goes no further in describing design, certification or standardisation. Nevertheless, as part of the historical trajectory outlined so far, it is important to note that cleanroom technology has continued to accelerate at a rapid pace and it is arguable that, since 2000, there has been a more rapid progression with cleanroom technological developments than those seen previously. Many of these technological developments have been directed towards the manufacture of sterile products, particularly aseptically filled products. This has included use of computer modelling for the design and risk assessment of cleanrooms. There has been wide growth with barrier technology, centred on providing smaller areas of the highest level of cleanliness within the cleanroom, including better isolators with more robust decontamination cycles and the use of Rapid Access Barrier Systems (RABS). In terms of future steps, significant research effort has been placed into nanotechnologies.

2.9 Conclusion

Cleanrooms are an important feature of contamination control across a range of industrial sectors, and an understanding of how modern cleanrooms evolved requires a techno-historical overview of both industrial and medical advances. Cleanrooms, along with a multitude of other technological innovations, were a feature of the post-war reconstruction and industrial growth. The advances with cleanroom technologies were driven by a desire to minimise contamination in order to reduce costs, be that a damaged semiconductor or a rejected pharmaceutical preparation, and to protect people from harm, be that on an operating table or through a contaminated parenteral product. These drivers have led to the cleanroom technology and associated operating issues which this book goes on to discuss.

2.10 References

1. Smart, J. K. ‘History of the Army's Protective Mask’, U.S. *Army Soldier and Biological Chemical Command*: Aberdeen Proving Ground, MD, 2000, public domain.
2. Cook, T. *No Place to Run: The Canadian Corps and Gas Warfare in the First World War,* UBC Press, USA, 2000, p41.
3. Adams, G.W. Doctors in Blue. New York: Henry Schuman; 1952, pp. 113-144.
4. Weiss, E. D. ‘The Second Sacrifice: Costly Advances in Medicine and Surgery During the Civil War’, *Yale Journal of Biology and Medicine*, Vol. 74, 2001, pp169-177
5. Nuland, S. *Doctors: The Biography of Medicine*, New York: Knopf,1988
6. Lister, J. 'On the antiseptic principle in the practise of surgery', *British Medical Association Journal,* 1867, Vol. 2, p246.
7. Brock, T. D. *Robert Koch: a life in medicine and bacteriology*, ASM Press, New York, USA, 1999, p43
8. Anon. Obituary: Evangeline Johnson Merrill, 93, Prominent Supporter of the Arts, *New York Times*, June 18th 1990.
9. Bishop, W.J. *The Early history of surgery*, Barnes & Noble, 1995, pp. 169
10. Porter, R. *The Cambridge illustrated history of medicine*, Cambridge: Cambridge University Press, 2001, pp. 376
11. Austin, P.R. and Timmerman, S. W. *Design and operation of clean rooms*, Business News Pub. Co., Detroit, 1965

[12] Hart, D. 'Sterilization of air in the operating room by special bactericidal radiant energy', *Journal of Thoracic Surgery*, 6, 1936, p45.

[13] Goldstein, H.W., T. Baurer, and I.M. Pikus. Contaminant Removal from Optical Surfaces. *The Journal of Environmental Sciences,* 1973, 16 (5): 24-27.

[14] Straton, S.L. U.S. *Army Uniforms of World War II*, Stackpole Books, USA, 1995, p240.

[15] Berlinger, N. T. 'Why There Hasn't Been an Anthrax Outbreak?', *Invention & Technology*, Winter 2003, Vol. 18, No. 3

[16] Ramstorp, M. Introduction to Contamination Control Technology, Wiley-VCH, Weinheim, 2000, pp1-17

[17] McDade, J.J., Favero, M.S., Michaelsen, G.S. and Vesley, D. 'Environmental Microbiology and the Control of Microbial Contamination', in *National Aeronautics and Space Administration*: *Process Spacecraft Sterilization Technology*, 1966, NASA, Washington, D.C., USA, pp51-86

[18] Whyte, W. *Cleanroom Technology: Fundamentals of Design*, Testing and Operation, 2001, Whiley, Chichester.

[19] Anon. *National Fire Protection Association*, Code 56A, Boston: National Fire Protection Association, 1972.

[20] Lidwell, O.E. and Williams, R.E.O. 'The ventilation of operating-theatres', *Journal of Hygiene,* Vol. 50, 1960, pp449-464

[21] Int-Hout, D. and Cook, G. Hospital Air-Curtain Systems, *Krueger Air Systems*, on-line at: www.krueger-hvac.com (accessed on 1st March 2011)

[22] Whitfield, *et al*. Definition of surgical microbiological clean air, *American College of Surgeons Bulletin*, 1976.

[23] Holbrook, D. Controlling contamination: the origins of clean room technology, *History and Technology*, Volume 25, Issue 3, September 2009, pages 173 - 191

[24] Singer, N. 'Sandia engineer Willis Whitfield, whose invention made possible the modern electronic age, revisits Labs', *Sandia Lab News*, February 2005, p7

[25] Anon. Patents on Line. Patent 04/862430. At: www.freepatentsonline.com/3638404.html (accessed 1st March 2011)

[26] Anon. Standard and Guidelines for the Design and Operation of Clean Room and Clean Work Stations. USAF Technical Order 00-25-203.

[27] American Airforce, March 1961: Technical Manual AFTO 00.25-203: Standards and Guidelines for Design and Operation of Clean Rooms and Clean Work Stations, July 1, 1963. Reference: Military Custodial Services Manual, 1982, Departments of the Army and the Navy, Washington, D.C.

[28] Hall, I.B. and Bruch, C. W. 'Procedures Necessary for the Prevention of Planetary Contamination' in Florkin, M. (ed.) *Life Science and Space Research III,* North-Holland Publishing Company, Amsterdam, Netherlands, 1965, pp48-62

[29] Grose, V.L. Reliability Planning in Space Systems. *The Journal of Environmental Sciences*, 1960, 3 (5): 9-10, 23.

[30] Anon. NASA Unmanned Spacecraft Decontamination Policy, National Aeronautics and Space Administration Manual Number 4-4-1, Issued September 9th 1963, NASA, Washington, D.C.

[31] Portner, D.M., Hoffman, R.F., Decker, H.M., and Phillips, C.R. 'The Level of Microbial Contamination in a Clean Room During a One Year Period', National Aeronautics and Space Administration Report CR-60184, NASA, Washington, D.C., 1964

[32] Michaelsen, G.S. 'The Bacteriology of "Clean Rooms", National Aeronautics and Space Administration Report CR-63470, NASA, Washington, D.C., 1965

[33] Anon. Clean Room and Work Station Requirements. Federal Standard 209. Federal Drug Administration, Washington D.C., USA

[34] Whyte, W., Shaw, B. and Freeman, M. A. R. 'An evaluation of a partial-walled laminar flow operating room', *Journal of Hygiene*, Vol.73, Issue 61, 1974, pp61-74

[35] Kile, A. 'Clean air in the OR', American Academy of Orthopaedic Surgeons, on-line article, 2007: www.aaos.org/news/bulletin/oct07/research1.asp (accessed 1st March 2011)

[36] Laufman, Harold, M.D. *Surgeon's Requirements for Operating Room air Environment*. Healthcare Facilities Management Series, 1999.

[37] Irvine, R., Johnson, B. L., Amstutz, H. 'The relationship of genitourinary tract procedures and deep sepsis in total hip replacement', *Surgical Gynecology and Obstetrics*, 139, 1974, pp701-706

[38] Scott, C. Laminar/Linear Flow System of Ventilation: Its Application to Medicine and Surgery, *The Lancet*, Vol. 295, 1970, Issue 7654, pp989-993

[39] Whyte, W. And Shaw, B. H. ‘The effect of obstructions and thermals in laminar-flow systems’, *Journal of Hygiene*, Vol.74, 1974, pp415-423

[40] Weber B.G., Stühmer G., Meierhans R.: Die Bekleidung des Operationsteams in einer sterilen Operationskabine (Garmenting of the operating team in a sterile operat-ing cabin). In: Weihe W.H., Wanner H.U. (ed.): *Reinraumtechnik I*, Swiss Society for Contamination Control, Zürich, 1973, pp96-98.

[41] Anon. Standard Test Methods for Sizing and Counting Particulate Contaminant In and On Clean Room Garments, ASTM F51-68, issued 15th August 1968

[42] Stockdale, D. ‘Overview of Aseptic Fill/Finish Manufacturing’, *American Pharmaceutical Review,* 2004, on-line version (http://americanpharmaceuticalreview.com/ViewArticle.aspx?ContentID=73) (accessed 18th March 2011) unpaginated

[43] Anon. “Milestones in U.S. Food and Drug Law History,” FDA Backgrounder: Current and Useful Information from the Food and Drug Administration (FDA, Rockville, MD, August 1995), www.fda.gov/opacom/backgrounders/miles.html (accessed 15th February 2011).

[44] DeVisser, A.D. and Enzinger, M. ‘Microbiological Aspects of an Environmental Program’, Pro. Sec. PMA Seminar Program on Validation of Sterile Manufacturing Processes, 1979

[45] Clark, R. P. ‘The history of the development of microbiological safety cabinets. Part 1: the 1970s and 1980s’, *Clean Air and Containment Review,* Issue 1, 2010, pp5-7

[46] Anon. Centers for Disease Control and Prevention. Primary containment for biohazards: selection, installation and use of biological safety cabinets. Washington, DC: U.S. Government Printing Office, 2000.

[47] Waring, A. The design and management of cleanrooms in the late 1980s, *International Biodeterioration*, Volume 25, Issues 1-3, 1989, Pages 131-135

[48] Farquharson, G. J. ‘Picking a route to cleanliness’, *Manufacturing Chemist,* September 1987, pp39-41

[49] Parernteral Drug Association. *Validation of Aseptic Filling for Solution Drug Products*, PDA Technical Monogram, 1981, Number 2.

[50] Federal Drug Administration. Guideline on Sterile Drug Products Produced by Aseptic Processes, FDA, Rockwell, 1987.

[51] Peck, R. D. ‘The Proposed Revision of Federal Standard 209B’, *The Journal of Environmental Sciences*, Volume 31, Number 5/September-October 1988, pp43-47

[52] Akers, J. E. ‘Environmental monitoring and control: proposed standards, current practices, and future directions, *PDA Journal of Pharmaceutical Science and Technology*, 1987, Vol. 51, pp36-47

[53] Halls, N. *Microbiological Environmental Monitoring Explained,* Sue Horwood Publishing, Storrington, UK, 2002, p11.

[54] Agalloco, J. and Ackers, J. ‘Aseptic Processing for Dosage Form Manufacture: Organization and Validation’, Agalloco, J. ‘Isolator Decontamination’, pp277-285 in Agalloco, J.and Carleton, F.J. (Eds.) *Validation of Pharmaceutical Processes*, 2008, Informa Healthcare, New York, pp317-326

[55] Farquharson, G. ‘Using current environmental standards and guidelines in pharmaceutical clean room applications’, paper presented to Interphex 90 conference, 20th-23rd November 1990, Birmingham, UK

[56] Federal Standard 209E : *Airborne Particulate Cleanliness Classes In Cleanrooms and Clean Zones*. September 11, 1992

[57] Whyte, W., MacLeod, B. and Morrison, L. ‘Cleanroom classification standards’, *Cleanroom Technology,* February 2002, pp7-9

[58] Scottish Society for Contamination Control. Proceedings of a Meeting held in the National Exhibition Centre, Birmingham on Wednesday 20th October, 1993. S2C2, Glasgow, Scotland.

[59] International Standards Organisation. Document 243N31 ‘Classification of Cleanrooms and Clean Air Controlled Spaces by Airborne Particulate Content’, ISO, Geneva.

[60] Reeves, M. C. ’The European Contribution to World Cleanroom Standards’, paper delivered at a meeting held in the National Exhibition Centre, Birmingham on Wednesday 20th October, 1993, convened by the Scottish Society for Contamination Control.

[61] Donovan, R. P. “The coming debut of ISO cleanroom standards” PennWell: *CleanRooms* April, 1999

[62] Matthews, R.A. “209 is dead, long live 209”: *CleanRooms*, February 2002

[63] Anon. IEST-RP-CC003: garment system considerations for cleanrooms and other controlled environments, Institute of Environmental Sciences and Technology, USA.

[64] Kulie, W. ‘High efficiency filtration’, *Cleanroom Technology,* July/August 2002, pp18-19

[65] Reyniers, J.A. and Trexler, P.C. ‘The germ-free technique and its application to rearing animals free from contamination’, in Reyniers, J.A. (Ed.) *Micrugical and germ-free methods*, Springfield: USA, Thomas Publishers, 1943.

[66] Farquharson, G. J. ‘British and European Experience with Isolation Technology’, in Wagner, C.M. and Acker, J.E, (Eds.) *Isolator Technology*: *Applications in the Pharmaceutical and Biotechnology Industries*, Interpharm Press, Buffalo Grove, USA, 1995*,* pp77-96

[67] Wagner, C.M. ‘Current Challenges to Isolation Technology’ in Wagner, C.M. and Acker, J.E, (EDds.) *Isolator Technology*: *Applications in the Pharmaceutical and Biotechnology Industries*, Interpharm Press, Buffalo Grove, USA, 1995, pp3-36

[68] Trexler, P.C. and Reynolds, L.I. ‘Flexible film apparatus for the rearing of germ-free animals’, *Applied Microbiology,* Vol.5, pp406-412.

[69] Rosin, L.J. ‘Technologies and Training: Moving Sterility to New Levels’, *BioProcess Internaitonal,* November 2008, pp16-23

[70] Whyte, W., *et al.* ‘An evaluation of the routes of bacterial contamination occurring during aseptic pharmaceutical manufacturing’, *Journal of Parenteral Science and Technology, 1982, pp102-107.*

[71] Weatherhead, M. and Green, S. ‘Isolator Technology’ in Hodges, N. and Hanlon, G. *Industrial Pharmaceutical Microbiology: Standards and Controls,* Euromed, UK, 2003.

[72] Agalloco, J. ‘Isolator Decontamination’, in Agalloco, J.and Carleton, F.J. (Eds.) *Validation of Pharmaceutical Processes,* 2008, Informa Healthcare, New York, pp277-285.

Appendix I:

2.11 Historical milestones of cleanroom development

Time period	*Development*
1823	Development of the air filter as a 'respirator' for fire fighters, USA
1864	First papers citing the need for ventilation in hospitals
1880s	First commercial production of antibacterial chemicals for use in hospitals by Johnson and Johnson, USA
1890s	Improvements in hospital cleanliness, such as use of clean bandages
1914-1918	Improved development of air filters for gas masks used in World War I, Canada
1930s	Use of ultraviolet light to minimise contamination in 'clean' spaces
1939-1945	Use of 'white rooms' for assembling precision instruments under clean conditions using 'super interception' filters
1944-1945	Arthur D Little and the Atomic Energy Commission develop HEPA filter technology, USA
1950s	HEPA filters become widely used across many industries
1950s	Improved ventilation in hospitals, concept of 'air changes' applied
1961	Sandia Corporation develops first laminar airflow cleanroom
1961	First national standard for cleanrooms: Air Force Technical Order 00-25-203, USA
1961	Charnley-Howorth clean enclosure system for operating theatres
1962	FDA issues first Good Manufacturing Practices document
1963	NASA cleanroom standard is the first to make reference to microbiological testing
1963	Federal Standard 209 issued and the logarithmic classification system (class 100, 1,000 etc), USA
1966	Revised Federal Standard 209a issued
1968	First standard for cleanroom clothing, ASTM F51-68
1969	World Health Organisation issues its first Good Manufacturing Practices document
1971	UK 'Orange Guide' for Good Manufacturing Practices issued
Early 1970s	Microbiological Safety cabinets produced commercially
1973	Revised Federal Standard 209b issued
1976	Amendment to Federal Standard 209a issued
1976	British Standard 5295 issued, outlining all physical aspects of cleanroom testing
1981	PDA issue guideline on aseptic processing for pharmaceutical manufacturing
1987	FDA issue 'Guideline on Sterile Drug Products Produced by Aseptic Processing'
1987	Revised Federal Standard 209c issued
1988	Revised Federal Standard 209d issued
1989	British Standard 5295 revised
1992	Revised Federal Standard 209e issued
1997	Update to EU GMP includes isolation technology for the first time
1998	European standard for HEPA filters issued: EN 1822
1999	International cleanroom standard ISO14644 issued and adopted by most nations (excluding the USA)
2001	USA adopts standard ISO 14644
2004	FDA revise guideline on Aseptic Processing

Cleanroom standards and GMP requirements

Mark Hallworth, Particle Measuring Systems, Boulder, Co, USA
Madhu Raju Saghee, Assistant Manager – QA, Novartis India Ltd, Thane, India

3.1 Introduction

Pharmaceutical products are manufactured to meet exacting standards of both efficacy and quality. All aspects of quality are reviewed in light of the risks associated with the delivery method: injected, ingested etc.; and the manner in which they were produced: aseptic, terminally sterilised or under lesser controlled conditions. This chapter looks at one aspect in particular and that is the quality of the environment in which the product is manufactured and the standards that surround the particle concentration limits that determine the basis of a controlled environment.

This chapter examines the standards for physical testing (the current ISO 14644 series) and the standards for microbiological examination of cleanrooms (ISO 14698). In order to put these current cleanroom standards in context, the chapter provides an overview of the history of cleanroom standards.

3.2 History of cleanroom standards

The history of particle counting is not as complex, nor as long as that of microbiology testing of environments and only dates as far back as the 1950's when the first particle counters was used to determine the environmental conditions of cleanrooms (for a review of the development of cleanrooms, refer to Chapter 2).

3.2.1 USAF (T.O.) 00-25-203 (1961)

From these early applications, a common standard was written for the American Air Force in 1961 and was designated as the Technical Manual (T.O.) 00-25-203[1]. This document reviewed the requirements for cleanroom design, airborne particle standards, along with operating procedures associated with how to use the new cleanrooms, entry procedures, clothing, cleaning of materials and the cleanroom itself and the restriction of certain items (lead pencils) within the clean areas.

The new standard identified a procedure for determining the level of cleanliness of a cleanroom based upon the maximum number of particles within a fixed sample volume, which at that time was 10ft^3 or 283 litres of air. Sample locations were arranged uniformly within the cleanroom (**Figure 1**) being tested such that the cleanroom was deemed to be uniform across its entire floor area.

Testing was to be performed at sizes down to a minimum of 0.5m; it was deemed that at that time 0.5m was the only reliable and repeatable measurement using existing technology.

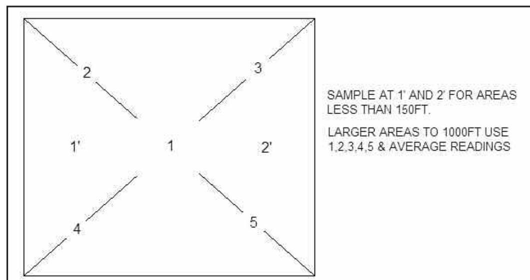


Figure 1: *Sample point locations for USAF (T.O.) 00-25-203*

3.2.2 Federal Standard 209

The first Federal Standard associated with cleanroom classification, Federal Standard 209 was published in the USA and issued in 1963 as the 'Cleanroom

and Work Station Requirements, Controlled Environments'[2]. It was subsequently revised in 1966 (FS209A), 1973 (B), 1987 (C), 1988 (D) and finally in 1992 (E) which included a metric conversion of the allowable room classifications. The standard also gave information on the type of device required to test the areas along with information relating to the location of the samples, the sample volume required to gain statistical significance and the maximum particle concentrations required for each given location. This standard was also based upon the 0.5m particle size although measurements smaller than this were available (0.1m laser particle counter from 1972) it was the only repeatable size that could be assured.

Sample point location was determined in one of several ways depending on the class and design of the cleanroom, again it was used to ensure that the cleanroom was not only below a prescribed maximum limit of particles in air, but also that it was uniform across the entire floor area.

For unidirectional cleanrooms the number of locations was determined by the lesser of either:

Floor Area/25

or

Floor Area/√Room Classification

So, for a Class 100 cleanroom of area 10ft x 15ft the result would be:

(10x15) = 150/25 = 6

or

(10x15)/√100 = 150/10 = 15

Therefore, in this example, 6 sample points would be chosen

The location of these points would be equidistant and at work height throughout the entire area (**Figure 2**).

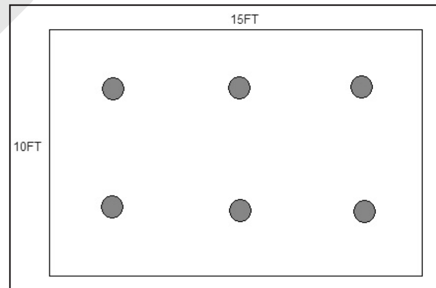


Figure 2: *Sample point locations for FS209 example*

For non-unidirectional cleanrooms (turbulent flow) the number of sample points is based solely on the Floor Area/√Room Classification.

The maximum concentration in these rooms was determined by a table (**Figure 3**) that used the formula:

Maximum particles = Room Classification x $(0.5/\text{particle diameter})^{2.2}$

Finally the sample volume was required such that a statistically valid number of particles was likely to be measured. This was also a formula based decision:

Volume = 20/Class limit

So for the above example of an FS209 Class 100 room, where 6 sample points were required, the sample volume at each location needed to be:

Volume = 20/maximum allowable concentration of 0.5m particles

Volume = 20/100 = 0.2ft^3

The Federal Standard became the *de facto* standard for cleanroom classification and is still today used by some to describe a room classification. Class 100, Class 10,000 etc., are common terms used in room classification, although as from 1999 the Federal Standard had become usurped as

Table 1: Maximum particle concentrations as indicated in FS209E

Class Name **		Class limits									
		0.1 m		0.2 m		0.3 m		0.5 m		5 m	
		Volume units		Volume units		Volume units		Volume units		Volume units	
SI	English***	(m^3)	(ft^3)	(m^3)	(ft^3)	(m^3)	(ft^3)	(m^3)	(ft^3)	(m^3)	(ft^3)
M1		350	9.91	75.7	2.14	30.9	0.875	10.0	0.283	–	–
M1.5	1	1,240	35.0	265	7.50	106	3.00	11.0	1.00	–	–
M2		3,500	99.1	757	21.4	309	8.75	100	2.83	–	–
M2.5	10	12,400	350	2,650	75.0	1,060	30.0	353	10.0	–	–
M3		35,000	991	7,570	214	3,090	87.5	1,000	28.3	–	–
M3.5	100	-	-	26,500	750	10,600	300	3,530	100	–	–
M4		-	-	75,700	2,140	30,900	875	10,000	283	–	–
M4.5	1,000	-	-	-	-	-	-	35,300	1,000	247	7.00
M5		-	-	-	-	-	-	100,000	2,830	618	17.5
M5.5	10,000	-	-	-	-	-	-	353,000	10,000	2,470	70.0
M6		-	-	-	-	-	-	1000,000	28,300	6,180	175
M6.5	100,000	-	-	-	-	-	-	3530,000	100000	24,700	700
M7		-	-	-	-	-	-	10000000	283000	61,800	1,750

the common standard. An ISO standard was released for the determination and classification of cleanrooms: ISO 144644. The standard was adopted by European nations in 1999 and the official recognition of FS209E was dropped by the Federal Standards in November 2002.

3.3 ISO 14644 Cleanroom Standard

The ISO 14644 standard comprises of a series of sub-parts, which are shown in the table below:

Table 2: Component parts of ISO 14644

Reference	Title	Year of last revision
ISO 14644-1	Cleanrooms and associated controlled environments - Part 1: Classification of air cleanliness	1999
ISO 14644-2	Cleanrooms and associated controlled environments - Part 2: Specification for testing and monitoring to prove continual compliance with ISO 14644-1	2000
ISO 14644-3	Cleanrooms and associated controlled environments - Part 3: Metrology and test methods	2005
ISO 14644-4	Cleanrooms and associated controlled environments - Part 4: Design, construction and start up	2001
ISO 14644-5	Cleanrooms and associated controlled environments - Part 5: Operations	2004
ISO 14644-6	Cleanrooms and associated controlled environments - Part 6: Terms and definitions.	2007
ISO 14644-7	Cleanrooms and associated controlled environments - Part 7: Enhanced clean devices	2004
ISO 14644-8	Cleanrooms and associated controlled environments - Part 8: Classification of airborne molecular contamination	2006
ISO 14644-9	Cleanrooms and associated controlled environments - Part 9: Classification of surface particle cleanliness	2008
ISO 14698-1	Cleanrooms and associated controlled environments - Biocontamination control - Part 1: General principles and methods	2003
ISO 14698-2	Cleanrooms and associated controlled environments - Biocontamination control - Part 2: Evaluation and interpretation of Biocontamination data	2003

Each of the component parts of ISO 14644 is next examined.

3.3.1 ISO 14644-1

From 1999 the new ISO 14644 room classification suite of standards became active, the first of which was ISO 14644-1[3] which determined the method by which a room should be classified and the maximum allowable particles within a fixed volume of air. The reader should note that although ISO 14644 has been adopted globally for cleanroom classification. there are differences for routine monitoring, particularly between ISO 14644 and EU and WHO GMP.

The certification state of the cleanroom must be defined in advance of testing; three states exist within the context of ISO 14644-1:

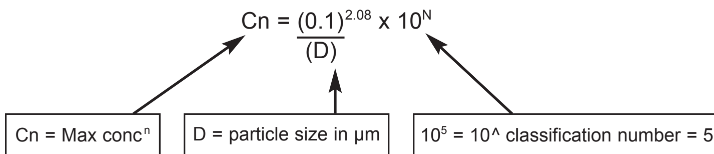
- **As built**, a completed room with all services connected and functional, but without production equipment or personnel within the facility.
- **At rest**, a condition where all the services are connected, all the equipment is installed and operating to an agreed manner, but no personnel are present.
- **Operational**, all equipment is installed and is functioning to an agreed format, and a specified number of personnel are present working to an agreed procedure.

The limits for the cleanroom concentration of particles greater than a prescribed size are defined and presented in Table 1 (above).

Table 3: Airborne particulate cleanliness classes for cleanroom and clean zones (as indicated in ISO 14644)

ISO classification number	Maximum concentration limits (particles per cubic metre of air) for particles equal to and larger than the sizes shown below (refer to ISO 14644-1 part 3.2)					
	0.1µm	0.2µm	0.3µm	0.5µm	1µm	5µm
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1,000	237	102	35	8	
ISO Class 4	10,000	2,370	1,020	352	83	
ISO Class 5	100,000	23,700	10,200	3,520	832	29
ISO Class 6	1,000,000	237,000	102,000	35,200	8,320	293
ISO Class 7				352,000	83,200	2,930
ISO Class 8				3,520,000	832,000	29,320
ISO Class 9				35,200,000	8,320,000	293,000

These limits have been defined in accordance with the calculation from the standard:



$$Cn = \left(\frac{0.1}{D}\right)^{2.08} \times 10^{N}$$

The relationship of particle size to its abundance within a population is therefore a function of $1/D^{2.08}$ and if the particle size is plotted against its concentration on a log/log scale, the slope of the curve for each class is 2.08; this relationship is shown in the table above.

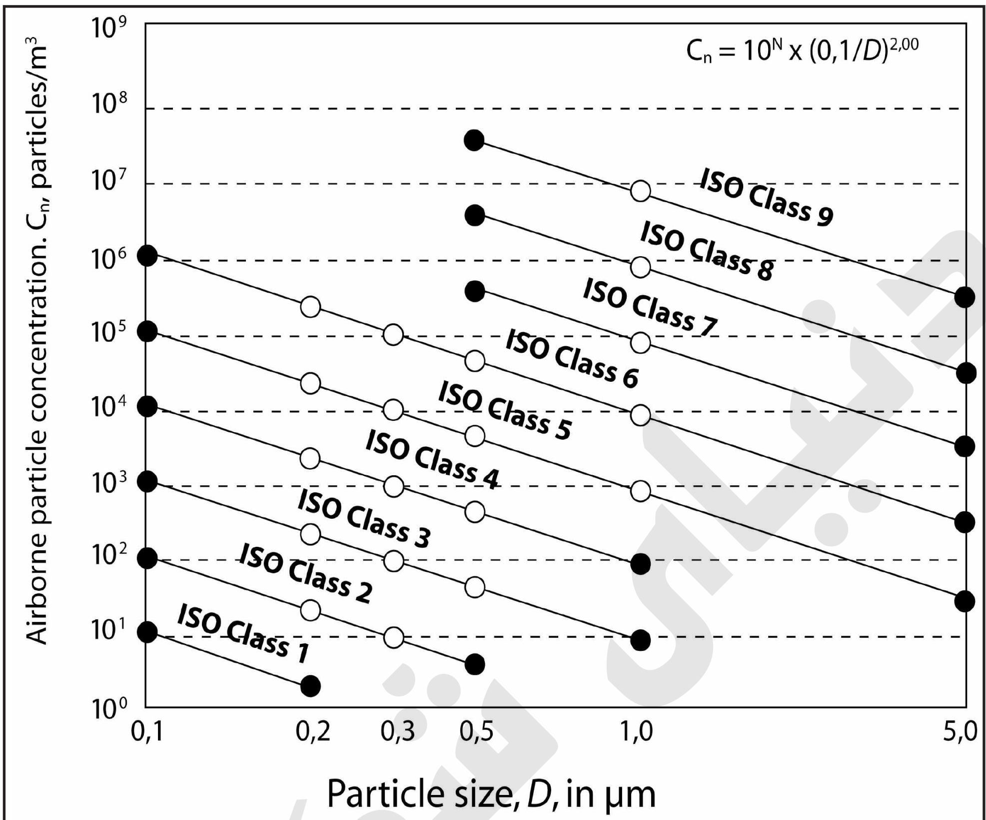


Figure 3: *Graphical representation of ISO class concentrations limits for selected classes.*

The designation for cleanroom or clean zone certification should also include the following elements:

- The room classification number expressed as "ISO Class N".
- The occupancy state.
- The considered particle size. It is also possible to certify a cleanroom at multiple sizes; if this is the case then the sample volume requirement for the largest particle size is used.

An example would be: unidirectional airflow device *x* is an ISO Class 5 clean zone at 0.5m (3520n/m^3), operational state.

The clean zone now needs to be tested to prove the statement, the ISO 14644-1 standard identifies each of the component steps required to prove compliance.

<u>Example:</u>
Assume we have a clean air device that we want to use for aseptic preparation area. This area needs to meet ISO Class 5 at 0.5m (=10,000/ft^3) in the operational state, how do we go about the process of determining the classification of this area?

The room is 12m by 5m and has a work table in the centre of the room.

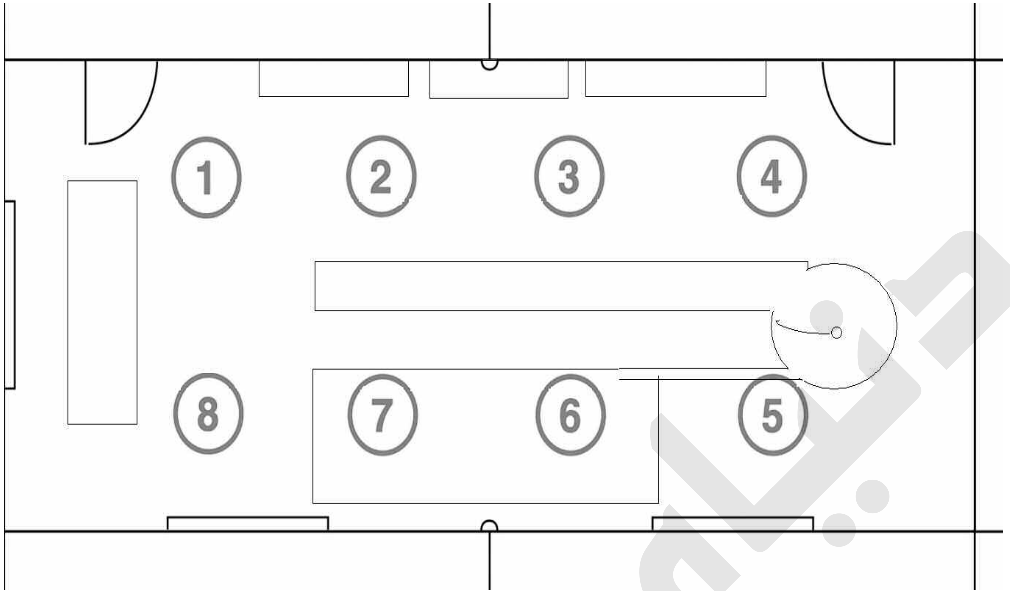


Figure 4: *Diagram showing the locations for monitoring within the clean zone.*

Step 1. Calculate the maximum permitted particle concentration

$$Cn = \left(\frac{0.1}{0.5}\right)^{2.08} \times 10^5 = 3517 \text{ rounded to } 3520 n/m^3$$

Cn = Max concⁿ

(0.5) = particle size in µm

10^5 = 10^ classification number = 5

Step 2. Calculate the number of sample locations

$$N_L = \sqrt{A} = \sqrt{12 \times 5} = \sqrt{60} = 7.74 = 8$$

Square root of floor area in m

Step 3. Calculate the sample volume

$$Vs = \frac{20}{C_{nm}} \times 1000$$

$$= \frac{20}{3517} \times 1000 = 5.69 \text{ litres}$$

From ISO 14644-1 Section B.4.2.2, Minimum Volume = 2 litres and Sample period = 1 minute. Standard particle counters run at 28.3l/min, therefore, if a 1 minute sample is taken the criteria is met.

So to meet the specification we shall take a 1 minute sample at each of the 8 locations.

Step 4. Take measurements at each location and record results

Table 4: Illustration of locations within the example clean zone and measurement results

Location	Number/ft^3	Number/m^3
1	20	708
2	25	885
3	43	1522
4	66	2336
5	95	3363
6	73	2584
7	65	2301
8	59	2089

Step 5. Perform the statistical analysis on the results

Step 5.1. Mean

$$x = \frac{(708 + 885 + 1522 + 2336 + 3363 + 2584 + 2301 + 2089)}{8}$$

$$= \frac{15788}{8}$$

$$= 1974 \text{ n/m}^3$$

Step 5.2. Standard Deviation

$$S^2 = \frac{1}{7} [(708-1974)^2 + (885-1974)^2 + (1522-1974)^2 + (2336-1974)^2] [+ (3363-1974)^2 + (2584-1974)^2 + (2301-1974)^2 + (2089-1974)^2]$$

$$= 5,545,600$$

$$S = \sqrt{5,545,600}$$

$$= 92355 \text{n/m}^3$$

Step 5.3. 95% UCL

$$95\% \text{ UCL} = 1974 + 1.9\frac{(2355)}{(8)}$$

Sample averages = 8 From. Table C1, 1.9 (in ISO 14644 Part 1)

Average (1974) — Std Dev (2355)

$$= 2533 \text{ n/m}^3$$

Step 6. Define report

Each Location max = 3,363n/m^3 <3,520 class limit = PASS
95% Upper Confidence Interval (UCL) = 2533n/m^3 <3,520 class Limit = PASS

This area meets the specification for an ISO class 5 clean zone at 0.5m and can now be used for the purpose that it was designed for. Room classification will need to be repeated on a frequency defined by ISO 14644-2.

3.3.2 ISO 14644-2: Specifications for testing and monitoring to prove continued compliance

The ISO 14644 Part 2[4] was released in 2000 and became a supplemental document for ISO 14644-1. It describes the frequency and requirements of a system to prove continued compliance for cleanroom classification.

Once a cleanroom has been fully classified there are certain aspects that need to be maintained such that the cleanroom operator can continue to claim that the cleanroom continues to meet the classification and in which state that classification was made.

Room classification will need to be repeated on a frequency defined by ISO 14644-2. For a Class 5 or cleaner environment this is defined as every 6 months, for a Class 6 or greater cleanroom the interval is every 12 months. This interval can be extended to the maximum permitted interval providing that the organisation shows that ‘no significant change’ has occurred in the control of their cleanroom, by evidence of continued compliance. Therefore Class 5 cleanrooms do not necessarily need to be re-validated on a 6 month basis and the period between re-classifications may be extended up to a maximum of 12 months.

Table 5: Recommended reclassification intervals

Class	Maximum Time Interval	Test Method
≤ ISO 5	6 months	ISO 14644-1 Annex B
≥ ISO 6	12 months	ISO 14644-1 Annex B

The document also outlines the requirements of a risk assessment to be performed in determining the details of a monitoring plan. These include: what level of monitoring is required?; what action and alert limits will be set?; and what to do should an action or alert limit be exceeded?

It is also required that the system being used to demonstrate control over the environment be based upon a written design and that design should take into account the risks associated with the activities being performed and the best practice to both monitor the environment and establish suitable control limits.

3.3.3 ISO 14644-3: Test methods

This part of ISO 14644 specifies the test methods for the performance of cleanrooms[5]. Tests are specified for two types of cleanrooms: unidirectional flow and non-unidirectional flow (or turbulent flow). The test methods recommend what test apparatus and test procedures are required and where appropriate an alternative procedure is suggested. For some of the tests, several different methods and apparatus are recommended.

A list of the required and optional tests and the associated reference is found on **Table 6** on the opposite page.

It is important to note here that the standard gives information relating to the calibration functions of a laser particle counter; these have been superseded by the standard ISO 21501-4 – Light Scattering Airborne Particle Counter for Clean Spaces (2007)[6].

3.3.4 ISO 14644-4: Design, construction and start-up

This part of ISO 14644 specifies the requirements for the design and construction of cleanroom installations[7]. It provides the details for all the important elements for the design and construction of a new cleanroom or clean facility.

Table 6: Test method requirements for room classification

Required test items	Required Test	Test Procedure	Test Apparatus	Referenced in ISO standard
Airborne particle count for classification and test measurement of cleanrooms and clean air devices	4.2.1	B.1	C.1	14644-1 and 14644-2
Airborne particle count for ultrafine particles	4.2.1	B.2	C.2	14644-1
Airborne particle count for macro-particles	4.2.1	B.3	C.3	14644-1
Airflow test	4.2.2	B.4	C.4	14644-1 and 14644-2
Air pressure difference test	4.2.3	B.5	C.5	14644-1 and 14644-2
Installed filter system leakage test	4.2.4	B.6	C.6	14644-2
Airflow direction test and visualisation	4.2.5	B.7	C.7	14644-2
Temperature test	4.2.6	B.8	C.8	7726
Humidity test	4.2.6	B.9	C.9	7726
Electrostatic and ion generator test	4.2.7	B.10	C.10	
Particle deposition test	4.2.8	B.11	C.11	
Recovery test	4.2.9	B.12	C.12	14644-2
Containment leak test	4.2.10	B.13	C.13	14644-1 and 14644-2

***NOTE**: recommended tests are not presented in order of importance. The order in which tests should be performed may be based upon the requirements of a specific document or after agreement between the customer and supplier.*

As the standard is only focused on the design and construction of a cleanroom or clean facility it is important to list important aspects which are not covered in the standard, these include:

- The user requirements of certain aspects of performance
- The final use of the cleanroom
- Fire and safety regulations, these are mandated through local regulations
- How the cleanroom will be used with respect to process media and utility services
- Operation and maintenance is excluded

The standard includes sections on defining requirement, the planning and design of the cleanroom, construction and start-up, the testing and approval of the delivered facility and the documentation required to hand over the facility to the end user.

There is also a series of annexes that list:

- How a cleanroom is controlled and how it establishes the required levels of cleanliness and how segregation of the different areas is maintained (Annex A).
- Different examples of cleanroom and their intended use (Annex B).
- The testing and approval of the completed facility (Annex C).
- The layout of the facility taking into account what activities need to be planned for and what services are required (Annex D).
- The selection of materials to be used in construction and the consideration of how they are likely to be used (Annex E).

- The environmental control of the cleanroom, including temperature, humidity, lighting, noise and energy usage (Annex F).
- The filtration of the facility (Annex G).
- A list of all aspects that need to be discussed as part of the facility design (Annex H).

3.3.5 ISO 14644-5: Operations

ISO 14644-5 provides a source for addressing the primary functions of how a cleanroom should be used and the operations that are essential to their use[8]. These include: operational systems, cleanroom clothing, personnel, stationary equipment, portable equipment and materials and also the cleaning and disinfection of the cleanroom. The standard includes these sections as being a requirement of cleanroom operations and as such must be followed or have a procedure against each one, it then expands on each section to give more information regarding how each can be implemented. These are discussed below.

Operational systems: this section defines the organisation of the cleanroom for producing quality products while following proper cleanroom practices. The elements covered include: following Standard Operating Procedures (SOP), risk assessments, training, documentation requirements, maintenance records, safety and methods for modification and improvements to the cleanroom. It also includes examples of accepted risks assessment methods and reviews various risk factors such as clothing, personnel, equipment and environmental conditions.

Personnel: this section reviews the training of operators to ensure that the cleanroom is operated in the correct manner. The section includes a sample gowning procedure and a guide to the establishing of internal standards for hygiene and safety.

Stationary equipment: This section focuses on the layout of permanently mounted equipment within the cleanroom, how and where it should be cleaned and located in the cleanroom and how it should be transported into place. It also discusses preventative maintenance and how it should be performed while maintaining a controlled environment.

Materials and portable equipment: the transfer of materials is possibly the second highest risk to the cleanroom and as such controls should be in place to control the transfer of contaminants into the cleanroom. This section covers many aspects of transferable materials including chemicals in pipe work, mops and buckets for cleaning, note pads and paper and defines criteria that specifies the properties that reduces the risk.

Cleanroom cleaning: this part reviews the role of cleanroom clothing and its protection of the operators from the potential harmful effects of cleanroom materials also the protection of the clean products from contamination from the personnel. People are the primary source of contamination in a cleanroom and so the choice of clothing is critical if one is to protect the environmental conditions and product being manufactured. Choice of clothing is based upon three key factors: product sensitivity, the classification of the cleanroom and safety. This annex assists the choice of clothing used covering a variety of clothing properties available. The requirements for cleaning a cleanroom include the methods, personnel, training, schedules and contamination checks. This section describes the procedures for routine cleaning and certain activities that may need control but are beyond a typical activity, such as construction. It defines the 10 stages of construction related cleaning programme (**Table 7**). For more detail about cleaning and disinfection the reader is advised to refer to Chapter 20 of this book.

3.3.6 ISO 14644-6: Vocabulary

The part (ISO 14644-6)[9] contains a full listing of all the terms and vocabulary required for the ISO

Table 7: The ten stages of cleanroom cleaning

Stage	Purpose	Responsibility	Method	Standard
1. Demolition	Prevent any concentration of dust that will be difficult to reach at a later stage.	Contractor	Vacuum clean	Visual clean
2. Utility installation	Remove local contaminants caused by installing primary utilities	Installation engineer	Vacuum clean Wipe down	Visual clean
3. Early construction	All visible contamination should be cleaned including floors, walls and ceilings	Cleaning contractor	Vacuum clean Wipe down Protective seal	Visual clean
4. Preparation for AHU installation	Clean any dust from ductwork and apply a positive pressure to cleanrooms	Installation engineer and cleaning contractor	Vacuum clean Wipe down	Wipe clean
5. Pre-installation of filters	Remove settled dust from ceilings, walls and floors	Cleaning contractor	Wipe down	Wipe clean
6. Mounting filters	Remove possible contamination caused by installation of filters	Cleanroom HVAC filter engineer	Clean and disinfect all surface edges on all sides	Wipe clean
7. Air conditioning adjustment	Remove suspended dust from system and over pressure the installation	Cleanroom HVAC filter engineer	Air conditioning air flushing	Wipe clean
8. Classified state	Removed all deposited and clinging dust from every surface	Professional cleaning by specially instructed personnel	Wipe down	Wipe clean
9. Validation	Verify the cleanroom to the required specification	Installation engineer and certification engineer	Monitor airborne and surface dust, air velocity, temperature and humidity	Wipe clean
10. Daily or periodic cleaning	Maintaining cleanroom in compliance with design	Cleanroom manager and cleaning contractor	Further information is given in Annex F1 of the standard see Annex F1 – F8	Customer specification (normally wiper clean is undertaken using detergent and disinfection)

Note 1. During stage 4-10 all high efficiency components such as filters should arrive on site protected from contamination.

Note 2. During stages 6-10 all activities should be done wearing cleanroom clothing.

14644 documents along with those required for the ISO14698 set of documents for microbiological control of cleanrooms.

3.3.7 ISO 14644-7: Separative devices

This part of ISO 14644 (part 7)[10] specifies the minimum requirements for the design, construction, installation, testing and approval of separative devices in those respects where they differ from cleanrooms as described in ISO 14644-4 and 14644-5. Separative devices range from open to closed systems and include isolators and Rapid Access Barrier Systems (RABS).

The limitations are:

- Application-specific requirements are not addressed

- User requirements are as agreed by customer and supplier
- Specific processes to be accommodated in the separative device installation are not specified
- Fire, safety and other regulatory matters are not considered specifically; the appropriate national and local requirements shall be respected
- Full-suits are not within the scope of this standard

This ISO document includes a series of Annexes that give more information relating to the requirements of the ISO standard. The separation continuum concept, the idea that the boundary creates an improved separation between the inside and outside conditions of a defined volume using varying degrees of separation based upon a leakage rate is covered in Annex A and a schematic of the barrier types is shown below.

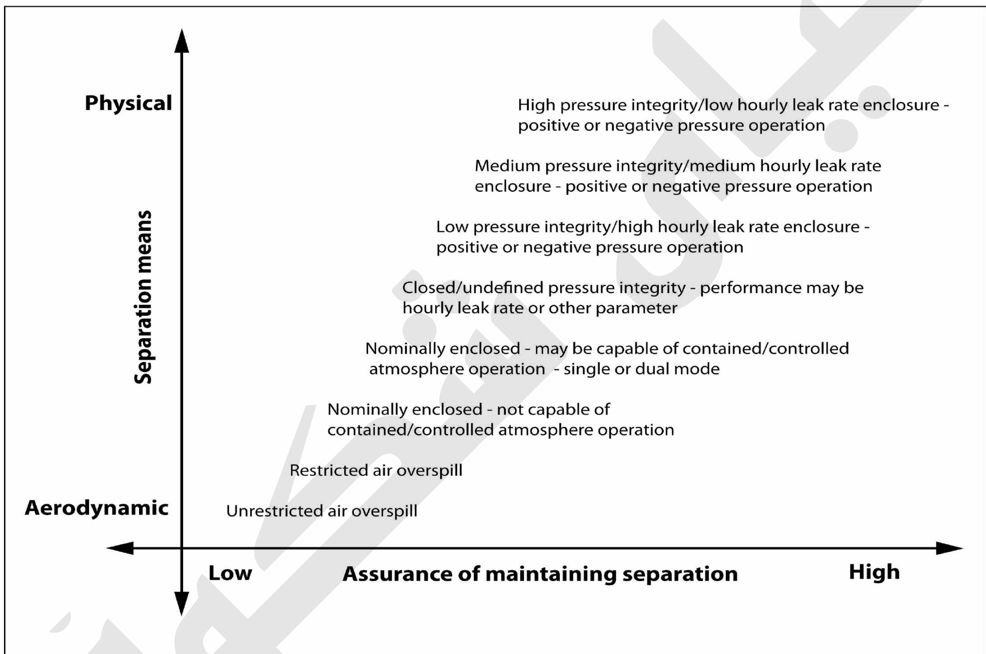


Figure 5: *Schematic of the Separation Continuum, with the increase in separation moving from aerodynamic to physical.*

Air-handling and inert gas systems are covered in Annex B; access devices including gloves and half suits are covered in Annex C, with different examples described in Annex D. The assurance of containment is maintained by ensuring a sealed enclosure and leak testing and the Parjo leak test methods are described in Annexes E and F respectively. The reader should note that although the Parjo method is described in the standard it is only suitable for small leaks and it is not widely used for the assessment of pharmaceutical grade isolators[11].

3.3.8 ISO 14644-8: Classification of airborne molecular contamination

This standard (part 8)[12] covers the classification of airborne molecular contamination (AMC) in cleanrooms for chemical substances and provides test methods, analysis and time weighted factors for classification. The standard is described in this chapter for completeness, although it does not normally apply to pharmaceutical operations. The chemical substances considered typically belong to a group of contaminants that manifest themselves as either: an acid, a base,

biotoxic, condensable, corrosive, a dopant[A], organic, an oxidant or a group of substances. The classification of an environment is defined from a formula:

ISO AMC class = *N(X)*

Where *N* = logarithmic index of concentration c_X expressed in grams per cubic meter and falls within a range of 0 to 12.

And *X* = one of the substances noted above.

So an airborne contaminant would be expressed as "ISO-AMC class -6 (NH_3)"

The range of classes is shown below.

ISO-AMC Class	Concentration (in g/m^3)	Concentration (in ug/m^3)	Concentration (in ng/m^3)
0	100	1,000,000	1,000,000,000
-1	10^{-1}	100,000	100,000,000
-2	10^{-2}	10,000	10,000,000
-3	10^{-3}	1,000	1,000,000
-4	10^{-4}	100	100,000
-5	10^{-5}	10	10,000
-6	10^{-6}	1	1,000
-7	10^{-7}	0.1	100
-8	10^{-8}	0.01	10
-9	10^{-9}	0.001	1
-10	10^{-10}	0.0001	0.1
-11	10^{-11}	0.00001	0.01
-12	10^{-12}	0.00000001	0.001

Figure 6: *ISO-AMC classes*

The main body also describes the demonstration of compliance as being verified by following a specific procedure for sampling, testing and documenting the result and conditions of test.

There are four Annexes:

- The parameters of the test environment (Annex A)
- The typical contaminants (Annex B)
- The methods of measurement (Annex C)
- The considerations of specific requirements for separative devices

3.4 ISO 14698 Cleanroom biocontamination standards

Cleanroom biocontamination control is an important part of the control of cleanrooms. The applicable ISO standards are outlined here. For a more detailed overview of environmental monitoring in cleanrooms, readers are referred to Chapter 19 in this book.

3.4.1 ISO 14698-1: Cleanrooms and associated controlled environments – Biocontamination control – Part 1: General principles and methods

ISO 14698 is a set of two documents that change direction slightly on the concept of cleanroom contamination control, in as much as they examine microbiological contamination as a separate component of the overall risk. Biocontamination has a significant impact on medical and pharmaceutical applications as the ingress of microbiological, viable contaminants can have

serious deleterious effect on finished products, including deterioration of a product such as taste, smell and appearance; destruction of potency and the potential for causing infection and disease. Although ISO 14698 is well established it is not referenced in EU GMP or the FDA aseptic filling guidance and is not yet received widespread adoption by microbiologists.

The first part of ISO 14698[13] establishes the principles and basic methodology of a formal system for biocontamination control and for assessing and controlling biocontamination. It includes sections relating to the principles of biocontamination control; establishment of a formal system including the setting of alert and target levels and general advice on the conditions of a formal system including:

- Appropriate sampling methods
- A suitable air-sampling sampling device, taking into account the type of particle for collection; the sensitivity of the microorganism to the sampling procedure; the concentration; the time required to take a meaningful sample and the collection accuracy and efficiency of the instrument
- The sampling plan developed according to site requirements
- The design of the sampling plan, taking into account the expected cleanliness levels
- The frequency of sampling
- The sampling sites
- The identification of the sample: who, what, where, when
- The processing of the sample once taken into account the requirements for culturing the sample
- The evaluation of the final data

The annexes associated with the standard include:

- Guidance on determining airborne biocontamination (Annex A)
- Guidance on validating air samplers including the establishment of biological and physical efficiencies (Annex B). The determination of biological and physical efficiency is performed following test methods on specific strains of microbiology under controlled conditions against a reference instrument No specification is set for pass/fail criteria as the choice of sampler depends on the application, however, it is important to understand how to make the suitable choice required in the main body of the document
- Guidance on determining the biocontamination levels of surfaces (Annex C) is given along with that for textiles (Annex D), laundering processes (Annex E) and in liquids (Annex F)
- Guidance on training is also given as the training of personnel in understanding, sampling and reading samples is important (Annex G)

3.4.2 ISO 14698-2 Cleanrooms and associated controlled environments – Biocontamination control – Part 2: Evaluation and interpretation of data

Part 2 of ISO 14698[14] gives guidance on methods of microbiological data and the estimation of results obtained from sampling for micro-organisms in risk zones. It includes information on the setting of action, alert and target levels on the evaluation of data. This should be performed in two parts, the initial monitoring phase and then as part of the routine sampling plan.

Data from the initial phase require sampling an adequate number of samples to establish the composition of micro-organisms along with the time and effect of stress and injury. The data from the routine sampling plan should then establish the action levels for demonstration of control.

3.5 Global GMP requirements for pharmaceutical cleanrooms

3.5.1 EU GMP Annex 1

The European Union GMP guidance[15] for sterile manufacture was revised in 2003 to accommodate the changes from various cleanroom standards to a single unified cleanroom standard, ISO 14644-1. The front page makes note of this:

> *"Annex 1 of the EC Guide to Good Manufacturing Practice (GMP) provides supplementary guidance on the application of the principles and guidelines of GMP to sterile medicinal products. The guidance includes recommendations on standards of environmental cleanliness for clean rooms. The guidance has been reviewed in the light of the international standard EN/ISO 14644-1 and amended in the interests of harmonisation but taking into account specific concerns unique to the production of sterile medicinal products."*

Specifically the means by which a cleanroom was certified needed to comply with the rules and format of the ISO 14644-1 guidance, but, the ISO standard was modified with respect to sterile medicinal products. To that end a table of cleanroom certification values that roughly translated to ISO 14644-1 was defined.

For clarity a series of notes appended the table, unfortunately the first of which, Note 'a', caused certain confusion. This confusion was remedied in the 2008 release of the EU GMP Annex 1 which clearly outlines three phases that need to be performed. Each cleanroom and clean air device should first be classified; it should then be monitored to verify that conditions are being maintained relative to product quality and that the data accrued from said monitoring be reviewed in the light of risk to finished product quality.

Table 7: EU GMP Annex 1 room classification

EU GMP Grade	At rest		In operation	
	Maximum number of particles permitted/m³		Maximum number of particles permitted/m³	
	0.5-5.0µm	>5.0µm	0.5-5.0µm	>5.0µm
A	3520	20	3520	0
B	3520	29	352,000	2900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Not Defined	Not Defined

The reader will note as indicated above, that the limits in the EU GMP table differ slightly from those in the ISO 14644 standard. To perform the required certification it is important to know the workings of ISO 14644-1 and how to certify a cleanroom in accordance with that standard Rules on number of sample points, sample point location, and volume of sample to be taken at each location, along with the rules on statistical analysis of cleanroom data need to be followed. However, rather than use the table for classification limits prescribed in ISO 14644-1, one should be using the table shown above, as printed in the revised guidance document.

Other expectations are also defined by the GMP, such as the sample volume for Grade A should be 1m^3 per sample location, and that a minimum length of sample tubing is to be used due to the high precipitation of 5.0m particles in transport tubing (ideally no sample tubing should be used). Also recertification of the cleanroom should follow the guidance given in ISO 14644-2, i.e. once per year for ISO Grade 6 and greater and once per six months for ISO Grade 5. Concessions are made for extending the ISO Grade 5 areas if a continuous monitoring system has been implemented. Suitable times to perform certification are media fills, or simulated filling runs.

Monitoring of cleanrooms

Once a cleanroom or clean air device has been certified to meet compliance, the room must now be monitored, relative to risk, to prove that the aseptic manufacturing environment can be maintained, and proven to be maintained. This section focuses on particle counting only; for information relating to viable microbiological monitoring the reader should consult Chapter 19 of this book.

The ISO Class 5/Grade A zone, which is the environment of greatest potential risk to the finished product, should be monitored for the full cycle of production, including set-up. The frequency of monitoring should be such that any interventions, short duration events or general deterioration in conditions be measured and alarms triggered if alert/action limits be exceeded. This requirement of all events essentially precludes the use of manifolds in these areas due to the sequential nature of the sampling being performed; concessions are made for the use of manifolds providing they have been sufficiently validated as suitable for the manufacturing type being carried out.

ISO Class 7/Grade B areas follow the same rules as ISO class 5/Grade A, in terms of the requirement for continuous monitoring. When one looks at the type of environment and relative risk in each of the areas; ISO class 5/Grade A is maintained under unidirectional airflow, and so short burst events may be very localised and of a very short duration – excluding some catastrophic failures, where as ISO class 7/Grade B is turbulent mixed air flow and reflective of the general environment in which the operators occupy, a low level of continuous particulate activity in this area would be normal and the systems response would be more to alarm should general control of this area be found to be out of tolerance, so an immediate spike is less likely to have a significant impact on product quality due to the dilution effect of the cleanroom design. This becomes more pronounced when one looks at background support areas beyond the zone in immediate proximity to the filling line or other ISO Class 5/Grade A areas.

There had been much confusion over the sample required for monitoring the ISO class 5/Grade A and ISO class 7/Grade B areas due to the phraseology used in the 2003 edition of the GMP guidance. The $1m^3$ sample was to meet the calculation required by ISO 14644-1 and not a risk based monitoring value, however several attempts at combining the two have been implemented, clarity is given in the revised guidance (2008 edition onwards).

> *"The sample sizes taken for monitoring purposes using automated systems will usually be a function of the sampling rate of the system used. It is not necessary for the sample volume to be the same as that used for formal classification of clean rooms and clean air devices."*

Therefore a system utilising a 28.3l/min particle counter would ideally sample continuously, from set-up through the entire filling period and slightly beyond, taking minute-by-minute samples, normalising this data to counts/m^3 and setting appropriate alarm and alert limits on the normalised values. The key to monitoring is to be able to respond in a timely manner to events that would be deemed to show the area is no longer in environmental control.

3.5.2 FDA Guidance on sterile manufacture

A document produced by the FDA - Guidance for Industry, Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice, 2004[16], defines two areas. A "critical" area is where the sterilised drug product, containers, and closures are exposed to environmental conditions that must be designed to maintain product sterility, and a "supporting" clean area is where non-sterile components, formulated products, in-process materials, equipment, and container/closures are prepared. The environmental requirements for these two areas are given in the FDA Guide Table 8 on the following page.

Critical area: This area is defined as critical because it contains sterilised products that, if exposed, are vulnerable to contamination. To maintain product sterility, it is essential that the environment in which aseptic operations are conducted be controlled and maintained at an appropriate quality. One aspect of environmental quality is the particle content in the air. Particles are significant because they can enter a product as an extraneous contaminant and can also contaminate it biologically by acting as a vehicle for micro-organisms. As the FDA guidance states:

Table 8: FDA aseptic processing room classification

Clean Area Classification (0.5 um particles/ft^3) This refers to former FS209E standard (now withdrawn but included for comparative purposes)	ISO Designation[b]	≥ 0.5 µm particles/m^3
100	5	3,520
1000	6	35,200
10,000	7	352,000
100,000	8	3,520,000

- All classifications based on data measured in the vicinity of exposed materials/articles during periods of activity.
- ISO 14644-1 designations provide uniform particle concentration values for cleanrooms in multiple industries. An ISO 5 particle concentration is equal to the former Class 100 designation and approximately equals EU Grade A.

> *"Air in the immediate proximity of exposed sterilised containers/closures and filling/closing operations would be of appropriate particle quality when it has a per-cubic-meter particle count of no more than 3,520 in a size range of 0.5µm and larger when counted at representative locations normally not more than 1 foot away from the work site, within the airflow, and during filling/closing operations. This level of air cleanliness is also known as Class 100 (ISO Class 5).*
>
> *We recommend that measurements to confirm air cleanliness in critical areas be taken at sites where there is the most potential risk to the exposed sterilised product, containers, and closures. The particle counting probe should be placed in an orientation demonstrated to obtain a meaningful sample. Regular monitoring should be performed during each production shift. We recommend conducting nonviable particle monitoring with a remote counting system. These systems are capable of collecting more comprehensive data and are generally less invasive than portable particle counters."*

Where the process is likely to interfere with the particle counting aspect of the measurements, such as powder filling or the filling of a radioactive material, then a slightly different philosophy is adopted. Readers are advised to refer to Chapter 18 for information on this philosophy.

HEPA-filtered air should be supplied in critical areas at the point-of-use. The air flow should be at a velocity sufficient to sweep particles away from the filling/closing area and maintain unidirectional airflow during operations.

Supporting clean areas: Classification of a supporting clean area is explained by the FDA as follows:

> *"The nature of the activities conducted in a supporting clean area determines its classification. It is recommended that the area immediately adjacent to the aseptic processing line meet, at a minimum, Class ISO 7 standards under dynamic (operational) conditions. Manufacturers can also classify this area as Class ISO 6 or maintain the entire aseptic filling room at Class ISO 5. An area classified at a Class ISO 8 air cleanliness level is appropriate for less critical activities (e.g., equipment cleaning).*
> (Guidelines on Sterile Drug Products Produced by Aseptic Processing, CDER, FDA 2004)".

Environmental monitoring

In addition to particle monitoring, like the EU GMP Guide, the FDA guidance document refers to microbiological environmental monitoring in relation to aseptic manufacturing[17]. The FDA document cross-refers to the Code of Federal Regulations (such as 21 CFR 211.42)[18]. Readers seeking further information on microbiological monitoring should refer to **Chapter 19** of this book.

3.5.3 The Pharmaceutical Inspection Co-operation Scheme (PIC/S)

The Pharmaceutical Inspection Co-operation Scheme (PIC/S) was established in 1995 as an extension to the Pharmaceutical Inspection Convention (PIC) of 1970.

PIC/S is an informal co-operative arrangement between Regulatory Authorities in the field of Good Manufacturing Practice (GMP) of medicinal products for human or veterinary use. PIC/S comprises Participating Authorities mainly coming from Europe but also from Africa, America (including the FDA from 2011), Asia and Australasia. PIC/S aims at harmonising inspection procedures worldwide by developing common standards in the field of GMP, including the harmonisation of Annex 1 for sterile manufacture. To that end PIC/S has fully embraced the current revision of EU GMP and for guidance one should review the section above for details.

3.5.4 World Health Organisation

The WHO GMP limits for particles follow the limits described in the EU Annex 1 (refer to 3.5.1 above), stating that no macro-particles should be allowed in the critical areas where product is exposed directly to the environment in which it is processed. Thus WHO refers to Grades A, B, C and D. They do not offer guidance on the frequency of monitoring, only that monitoring to prove compliance must be performed.

3.5.5 Comparing international standards

The direct relationship between the WHO and EU GMP classifications with those of ISO are summarised in the table below[19]:

Table 9: Comparison between EU GMP/WHO cleanroom grades and ISO 14644 cleanroom classes

EU GMP Grade	ISO 14644 Class
A	5[B]
B	7
C	8
D	9

The similarity of the classifications is evident, such as with the association of Grade A areas with ISO 5. With the FDA now a member of the Pharmaceutical Inspection Cooperation Scheme, harmonisation between the various cleanroom standards is now a closer possibility.

3.6 Conclusions

The regulatory landscape for environmental control is well defined under the ISO14644 family of documents and the GMP requirements from the EU and FDA for regulatory control. This chapter has provided the reader with an overview of current standards and regulatory guidance in relation to cleanrooms used for pharmaceutical processing.

It is a requirement that a cleanroom be first classified in accordance with the prevailing standard and once proven to meet the standard it is to be monitored in conjunction with a risk-based monitoring plan, covered in Chapter 10 of this book.

3.7 References

[1] American Air Force Technical Manual (TO) 00-25-203, 1961.

[2] FS 209E, Federal Standard Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones, Rockville: USA, 1988.

[3] EN ISO 14644-1, Cleanrooms and associated controlled environments – Part 1: Classification of air Cleanliness, ISO, Geneva, Switzerland, 1999.

[4] EN ISO 14644-2, Cleanrooms and associated controlled environments – Part 1: specifications for testing and monitoring to prove continued compliance with ISO14644-1, ISO, Geneva, Switzerland, 2000.

[5] EN ISO 14644-3, Cleanrooms and associated controlled environments – Part 3: Metrology and test methods, ISO, Geneva, Switzerland, 2005.

[6] ISO 21501-4 – Light Scattering Airborne Particle Counter for Clean Spaces, ISO, Geneva, Switzerland, 2007.

[7] EN ISO 14644-4, Cleanrooms and associated controlled environments – Part 4: Design, construction and start up, ISO, Geneva, Switzerland, 2001.

[8] EN ISO 14644-5, Cleanrooms and associated controlled environments – Part 5: Operations, ISO, Geneva, Switzerland, 2004.

[9] EN ISO 14644-6, Cleanrooms and associated controlled environments – Part 6: Terms and definitions, ISO, Geneva, Switzerland, 2007.

[10] EN ISO 14644-7, Cleanrooms and associated controlled environments – Part 7: Separative devices (clean air hoods, gloveboxes, isolators and mini-environments, ISO, Geneva, Switzerland, 2004.

[11] Midcalf B, Phillips WM, Neiger JS and Coles T. *Pharmaceutical Isolators,* London: Pharmaceutical Press, 2004.

[12] EN ISO 14644-8, Cleanrooms and associated controlled environments – Part 8: Classification of airborne molecular contamination, ISO, Geneva, Switzerland, 2006.

[13] EN ISO 14698-1, Cleanrooms and associated controlled environments – Biocontamination control – Part 1: General principles and methods ion, ISO, Geneva, Switzerland, 2003.

[14] EN ISO 14698-2, Cleanrooms and associated controlled environments – Biocontamination control – Part 2: Evaluation and interpretation of Biocontamination data, ISO, Geneva, Switzerland, 2003.

[15] Eudralex. The Rules Governing Medicinal Products in the European Community, Annex 1, published by the European Commission, Brussels: Belgium, 2009.

[16] FDA. 'Guidance for Industry. Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice,' (FDA, Rockville, MD, August 2004).

[17] Sandle T. (2011): 'Environmental Monitoring' in Saghee R, Sandle T and Tidswell EC (Eds.) (2011): *Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices*, New Delhi: Business Horizons, pp293-326.

[18] 21 CFR 211, Food and Drug Administration Department of Health and Human Services, Current Good Manufacturing Practice for Finished Pharmaceutical, Rockville: USA, 2008.

[19] Sandle T. 'Advances in Cleanroom Technology'. *Process India* 2011; **1**(3):42-44.

Notes:

[A] A dopant, sometimes called a doping agent, is a trace impurity element that is inserted into a substance (in very low concentrations) in order to alter the electrical properties or the optical properties of the substance.

[B] Due to the difference in the maximal level of permitted particle count concentration, EU GMP Grade A is actually equivalent to ISO 4644 class 4.8.

Design and construction of pharmaceutical cleanrooms

Alexander E Fedotov, Managing Director, Company "Invar-project", Russia

4.1 Introduction

This chapter examines the key points in relation to the design and construction of pharmaceutical grade cleanrooms. The aim of the chapter is to provide those commissioning cleanrooms and those tasked with the design works an understanding of the important points to be covered, as well as providing the general reader with an understanding of the process of cleanroom design. The chapter discusses the following topics:

1. What is a design?
2. Design specification
3. Concept of design
4. Basic design
5. Working drawings
6. Construction of cleanrooms
7. Modular and flexible cleanrooms
8. Design qualification
9. Examples of typical design mistakes

In discussing these topics, the chapter draws upon practical examples.

4.2 What is a design?

There are different definitions of the term "design". In the context of cleanrooms, design is a set of documents containing explanatory notes (texts) and drawings that allows the user to understand a future manufacturing site, to estimate expenses and how to construct it. There are two main stages of design:

- Basic design and
- Working drawings

The purpose of basic design is to check compliance in relation to the Design Specification, to GMP and other normative requirements. At this stage the design may need to be submitted to authorities for approval, where necessary, and to make approximate budget estimation. The design specification provides explicit information about the requirements for the cleanroom and how the cleanroom will be assembled. A design specification must include all necessary drawings, dimensions, terms, definitions of non-standard terms, etc.

Before undertaking the basic design, the design concept should be considered. This allows complicated tasks and processes to be considered in advance. Working drawings are designated for construction and contain detailed budget calculations. For undertaking the design and working drawings, the design specification and normative documents are the starting point of the design.

Design is a part of the whole process of creating a new facility (**Table 1**):

Marketing study ➙ Design specification ➙ Design ➙ Construction (installation) ➙ Start-up ➙ Testing ➙ Operation

Table 1: The cleanroom design process

Stages of creating a new facility cleanrooms					
No	Stage	Who makes?			
		Customer	Design company	Construction company	Independent consultant/testing companies
1	Marketing study	+	–	–	–
2	Design specification	+	–	–	–
3	Design:				
	– Concept, if necessary	–	+	–	–
	– Basic design	–	+	–	–
	– Working drawings	–	+	–	–
	– Design qualification	+	–	–	+
4	Construction:				
	– installation drawings	–	–	+	–
	– executing of construction/installation	–	–	+	–
	– field supervision	–	+	–	–
	– executive drawings	–	–	+	–
5	Start-up	–	–	+	–
6	Testing	+	–	–	+
7	Commissioning	+	+	+	+
8	Operation	+	–	–	–

This is a basic table. The consequence of executing some stages may be flexible. For example, developing a design specification is not always simple or straightforward. It may be necessary to make the 'concept of design' to create a more precise specification or to adjust the marketing part. It may be that the customer will only start to understand what they really want when they study the overall concept. So the link between two elements of the whole chain can move in two opposite directions and the process of reaching a solution, is iterative:

Design specification ⇄ *Concept of design*

Normative documents

This is a key section of design. The customer needs to specify to which markets (which countries) products shall be delivered, so that the design complies with the requirements of these countries. The best solution is to arrange for flexible manufacturing, which will allow products to be supplied to all major markets (USA, Europe and others). Here, the US and EU GMP requirements cover most demands. If manufacture is designed according to both US and EU norms, then products can be sold to almost any country. However, it is recommended that the local requirements of the countries of interest be understood to avoid unexpected surprises. In particular, national fire, environment protection and other safety issues must be understood. For this, consulting with local design companies is useful. In addition, it may be necessary to obtain local licenses or to seek permission for design services in some countries.

The design process can be compared with construction of a tunnel, when two teams work from the opposite sides of the mountain and aim to meet each other at the same point in the middle. In order

to meet in the middle, this is dependent upon the skill of the designers. No testing, qualification or validation can improve poor design. These factors can only indicate whether the design is in compliance with the specification and GMP requirements or not. This is a very important point. Sometimes it was assumed in earlier times (and maybe even now) that poor design, premises or equipment can be improved by qualification or validation. In fact, qualification and validation can only confirm compliance or reject it. Thus the main attention shall be paid to the primary process, not to its validation. This important point is stressed in FDA Guidance on validation[1] and ASTM E2500 standard[2].

The facility will have the same quality as the design, not better.
Thus the quality of design is the first priority at the beginning stage of creating the facility. Of course, qualification is necessary because it gives assurance that the design is correct. Even the best designs may have errors, oversights or some improvements that can be made by the third party that provides the Design Qualification (DQ).

4.3 Design specification

Design specification describes user requirements for future facility and includes the following:

1. Name and address of manufacturing site
2. Kind of construction (new, reconstruction)
3. Nomenclature of products to be manufactured
4. Building characterisation (sizes, number of floors, heights etc.)
5. Stages of design to be developed and content of each stage
6. Normative documents
7. Special requirements of the process:
   - Cleanrooms for example, modular, isolator, etc.
   - HVAC
   - Constructions
   - Utilities and other equipment
   - Waste disposal
8. Present resources for supply with:
   - Energy
   - Hot water
   - Cold water
   - Gas
   - Plant steam
   - Others
9. Other conditions:
   - Annexes to Design specification:
     - Manufacturing formulae (process manual) that includes detailed description of the process with norms for consumption of materials, process media, wastes, etc.
     - Report on testing the present state of the building.

Content of design and scale of work differ significantly for ***reconstruction*** of an existing building or construction of ***a new building***. Reconstruction means changing of layout and equipment in the existing building with or without changing products. It is much easier to design a new building as in this case the designer has the freedom to develop layouts without many restrictions.

If the customer wishes to design a facility in the existing building, then the task can become much more difficult. It may be impossible to arrange the required process at all; or it may be that additional spaces outside, on the roof or beneath the building need to be considered. If it is necessary to go outside the external walls of the existing building, then the relevant estimation shall be done and the necessary permissions obtained.

4.4 Concept of design

Developing the design specification is not a simple task. In many cases it is necessary to undertake preliminary work to understand the requirements for design. For example, it can be unclear how to arrange production of several oral dosage forms: to use dedicated equipment for each product or to use the same equipment. The solution depends on the nature of product, the scale of manufacturing, the possibility of reliable cleaning of equipment, protective solutions against cross contamination, etc. The second example is deciding how to manage aseptic production using common cleanrooms or with isolator technology.

The ‘concept’ is the shortened part of basic design and includes only those parts that are necessary to make basic decisions and to estimate expenses very roughly.

Content of concept may include the following:

- Introduction with the name and address of manufacturing site
- Normative references
- Nomenclature and quantities of products to be manufactured; forecast of sales if applicable
- Process flow charts
- Cleanroom or separation concept
- Layouts of premises with room specifications
- Specifications for Technological and control equipment
- Personnel (number and qualification) for each room
- Number of shifts
- Where to wash/sterilise garments (outsourcing or laundry at the manufacturing site)
- Monitoring
- Utilities (electricity)
- Wastes
- Safety requirements if necessary.

The results of the concept may include the following recommendations:

- Conclusion on the possibility of making the design according to design specification in given building or on given territory; if it is not possible, then recommendations what to do should be offered
- Key decisions on execution of the process (dedicated equipment or not; isolator or common cleanrooms for aseptic processes, etc.)
- Basic layouts
- Specifications for main process equipment
- Cleanroom concept with principles of separation of premises/zones, cleanliness classes or GMP EC grades, pressure differences, etc.
- Basic HVAC solutions (number of AHUs, designation of rooms which every AHU serves, concerns on recirculation, heat recovery, etc.)

- Estimation of energy consumption
- Very approximate cost estimation
- Recommendations to correct or to precise the design specification if necessary or draft of a new design specification, namely:
  – The extent and content of the concept may differ depending on the project
  – Design specification or précised design specification can be the main result of the concept

4.5 Basic design

The basic design gives a complete understanding to the customer as to what they will get. It also allows all the necessary approvals to be obtained and for expenses to be estimated (with about 20% precision for good basic design), on the basis of quotations obtained from contractor/suppliers. It is possible also to start, at this stage, contracting for supplying long term delivery equipment, such as vessels, reactors, water stills and sterilizer that may require 6-8 months to manufacture.

The purpose of basic design is to obtain solutions for:

1. Design specification compliance
2. Compliance with GMP and other norms
3. Compliance with safety, environment protection, energy saving and other regulatory requirements to allows the design to be submitted to relevant authorities for approval.

For this, the basic design should include the following parts:

– General description
– Layout of ground area (general ground plan) with all buildings, roads, external networks systems for electricity, water, sewage, plant steam, gas, etc.
– Processing
– Architecture of building
– Construction of building, including layouts and cross sections of building, structure of walls, ceilings, floors, doors, windows, finishing of surfaces, etc.
– Cleanrooms
– HVAC systems
– Process and HVAC control, monitoring
– Utilities
  - Plumbing and sewage
  - Electricity (lighting and power)
  - Process water, cleaning water
  - Process steam, etc.
– Communication, security, control of admission to certain premises, video surveillance, etc.
– Energy consumption calculations; developing of external sources of energy (electricity, steam, hot water, etc.) may be necessary and special design may be requested
– Safety (fire and explosion protection, etc.)
– Environment protection and reducing or elimination of contamination of air, sewages procedure to deal with dry litter and scrap
– Measures for disabled persons
– Budget calculation
– Others.

Each part consists of text notes and drawings if necessary.

Features to be considered

Pharmaceutical manufacturing can have some specific features to be considered in the design. The main features depend on the following:

- Types of products (medicinal products, APIs, excipients)
- GMP and safety issues that can fall in contradiction sometimes
- Types of process: non-sterile, sterile (terminal sterilisation, isolators)
- What is to be protected (product only, environment and personal, safety and GMP issues)?
- Should the equipment be dedicated or not?
- What is included in manufacturing: the whole process or only the part of it, such as packaging?
- Manufacturing of medicinal products and Active Pharmaceutical Ingredients shall comply with GMP requirements

Basic process solutions

The process part is the core part of the whole design. The technologist develops or specifies the following:

- Process flow charts and layouts of premises
- Personal, material and product flows
- Process equipment and its places on layouts
- Cleanroom classes and pressure differences
- Number of people in every room
- Air exhausts, toxic and other harmful materials and wastes
- Consumption of energy, water, steam etc. for process equipment
- And many others

The technologists not only develop their own part of design, they also give detailed specifications for the other specialists: civil engineering, HVAC, electrical, plumbing and sewage, environment protection and so forth. These specifications are to be written in a clear and unambiguous way and set the task for relevant specialists or parties.

The following flow charts show the key operations and rooms with cleanliness classes, utilities used, etc. It gives visual presentation of technological process and serves as a bridge to develop layouts that can be created, knowing the sizes and weights of the process equipment (**Figures 1** and **2**).

GMP rules specify following ***requirements for layouts:***

- Logical consequence of process and packaging operations
- Excluding mixing of materials and products
- Avoiding cross-contamination
- Absence of crossing of personal, starting and packaging materials, intermediate or final products flows or time separation of these flows (it may possible, for example, for small scale production with a small number of people involved)
- Separating process zones and zones for meals
- Special conditions for processing and handling of toxic and sensitising drugs, vaccines, radiopharmaceuticals and other products with special requirements
- Handling of air pressure differences between rooms, if necessary
- Entry and exit procedures, garment changing and hand washing, procedures for material

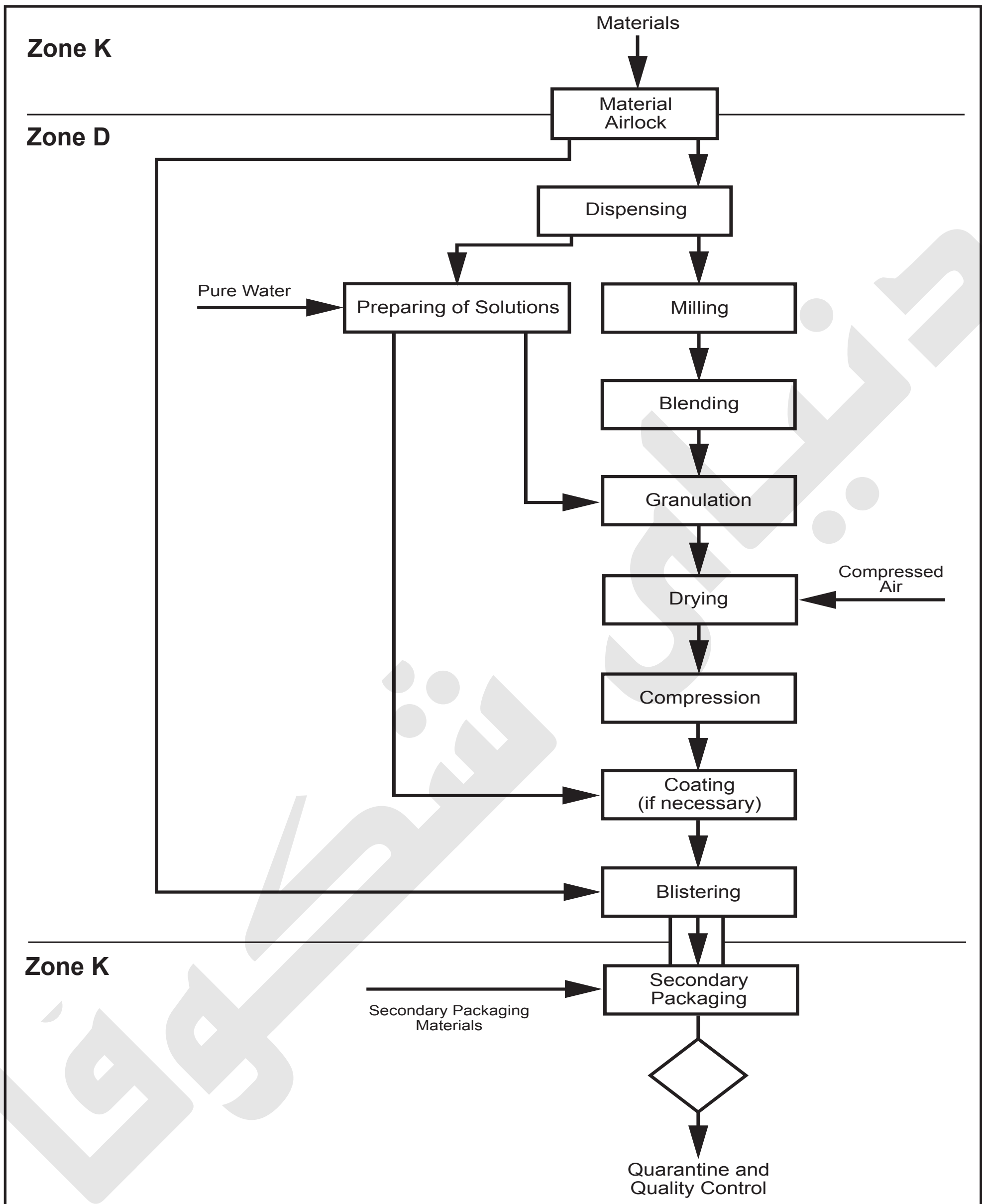


Figure 1: Process flow chart for tablet processing.

and production transferee
- Providing of specified cleanliness classes
- Rational disposition of equipment, proper areas for operation, maintenance and repair
- Convenience for handling and maintenance

Examples of layout are shown in **Figures 3** and **4**, on the following pages.
It is not sufficient to create good layouts. It is also necessary to calculate a material balance and balance of equipment productivities.

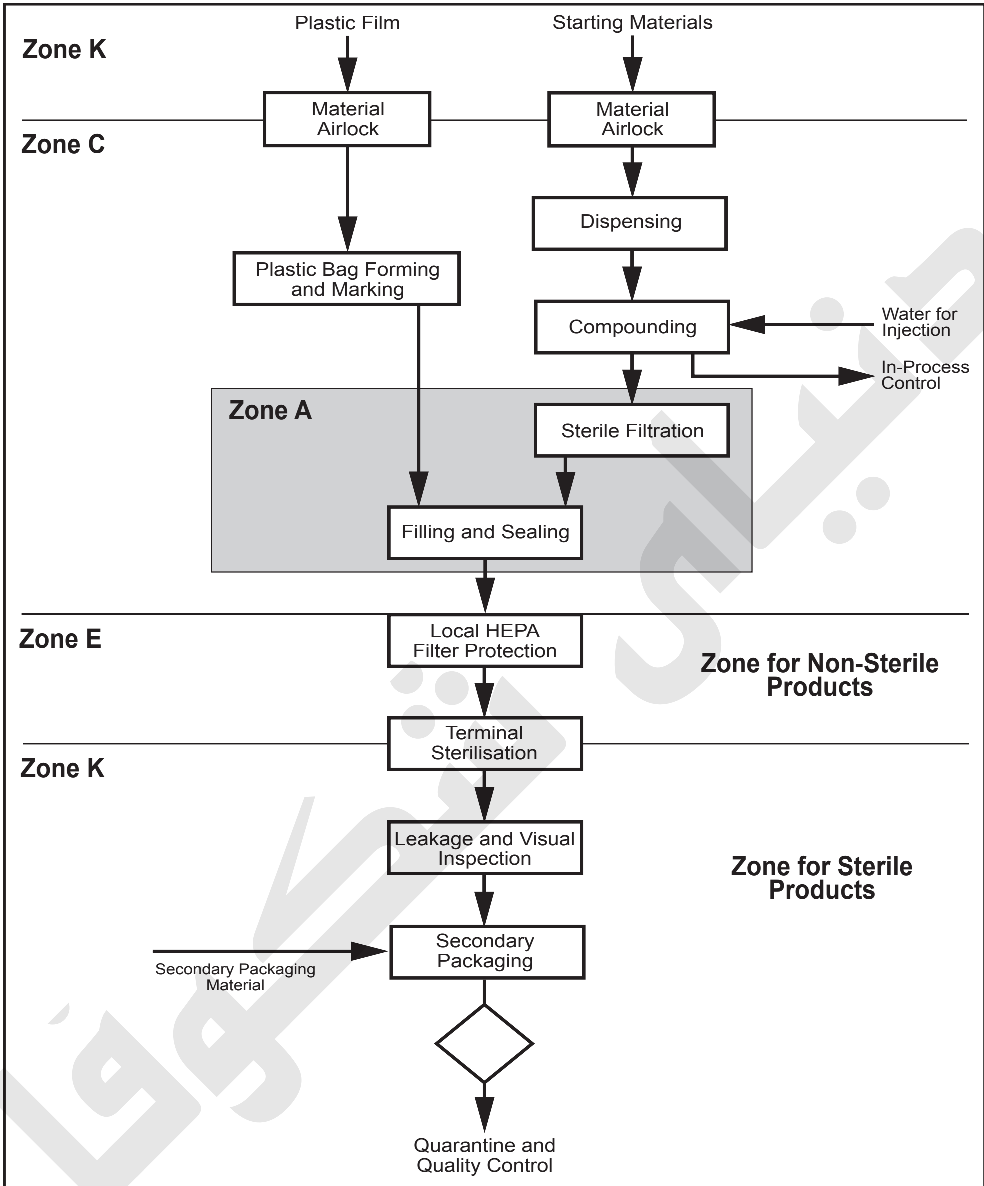


***Figure 2:** Process flow chart for processing of sterile solutions in plastic bags with terminal sterilisation.*

Constructing a process time diagram is very important at the early stages. It can show narrow places, demonstrate the necessity for additional equipment, etc. The process is most efficient when all pieces of equipment in the process chain have equal capacity. But it seldom happens. Different units in the same chain such as reactors, filling lines and sterilizers have different capacities. The capacity of the whole chain is limited by the element with lowest capacity. Adjustments of numbers of similar units and an iteration process can help develop proper solutions.

A process time diagram allows understanding of the real scheme of work of the whole manufacturing site and its real capacity, to discover narrow elements and crosses of process flows in a timely

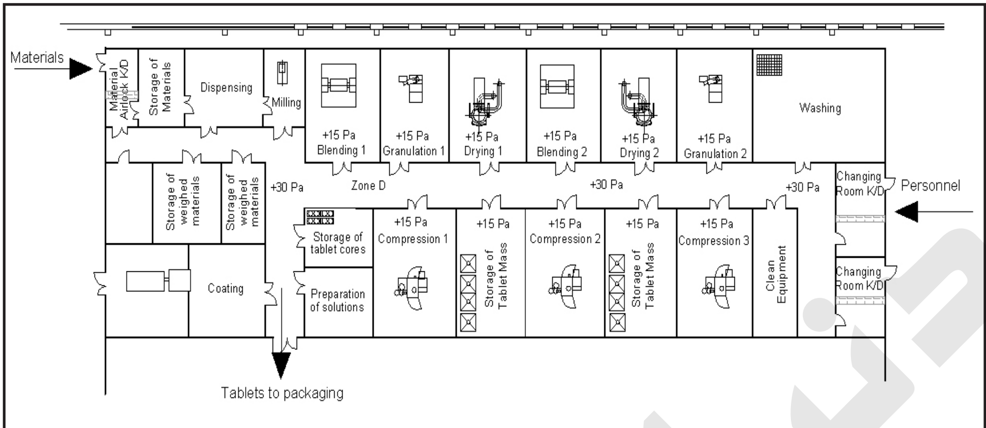


Figure 3: *Layout of tablets processing facility.*

*Zone E – Air cleanliness as in Zone D but no other measures (airlocks, pressure differences are specified)
IPC – In-process Control

Figure 4: *Layout of facility for processing of sterile solutions in plastic bags with terminal sterilisation.*

manner. It is possible that this diagram will not only prompt improvement of the layout, but will indicate that this layout should be redesigned completely or even that manufacturing should be arranged at some other place.

4.6 Working and other drawings

Working drawings are to be developed after approval of the basic design. They are to be used by construction companies to arrange all works, either according to the design or by developing installation documentation if necessary. Installation documentation can be helpful or even

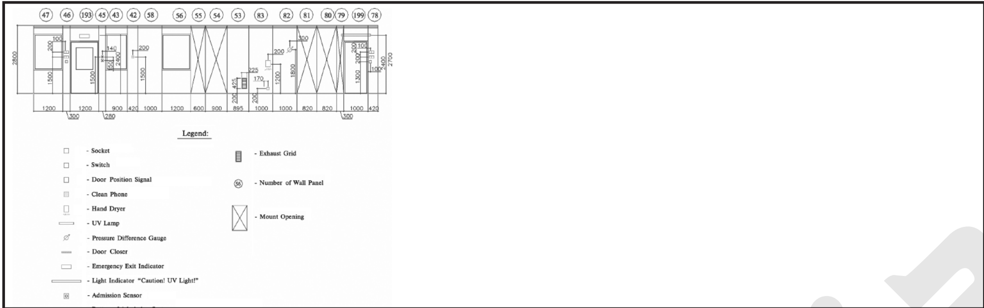


Figure 5: *Schematic view of four walls of cleanroom.*

necessary in many cases. For example, installing cleanrooms is much easier and better if the constructor has precise and clear drawings (**Figure 5**).

It is not possible to foresee all details in the design. Some changes may need to be done during the construction phase. The design company should provide field supervision and make necessary notes/approvals.

As built drawings shall be prepared after the competition of the cleanroom construction. This can be done by correction of working drawings with the signature of an authorised person.

4.7 Construction of cleanrooms

It is possible to define several stages of cleanroom construction process. These are:

- Common building works (civil construction)
- Installing air ducts, utilities and energy networks
- Installing air handling units and other elements of HVAC
- Construction cleanroom enclosure (walls, ceiling, etc.)
- Installing process equipment and ancillary systems

Common building works include improvements and cleaning of external territory, construction of buildings, arranging of external and internal networks and finishing and facing of internal walls, ceilings and floors for cleanroom construction.

Utilities and energy networks (electrical power and lighting, hot and cold water, steam, etc.) shall be finished and supplied to points of use/distribution.

Cleanrooms consist of enclosures (walls, ceilings, floors, doors, windows, transfer chambers, etc.), HVAC (Heating, Ventilation and Air Conditioning) systems with control, lighting, plumbing and other equipment inside.

Process, support and utility equipment/systems should be installed after finishing cleanroom construction. Heavy equipment and piping can be an exception. They can be installed before construction of the cleanroom enclosure. The cleanroom should also have a mount opening, to enable the works, although this must be sealed for normal operation. Requirements for transfer of equipment in and out of cleanrooms are described in ISO 14644-5 Standard[3].

The example of such a schedule is shown in **Table 2**. This schedule should be agreed in advance with all parties. It presents a basic plan. In practice, several corrections and deviations from the plan can occur. The most dangerous places are the boundaries between different teams. Every finished stage must be accepted and documented to comply with GMP.

Table 2: Typical schedule of cleanroom constuction

S. No	Stages	2011 г.		2012 г.				2013 г.		
		III	IV	I	II	III	IV	I	II	III
1	Working Drawings									
2	Civil construction and facing									
3	Installing of air ducts									
4	Installing of air handling units, chillers etc.									
5	Installing of HVAC control system									
6	Installing of floors									
7	Cleanroom enclosure construction									
8	Start-up of HVAC									
9	Installing of PW And WFI systems									
10	Installing of compressed air system									
11	Installing of process gases system									
12	Installing of process equipment									
13	Training of personal									
14	Start-up of cleanroom									
15	Cleanroom testing									

KEY: HVAC = Heating Ventilation and Air Conditioning; PW = Purified Water; WFI = Water for Inspections

Good construction management and defining of responsibilities are very important. Wasting time because of delays of equipment or cleanroom parts supply not only means increased expenses. It also means decreased quality. The best manner is rapid construction without delays between stages. Endless changing of tasks and working places for construction teams does not help to reach the desirable quality of the cleanroom.

The installer of a cleanroom enclosure should start work only after all civil construction, air ducts and other preparations have been completed. All contractors must complete their work and leave the site before cleanroom construction can start. The presence of many different people and companies, the mixing of works and packaging materials, leads to “*bad construction practice”* that should be avoided.

To work 'clean' is the golden rule for all cleanrooms constructors. The installation of air ducts should not commence until all civil works have been completed and all waste material has been removed. The cleaning of the site, correct behaviour of personal and wearing proper garments are each of key importance. Every member of the construction team should understand that he came to make the area cleaner and not to make it worse.

Requirements for cleanliness and increasing clean demands should be documented in a clean construction protocol (cleanliness protocol). **Table 3** presents an example of such a protocol. It may be shortened or modified but the main idea of increasing cleanliness during construction should be followed. The golden rule “***to work clean”*** is of critical importance for aseptic and other sterile manufacturing facilities.

4.8 Modular and flexible cleanrooms

Modular cleanrooms are cleanrooms assembled from prefabricated modules. The process of cleanroom construction differs from common cleanrooms in that:

- Common cleanrooms are assembled at the construction site from many elements

Table 3: Clean construction protocol.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6
	Civil Engineering	Installing of air ducts, electricity net, piping etc.	Construction of Cleanrooms	Adjustment of HVAC system	Cleanroom testing	
					As-built	At-rest
Purpose	Preparing of the site for construction of networks and cleanrooms	Readiness for cleanrooms construction	Readiness for switching HVAC on	Readiness of cleanrooms for testing	Testing as-built	Testing at-rest
Scale of works	Construction or reconstruction of building, changing of layouts, wall covering etc.	Installing of ducts, piping, HVAC etc.	Construction of cleanrooms (walls, ceiling, etc) and finishing HVAC	Balancing of air rates, adjustment of HVAC, installing of HEPA filters	Testing as-built	Testing at-rest
Document	Design	Design and Installation drawings	Design and Installation documents	Design, Installation and Executive drawings	As-built testing program	At-rest testing program
Result	Readiness for installing of ducts etc.	Testing of air ducts (air tightness, cleanliness), testing of communications	Visual testing of cleanrooms, testing on drawings	HVAC testing report	Report	Report
Who makes	General construction company	Specialized construction companies (HVAC etc.)	Cleanroom construction company	HVAC construction company	Customer or testing company	Customer or testing company
Garments:						
- purpose	Personal protection	Personal protection	Personal and cleanroom parts	Clean room air	Clean room air	Clean room air
- content	Overall for civil construction	Overall for civil construction	Clean overall	Coat (suit), cap, clean shoes (shoe covers)	Coat (suit), cap, clean shoes (shoe covers)	Cleanroom garments for specified class
Admission system	Common	Only for authorized personal. Entrance/exit control. Unpacking in dedicated zones	Stage 2 + entrance/exit only along specified routes	Stage 3 + continuous supervising	Stage 4 + instruments treatment before entrance in cleanrooms	Admission as specified for cleanroom operation
Cleaning	Daily waste removed	Daily cleaning	Daily vacuum cleaning	Stage 3 + cleaning wiping	Stage 4 + particle counting, testing	Cleanroom cleaning procedure
Control		Visual cleanliness	Visual cleanliness	Stage 3 + testing with clean wipe	Stage 4 + particle counting; as-built testing	At rest testing
Limitations	No smoking	No smoking, no eating, no drinking	Stage 2 + no welding, no cutting, no taking off external packaging	Stage 3	Stage 4 + personal hygiene	Cleanroom behavior

- For modular cleanrooms a significant part of assembly work is done at the factory that produces modules. Only the assembly of complete modules remains at the customer's site.

Common cleanrooms are ***tailor-made*** cleanrooms. Their design follows specific layouts drawn by the technologist from understanding specific processes. The technologist tries to consider only necessary elements according to the design specification. Existing buildings may have limited spaces which can cause restrictions, and these need to be considered by the designer.

With modular cleanrooms other restrictions can appear. This is because the cleanroom construction process is separated into two parts, executed in two different places: the modules' manufacturer and the customer's site. The distance between these places may be thousands of kilometers. So a new chain of cleanroom construction appears and this is a transfer chain. This new chain leads to three important features that should be considered:

1. It sets new restrictions for sizes of modules that are limited by transfer conditions. The space inside the cleanroom is always a subject for debate between technologists and constructors. It is a rare case when a poor or small space can be accepted. To construct a bigger cleanroom from small modules then the problem of reliability of joints arises. The sealing of joints between the modules also needs to be considered.
2. The cleanroom construction protocol must be followed both for common and modular cleanrooms. However, for modular cleanrooms the clean transfer of modules from manufacturing to construction site must be factored in. This may not be straightforward and will add to the budget.
3. Universal modules often lead unavoidably to ***additional expenses*** and ***redundancy*** (or superfluity) in comparison with a tailor-made approach. Extra expenses are the consequences of moving a part of the manual construction work from the site to the modules-making factory.

The assembly of the cleanroom enclosure and equipment is only a part of the whole process of construction of the manufacturing site. For correct budget estimation it is necessary to bear in mind all factors of construction of the facility. This whole picture may be quite different from the first impression when only a conventional cleanroom budget was estimated.

Module solutions can be effective for restricted zones, for example, for operating rooms in hospital or zones with unidirectional air flow. Module solutions can be regarded as flexible cleanrooms. This means that working places can be changed by moving fan-filter units in the ceiling without much reconstruction of the cleanrooms.

Modifying cleanrooms

When the process is to be changed, it is desirable to use the existing cleanroom as far as possible and to avoid considerable reconstruction of the facility. The extent to which this is possible depends on the process.

Major changes, product/process changes

If the type or quantity of products needs to be changed, then the process also requires changing. This causes changes to layouts, HVAC systems, energy consumption, etc. Cleanrooms may be reconstructed completely as a consequence. Using old air handling units is often not practicable because air supply and exhaust rates often differ greatly. Only limited parts such as doors, transfer chambers and some walls can be used again. The extent to which materials are recycled is a matter of cost estimation and construction management will need to decide if it is worth doing.

Minor changes

It is quite possible to avoid reconstruction of cleanrooms when minor modifications do not lead to

changes to air handling units, air ducts and layouts. An example is the movement or rearrangement of localised clean areas, like unidirectional airflow units. This can be done without significant issues.

4.9 Design Qualification

A GMP inspector does not check the design of a facility or a cleanroom. The inspector visits a facility when all construction and testing works are complete and when it is too late to correct design mistakes. Since all mistakes will be realised in practice, then this presents a big risk for the customer. Therefore it is critical to make the right selection of a design company. If the choice is correct, then no unpleasant surprises should appear during inspector's visit or testing of processes/equipment.

It is useful to check the design and its compliance with the design specification and GMPs. The process of such a check is named as ***Design Qualification (DQ).*** DQ can be arranged at different stages of design, as shown below (**Table 4**).

Table 4: Design and Design Qualification stages

Design stage	Qualification stage	Purpose of qualification
Design specification	Specification Qualification – **SQ**	Checking of correctness of Design specification
Concept of design (if it were developed)	Concept Qualification – **CQ**	Estimation of design feasibility, different variants, if specified, and better understanding the whole task
Basic design	Design Qualification 1 – **DQ1**	Checking compliance with design specification, GMPs and other norms if any
Working drawings	Design Qualification 2 – **DQ2**	Checking compliance with Basic design, checking of matching of networks, etc.
Changes in documentation	Change Control	Checking of correctness of changes of documentation done

This table shows the complete set of qualification activities. The key stages are Concept Qualification (CQ) and Basic Design Qualification (DQ 1 or simply DQ). It is not necessary to pay attention to the whole design. Care should be taken for core factors: processes, layouts, pressure differences concept, cleanrooms concept and HVAC basic solutions. It is recommended to inbuild into the design the need to avoid the mixing of products; to achieve protection against contamination and cross-contamination; to have good process control; to allow for the monitoring of critical parameters and other GMP issues.

4.10 Examples of typical design mistakes

Any design can have small errors or oversights. Different authors have different styles that can be a subject for discussion. But it is necessary to avoid mistakes that cause non-compliance with GMP, to find and correct some of them at an early stage. Even in the past, people understood the importance of quality of design and paid attention to it. Protective measures were different, some of them were impressive and effective. Russian Tsar Peter the Great manifested the decree in 1706:

"All projects shall be done in a very good order so as not to ruin the treasure in vain and not to cause harm to the Fatherland. I'll deprive of the rank and will bid to flog with a whip all those who will fudge projects anyhow".

These words reflect the problems of today too. Some examples of mistakes are given below, courtesy of the company "Invar-project", which provides relevant audit services.

Example 1 – Large volume parenteral solutions (infusions) facility
GMP requires avoidance of mix-ups of materials and products. It should be done by proper layout of premises and proper arranging of the material, product and personnel flows.
During the audit the following was discovered:

- Two one-door (not pass through) sterilisers were used for terminal sterilisation
- Layout of premises and positions of sterilisers permitted mixing of sterile and non-sterile products
- Flows of sterile and non-sterile products were arranged through the same door
- The space in the room was very limited, so both sterile and non-sterile products were positioned in close proximity
- The output was rather large and the plant worked continuously for three shifts of 24 hours

This manufacture is not GMP compliant. It is very difficult to make any correction to the existing plant, but quite possible to make a correct solution at the design stage.

Example 2 – Zoning for aseptic process
The designer offered personnel flow to zone ***C*** through zone ***B*** but forgot to arrange the changing room. Thus facility was constructed but could not pass the qualification stage. Reconstruction was difficult to be do because it was necessary to remake walls, doors, air ducts, etc. The second mistake of this design was that zone ***B*** could not serve as a pass-through room. Only a limited number of personnel involved in an operation is allowed to be present in zone ***B***.

Example 3 – Conveyor in aseptic process
The conveyor belt passed the boundary between zones ***A*** and a non-classified area, extending into it for 2m. So the belt went from zone ***A***, traveled 4m in a non-classified area and returned to zone ***A*** again. This is a clear violation of GMP requirements.

Item 56 of GMP EU Annex 1 states: “*A conveyor belt should not pass through a partition between a grade A or B area and a processing area of lower air cleanliness, unless the belt itself is continually sterilised (e.g. in a sterilising tunnel)”.*

Example 4 – Tablet production, start of process
Sampling of starting materials before placing in quarantine and weighing for the batch were done in processing room without separation. Such design gave a possibility for mix-ups of materials that were waiting for results of testing by the Quality Control unit and those that had already passed testing.

Example 5 – Tablet production – end of process
Several tablet blistering lines entered into the same room very close to each other without separation. It gave a chance for mixing products from different lines.

Example 6 – Dedicated equipment
This is example discusses how to ***exclude the possibility of mixing*** different products at design stage by the application of ***dedicated equipment***.

One customer decided to start manufacturing two different products:

- topical solutions
- syrups for children and adult

For budget reasons it was decided to prepare these products in one reactor with, of course, time separation. The auditor approved it on condition that the cleaning procedure was validated. But the situation is not so simple.

Validation or testing can improve nothing by itself. It only shows whether the process is acceptable or not. But how true can this testing/validation conclusion be? This conclusion is based on samples taken from ***several parts*** of the inner surface of the reactor, possibly just the “worst case places”, ***not from the whole surface***. But the result of analysis have been applied to the whole surface. Such 'spreading' of data is only an assumption, nothing more. There is no guarantee that it is valid for the whole surface. Risk of variation from validated status always exists. The greatest risk is for manual cleaning, surfaces with a complex form, “dead legs” and other places that are difficult to reach and clean. So it is necessary to estimate for every given case that such an assumption can be valid.

First, one should look at which products are to be produced, what are the methods of administration and how toxic they are. In the case observed, stilling of substance for preparing topical and syrups for children in the same reactor was offered to the customer. The substance was extremely toxic and its allowable concentration was not specified. This fact was enough to conclude that the cleaning process ***cannot be validated*** and two processes in one reactor ***cannot be permitted***. Syrup is a product for oral administration. Risk of toxic contamination was too high and could not be accepted. So the only possible solution was to use dedicated reactors, two different reactors in this case.

Second, successful validation shall be foreseen by the equipment structure. Equipment should be fit for reliable cleaning, but the reactor observed had places that were difficult to clean. Of course, it is possible to reassemble, clean and assemble the reactor each time after changing product but this is practically not realistic and difficult to control.

4.11 Summary

This chapter has presented a practical approach to cleanroom design and has provided best practices approaches based on the experiences of the author. The chapter has placed considerable emphasis upon the planning and review stages. This was done because it is very difficult to rectify mistakes at a later date.

4.12 References

[1] Guidance for Industry. Process Validation: General Principles and practices. Food and Drug Administration, Rockville: MD, USA.
[2] ASTM E2500 Standard Guide for Specification, Design and Verification of Pharmaceutical and Biopharmaceutical Manufacturing Systems and Equipment.
[3] ISO 14644-5 “Cleanrooms and associated controlled environments. Part 5. Operation”. International Standards Organisation, Geneva.

Air handling systems for the protection of pharmaceutical manufacturing processes

Hans H. Schicht, Dr. sc. techn., Contamination Control Consulting, Zumikon, Switzerland

5.1 Introduction

Air handling systems for pharmaceutical cleanrooms, also called HVAC, i.e. heating, ventilation and air-conditioning systems, are responsible for providing the rooms of a contamination controlled facility with a supply of filtered air, for establishing the appropriate airflow patterns in them and impeding cross-contamination between them. These are the decisive steps for ensuring product and process protection against detrimental effects from airborne contaminants. Not infrequently, HVAC technology also contributes to protect the facility's personnel and the external environment against airborne hazardous matter. This chapter intends to present on overview of the necessary technical knowledge and the corresponding regulatory requirements from different angles, with emphasis on the cleanroom-specific HVAC aspects and focussing on the peculiarities distinguishing the pharmaceutical application area of this technology. The objective is to introduce the pharmaceutical professional to the basics of HVAC technology relevant from the contamination control point of view, and to acquaint the HVAC technologist with the specific requirements and priorities he will have to address in the pharmaceutical application area. Thus, a common base of knowledge and understanding should result in order to enable them to speak a common language permitting them to co-operate efficiently and creatively in mutual understanding.

5.2 Objectives of pharmaceutical air handling systems

A **cleanroom** is defined[1] as a room in which the concentration of airborne contamination is controlled, and which is constructed and used in a manner to minimise the introduction, generation and retention of particles inside the room and in which other relevant parameters, e.g. temperature, humidity, and pressure, are controlled as necessary.

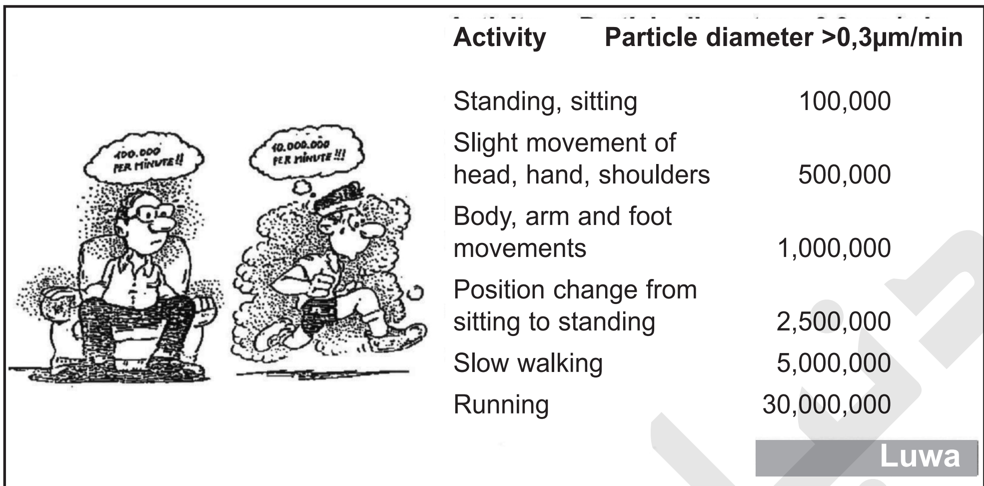
To these secondary parameters mentioned in[1] lighting and noise control may be added. All of them require control in all industrial air-conditioning applications and are not specific neither to cleanroom technology nor to the pharmaceutical industry. Therefore, they will not specifically be addressed in the following text.

Responsible for controlling all these parameters is the **air handling system**, also called **HVAC system** (**H**eating, **V**entilating and **A**ir **C**onditioning). From the contamination control and cleanroom technology point of view, three protection objectives may be distinguished:

- protection of processes and products against damaging effects caused by airborne contaminants;
- protection of personnel against exposure to airborne contaminants which may be detrimental to health;
- protection of the outside environment against noxious airborne emissions from a facility.

Most pharmaceutical manufacturing operations require process and product protection only. However, situations where hazardous substances such as cytotoxics are involved are becoming more frequent. They require combined product, personnel and environmental protection.

Two kinds of airborne contaminants must be distinguished: gaseous and particulate contaminants.



Activity	Particle diameter >0,3µm/min
Standing, sitting	100,000
Slight movement of head, hand, shoulders	500,000
Body, arm and foot movements	1,000,000
Position change from sitting to standing	2,500,000
Slow walking	5,000,000
Running	30,000,000

Figure 1: *Particle liberation by man. From Austin[3].*

In pharmaceutical applications of cleanroom technology, particles present the dominant contamination control task to which the following text is exclusively devoted.

Particles are minute pieces of matter in solid or liquid state with clearly defined physical boundaries, i.e. a clearly defined contour, dispersed in air as the surrounding continuous phase. An important sub-class of particles are **viable particles**, i.e. particles consisting of, or supporting, one or more live micro-organisms: single-cell organisms capable of multiplication under favourable conditions, i.e. in the presence of water and nutrients at an ambient temperature appropriate for multiplication. Bacteria, moulds and yeasts are single-cell organisms capable of multiplying into colonies under these circumstances. Therefore, they are also called **colony forming units** (**CFU**). Viruses can only multiply within cells: therefore, they can be considered as equivalent with non-viable particles from the contamination control point of view.

5.3 Sources of airborne particles

Three sources of airborne particles have to be taken care of by the air handling system of cleanrooms: the outside air, the air handling system, and dissemination in the working rooms.

Particles dispersed in the **outside air** originate both from natural causes and from the activities of man. Typical natural generators of particles are weathering processes, wind erosion, sandstorms, volcanic eruptions and forest fires. Traffic, industrial activities and heating are the predominant particle-liberating activities of man. Resulting from both contributory factors, the outside air will contain, depending on weather and the density of human population, 10 million to 10 billion particles ≥0.5µm per m^3 and temporarily even more. In addition, the outside air carries micro-organisms in concentrations which may well reach 1000 CFU/m^3 or more.

The **air handling system** generates particles through wear and tear of its mechanical components. In addition – and this is potentially much more dangerous – micro-organisms may multiply on its humid elements (air coolers, air humidifiers, cooling towers, or inside ductwork, if its insulation is damaged or insufficient) and subsequently be entrained into the supply air[2].

Inside **working rooms**, manufacturing and material handling processes are liable of liberating particles in considerable quantities. They may also penetrate into working rooms through uncontrolled infiltration of outside air into the building shell, or via airborne cross-contamination

from adjacent rooms. A particularly important dissemination source of particles is the human being (**Figure 1**): even during quiet activities a person will liberate per minute 100 000-500 000 particles 0.3µm and up to 500 colony forming units! **Figure 2** shows how densely the human body is populated with micro-organisms: its effectiveness as a dissemination source of colony forming units is thus easy to understand.

Part of the body/ Sampled material	Colony forming units
Skin of the head	$1{,}5 \times 10^6$ CFU/cm^2
Forearm	$1{,}0 \times 10^3$ CFU/cm^2
Hand and fingers	$1{,}0 \times 10^3$ CFU/cm^2
Nose secretion	10^6 to 10^7 CFU/g
Sneezing (once)	10^4 to 10^6 CFU
Ear wax	up to 10^8 CFU/g
Saliva	10^7 to 10^8 CFU/ml
Faeces	10^{11} CFU/g

Figure 2: *Micro-organisms and the human being. From Nieth[4].*

5.4 Controlling airborne contamination: general remarks

Where airborne contamination requires control, priority should be given above all to actions which aim at impeding particle generation and dissemination:

- process improvement towards reduced particle generation;
- improving machines and process equipment towards reduced particle dissemination, e.g. through construction measures and via suitable selection of construction materials;
- enclosures around dust-generating process steps;
- capture of dust at source of origin by means of suction or combined blowing/suction devices.

The contamination control task is then reduced to control of the airborne contaminants which remain after the potential of all the above steps has been exhausted. For controlling these remaining airborne contaminants, four key elements are required to interact:

- air filtration of the supply air and, where necessary, also extract air prior to exhausting it into the external atmosphere;
- appropriate airflow patterns for controlling particles disseminated in rooms;
- effective procedures for separating areas with different air cleanliness requirements from each other thus impeding cross-contamination;
- prevention of uncontrolled infiltration of outside air through the building shell into air cleanliness controlled areas.

The air handling system is indispensable for successfully dominating these particle sources and distribution mechanisms.

5.5 Key elements for ensuring air cleanliness in rooms

5.5.1 Air filtration

The air filters employed in cleanroom technology are fibre filters composed of a loose fleece of fine and finest fibres (**Figure 3**). Fibre diameter varies in function of the filtration performance of the fleece, and a lot of empty space is maintained between the fibres in order to enable particles to penetrate deeply into the filter material before capture so that a high dust storage capacity can be achieved. **Figure 4** shows a selection of air filters comprising all quality grades.

The following separation mechanisms interact in a fibre filter:

- The **sieve effect** (**Figure 5a**) which becomes effective when the distance between two fibres is smaller than the particle diameter.
- The **interception effect** (**Figure 5b**) which becomes effective when the streamline carrying

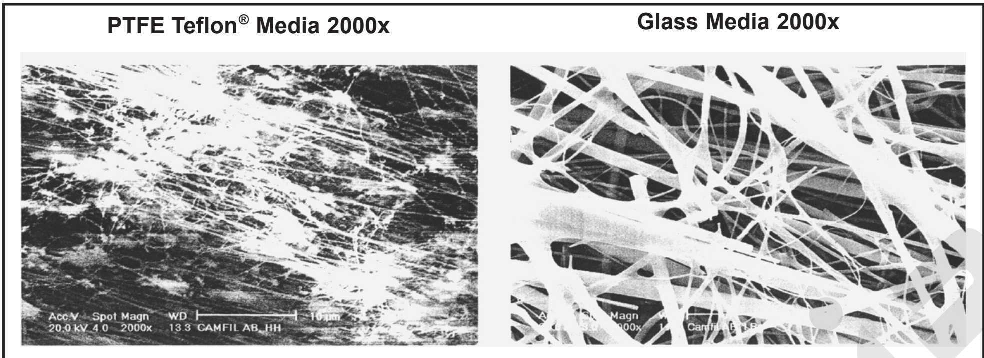


Figure 3: *Filter media for high-performance air filters: glass and PTFE (Teflon®). Copyright: Camfil Ltd.*

the particle passes so close to the filter fibre that the particle collides with it.

– The **inertia effect** (**Figure 6a**) which is effective above all for particles >1µm: due to their comparably high mass inertia these particles will be unable to follow the air streamlines as they are deflected around the fibre, and thus will tend to collide with the fibre.

– The **diffusion effect** (**Figure 6b**) which is of relevance for very small particles (below 0.5µm) with a correspondingly low mass: as a result of the constant collisions with the molecules of the gas surrounding them, these particles experience an irregular diffusional movement (*Brown*ian movement) around their streamline – the oscillations becoming more pronounced with decreasing particle diameter – thus increasing the probability that the particle will collide with one of the fibres of the filter.

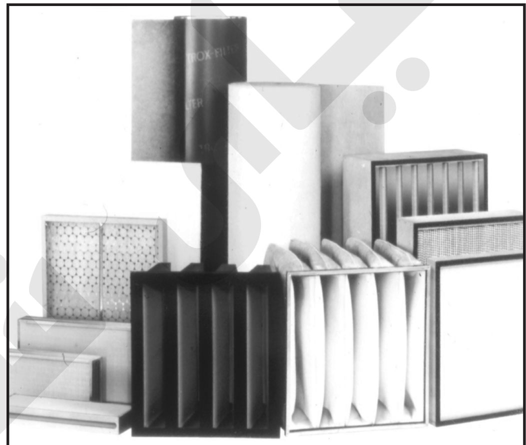


Figure 4: *A selection of air filters covering coarse dust, fine dust and HEPA filters. Copyright: Trox GmbH.*

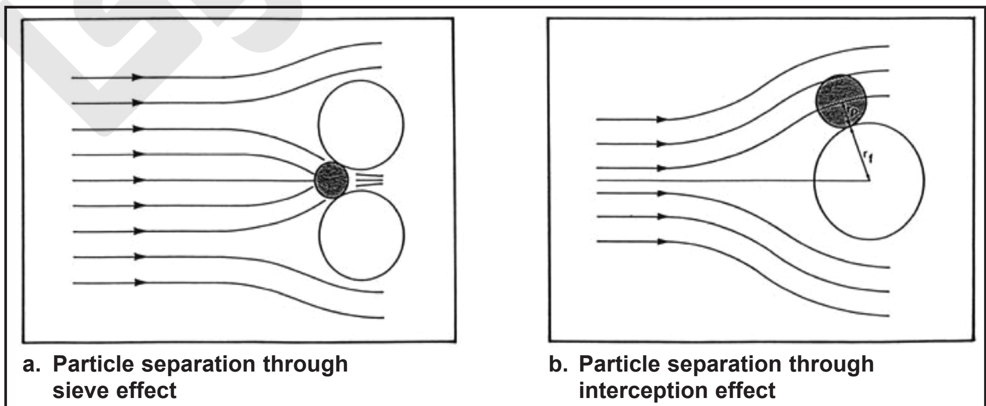


Figure 5: *Particle Separation mechanisms of fibre filters 1: sieve and interception effects.*

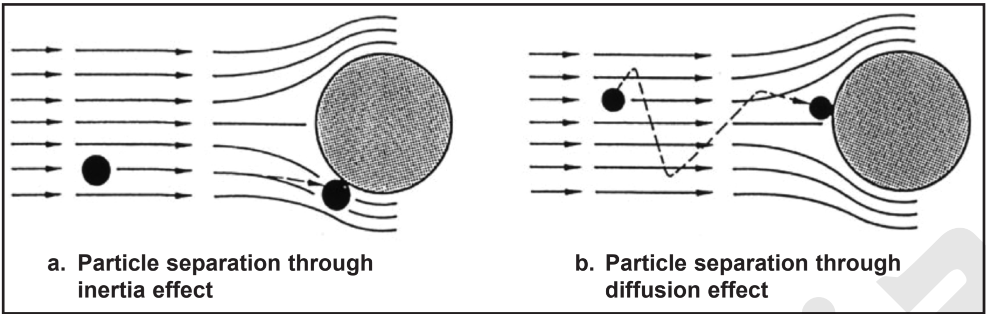


Figure 6: *Particle separation mechanisms of fibre filters 2: inertia and diffusion effects.*

The **integral separation efficiency E**, i.e. the over-all efficiency of a filter cell, is the sum of the effects mentioned above (**Figure 7**). It is normally indicated in percent (%).

Instead of the term integral separation efficiency, frequently the term **integral particle penetration P** is employed, i.e. the percentage of particles passing through the filter. The two terms complement each other as follows:

$$E + P = 100 \rightarrow P = 100 - E\ [\%]$$

Both the interception and the inertia effect become more effective with increasing particle diameter. The opposite is true for the diffusion effect: mobility of the particles and thus diffusion and the probability of capture will increase with diminishing particle size. As a consequence, the integral separation efficiency passes through a minimum, and integral particle penetration through a maximum. This particle penetration maximum is denominated **Most Penetrating Particle Size** (**MPPS**), and is a function of the air penetration velocity through the fibre fleece (**Figure 8**). As the air penetrates cleanroom filters at the very low velocity of a few cm/s, the filter medium requires to be pleated (**Figure 9**) for obtaining the desired filter face velocity of 0.4-0.5m/s. Numerically, the

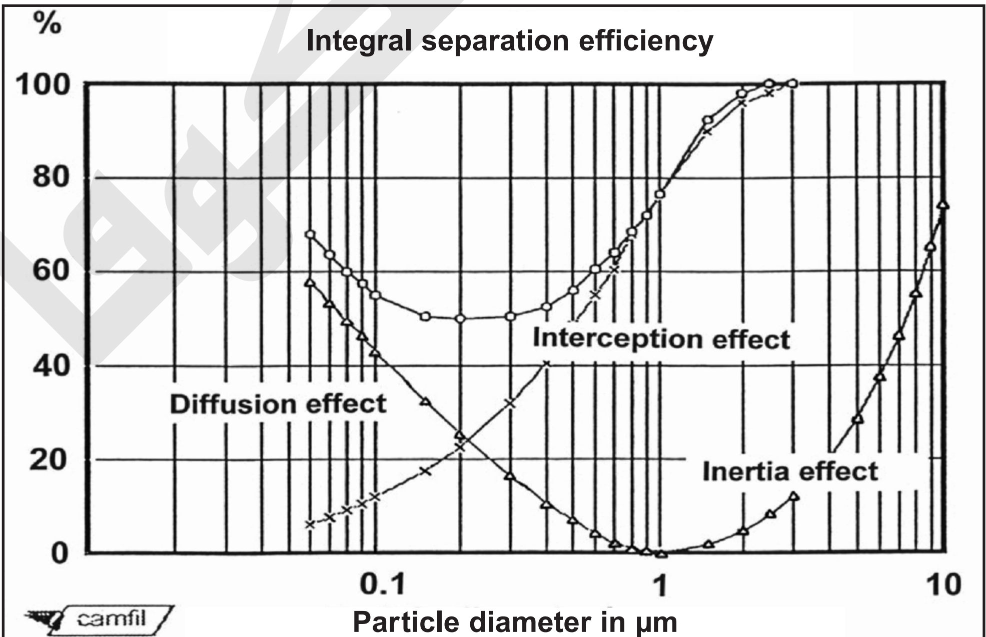


Figure 7: *Superimposition of separation mechanisms in fibre filters. Copyright: Camfil Ltd.*

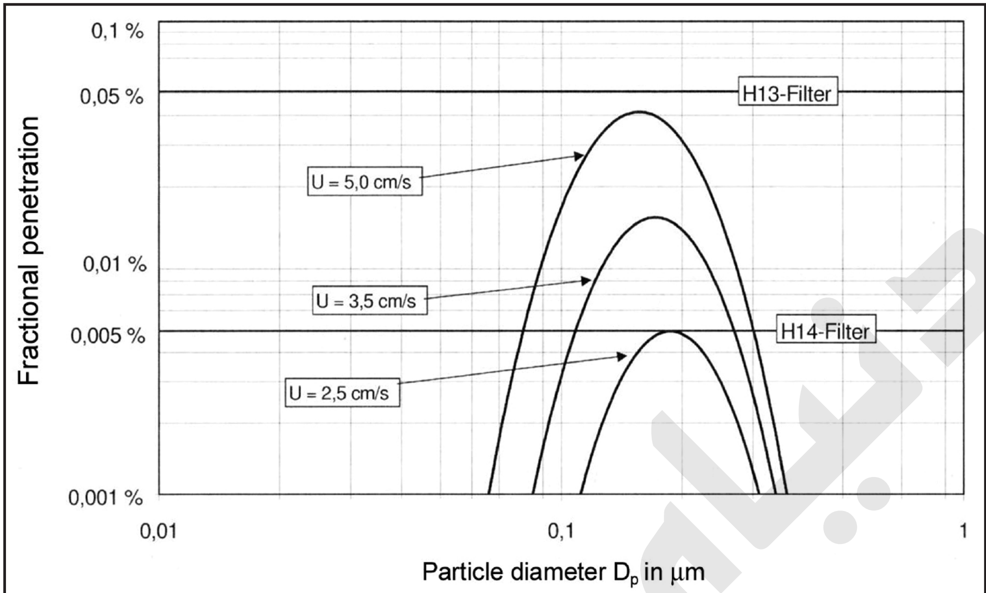


***Figure 8:** Fractional particle penetration characteristics of HEPA filters as a function of air velocity. From Förster/Schroers[5].*

MPPS diameter of high efficiency filters with glass fibre media is normally situated somewhere between 0.1 and 0.2µm.

As a consequence of these characteristics of fibre filters, both very small biological particles such as isolated virus cells with a typical diameter around 0.1µm and very large viable particles, i.e. micro-organisms which predominantly adhere to particles of 2µm diameter and above, are captured and retained most effectively by all quality grades of high efficiency air filters.

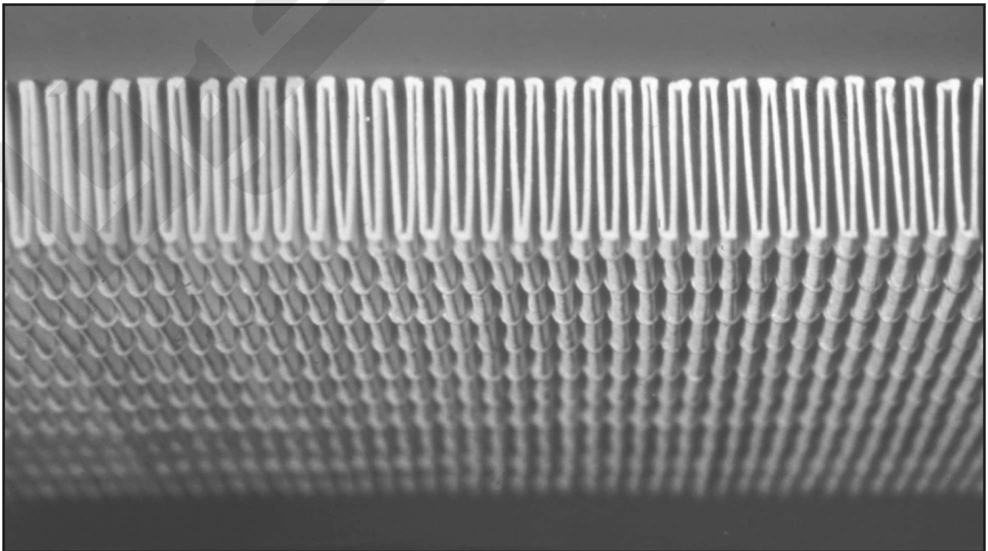


***Figure 9:** Minipleated filter medium. Copyright: Luwa/Vokes Air Ltd.*

Once deposited on the surface of the fibre, the particles will remain fixed there by **surface forces** (*van der Waals* forces).

The separation efficiency of air filters for HVAC systems covers a very wide range of particle sizes. Therefore, they are commonly subdivided into **performance groups** which are further subdivided into **air filter classes**. According to the European Standards EN 779[6] and EN 1822[7] five filter groups are distinguished:

– **coarse dust filters** (prefix G, classes G 1 to G 4);
– **fine dust filters** (prefix F, classes F 5 to F 9);
– **EPA filters**, i.e. **E**fficient **P**articulate **A**ir filters (prefix E, classes E 10 to E 12);
– **HEPA filters**, i.e. **H**igh **E**fficiency **P**articulate **A**ir filters (prefix H, classes H 13 to H 14);
– **ULPA filters**, i.e. **U**ltra **L**ow **P**enetration **A**ir filters (prefix G, classes U 15 and above).

Use of the EN filter classification scheme is also recommended by the *World Health Organisation* (WHO) in their guideline on pharmaceutical HVAC systems[8-9].

Air filter systems for cleanrooms normally are composed of three stages:

– **1st filter stage**: A medium performance fine dust filter for protecting the air handling unit against soiling;
– **2nd filter stage**: A high performance fine dust filter for maintaining the air ducts in a clean state;
– **3rd filter stage**: An EPA, HEPA or ULPA filter for guaranteeing the quality of the supply air entering the working rooms.

A three-stage air filtration system composed according to the recommendations given in **Table 1** will guarantee a long useful life for EPA, HEPA and ULPA filters.

5.5.2 Airflow patterns in cleanrooms and how to establish them

EPA, HEPA or ULPA filtration will guarantee the desired quality of the supply air entering the cleanrooms. This in itself, however, is not enough: for controlling the effects of particle and micro-organism dissemination in the working rooms to the required degree, and at the same time economically, the appropriate airflow pattern has to be selected. If room air cleanliness requirements are comparatively modest, it is sufficient to dilute the concentration of contaminants disseminated in the room by supplying it with sufficient quantities of EPA or HEPA filtered air according to the

Table 1: Recommended air filter combinations for cleanrooms of different air cleanliness classes

Air cleanliness class*	1st filter stage**	2nd filter stage**	3rd filter stage**
ISO 5	F 7	F 9	H 14
ISO 6	F 7	F 9	H 14
ISO 7	F 7	F 9	H 13
ISO 8	F 7	F 9	H 12

* Air cleanliness classes according to ISO 14644-1 for occupancy state *in operation*

** Filter classification according to EN 779:2002 (fine dust filters) and EN 1822:2009 (EPA and HEPA filters)

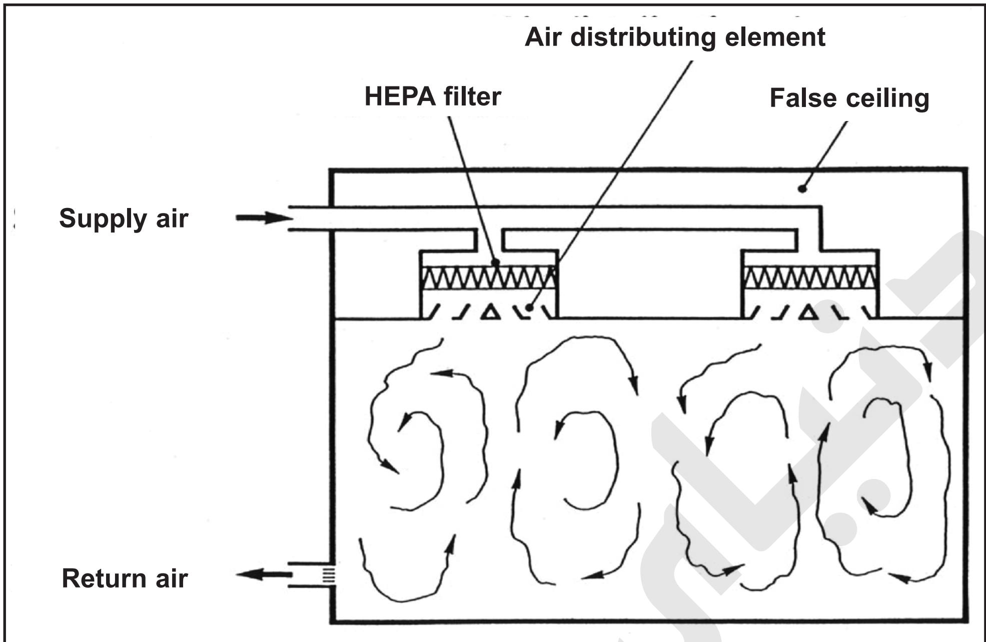


Figure 10: Airflow patterns in cleanroom 1: Turbulent mixing airflow.

principle of **turbulent mixing airflow**. This airflow pattern (**Figure 10**) is well known from air conditioning technology. With supply air filtration by means of EPA or HEPA filters, the requirements of the air cleanliness classes ISO 7 and ISO 8 according to the International Standard ISO 14644-1[1] in the occupancy state *in operation* can be fulfilled by a cleanroom during use with comparatively modest airflow rates. The ISO room air classification scheme is described in *Chapter 3*.

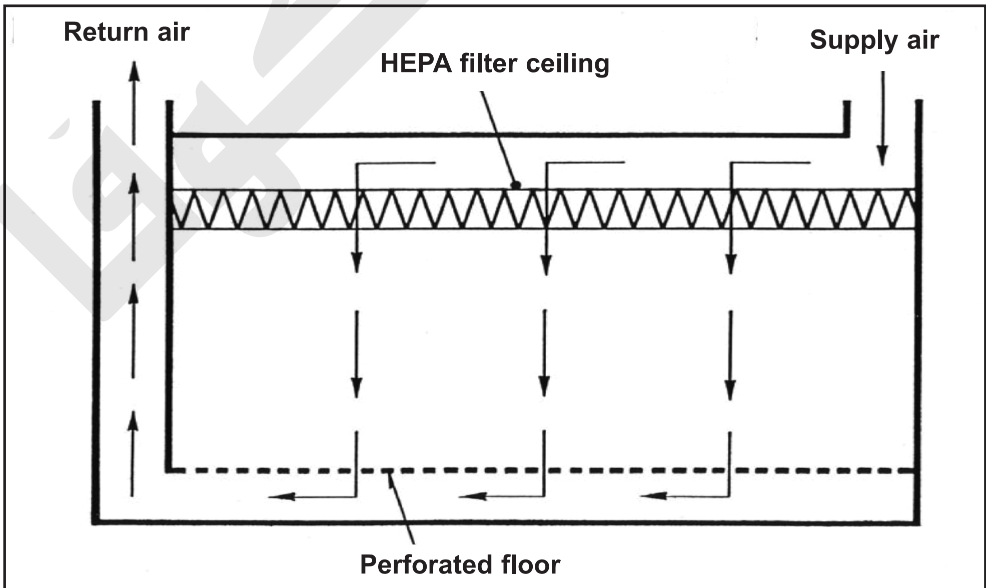


Figure 11: Airflow patterns in cleanrooms 2: Vertical unidirectional airflow.

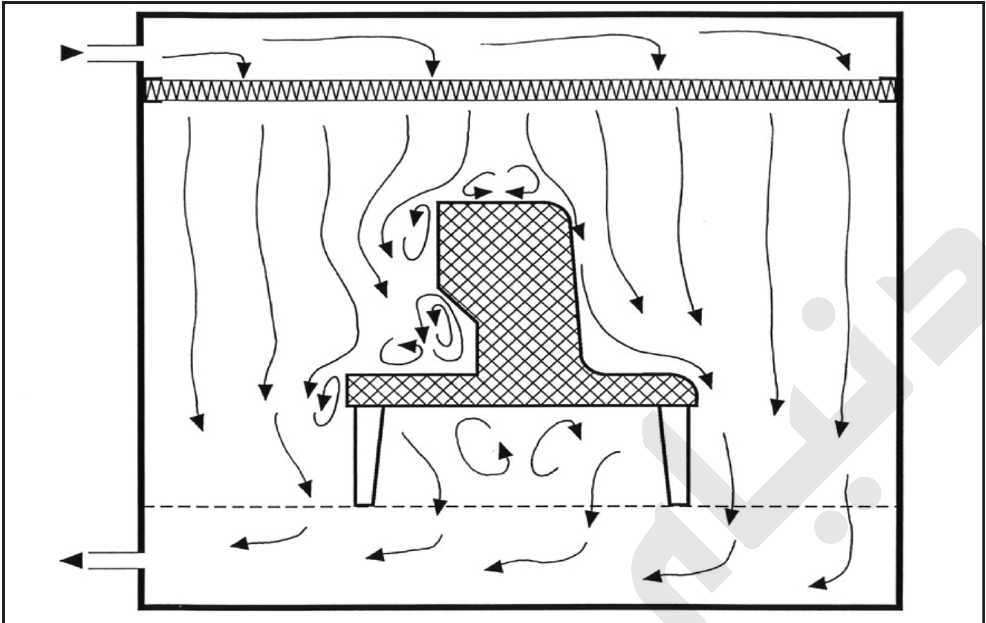


Figure 12: *Unidirectional airflow around obstacles – bad (left) and good (right).*

For higher and highest air cleanliness requirements a different airflow pattern must be employed: **unidirectional airflow** – also called, albeit not quite correctly from the scientific point of view, *laminar airflow*. Unidirectional airflow is normally established by means of a continuous HEPA or ULPA filter ceiling which will cause the airflow to move on more or less parallel streamlines and with a reasonably uniform velocity. Thus, this airflow pattern achieves removal of particles and micro-organisms disseminated into the room air by the most direct path – thus rendering them harmless. Unidirectional airflow is distinguished by very little lateral mixing and limits the detrimental effects of airborne particles to the immediate vicinity of the streamline along which they are carried. **Figure 11** presents an idealised example with the airflow directed vertically downwards and withdrawn from the room by means of a perforated floor. This kind of floor is unacceptable in the pharmaceutical industry for reasons of hygiene. Therefore the extract air will have to be withdrawn from the room through openings in the walls close to floor level: a somewhat modified air flow pattern results which is, however, still capable of sweeping particles away from their point of dissemination on a reasonably direct path. Unidirectional airflow requires terminal air filters of at least H 13 quality, i.e. a quality permitting testing for leaks to be performed after installation on site.

Unfortunately, the production equipment installed in clean areas with unidirectional airflow frequently does not take the aerodynamic requirements of this airflow pattern satisfactorily into consideration. Vortices and zones with stagnant air may thus be generated. Both are characterised by pronounced lateral transport of any contamination liberated there. Edges should be rounded, and recesses be avoided (**Figure 12**). Local heat sources generate thermal currents which again lead to zones with stagnant air (**Figure 13**). Horizontal or inclined airflow (**Figure 14**) are both capable of coping effectively with this phenomenon.

Stable unidirectional airflow requires an air velocity sufficiently high to minimise the effect of local heat sources. Even small temperature differences can cause surprisingly strong thermal currents: the heat liberated by a standing or sitting person, for example, of about 80W intensity, will lead to a thermal current with a velocity of 0.2m/s above her head!

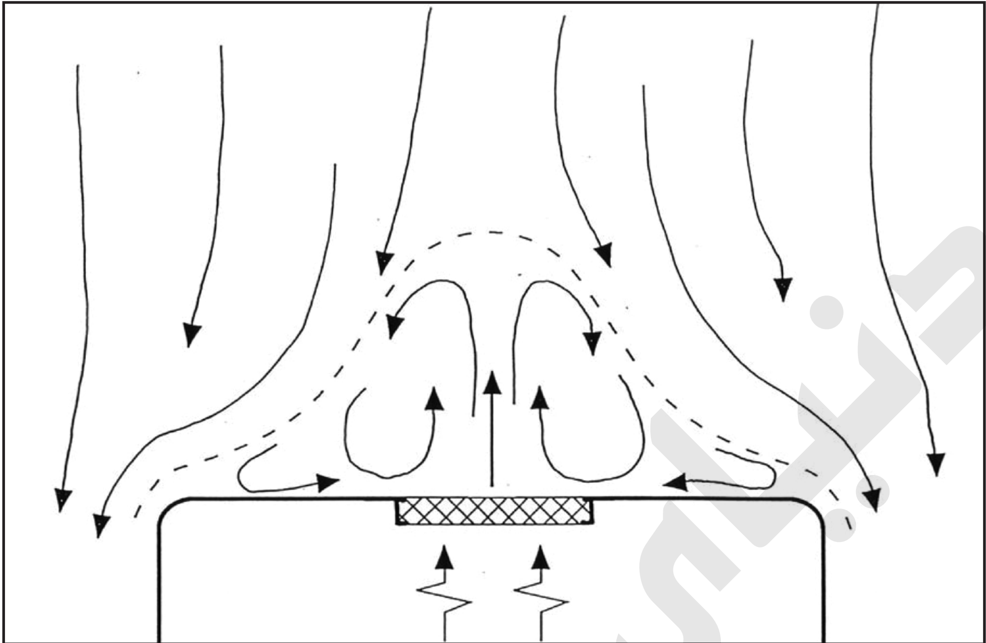


__Figure 13:__ Unidirectional airflow prejudiced by a heat source.

5.5.3 Area segregation concepts

Clean areas frequently consist of multiple rooms with differing air cleanliness requirements. In order to segregate clean areas from adjacent zones with a lower air cleanliness level, and to prevent infiltration of contamination from these zones, ISO 14644-4[10] distinguishes between three basic conceptual approaches:

– the **displacement** concept, i.e. spill-over of clean air into areas of lower air cleanliness level, providing an **aerodynamic barrier**;

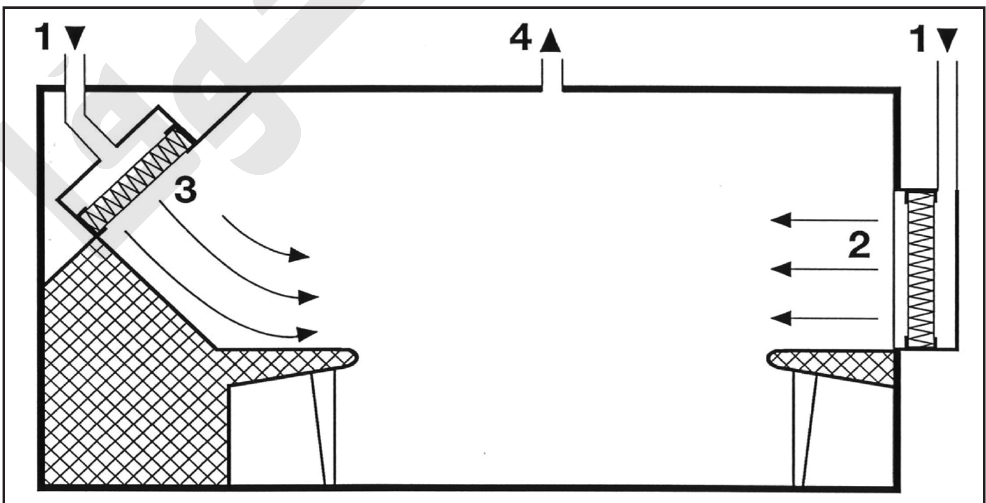


__Figure 14:__ Control of heat sources with horizontal or inclined unidirectional airflow.
KEY: 1 supply air; 2 horizontal unidirectional airflow; 3 inclined unidirectional airflow; 4 extract air.

– maintenance of a **pressure differential** between zones of different air cleanliness levels;
– and the **physical barrier** concept where an impervious physical barrier, i.e. a wall prevents contamination transfer to a clean zone from a less clean area.

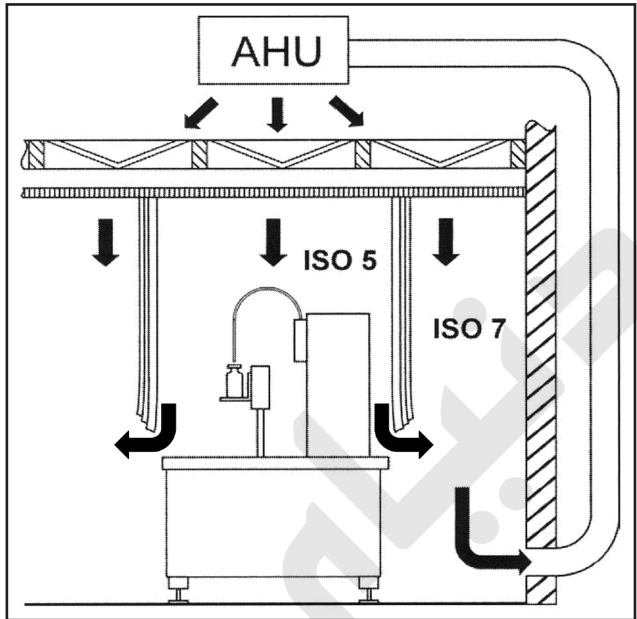


Figure 15: *Air spill-over from an aseptic grade A core area into the surrounding grade B area.*
The ISO air cleanliness class numbers refer to the occupancy state in operation. From Rauschnabel[11].

The **displacement** principle is mainly used when an area protected by unidirectional airflow is to be separated from a surrounding area of lesser air cleanliness level with turbulent airflow. An example is the protection scheme of a filling machine shown in **Figure 15**. Here the aseptic core is protected by unidirectional airflow maintaining air cleanliness class ISO 5 in operation. The air then spills over into the surroundings where turbulent airflow with an air cleanliness level according to ISO Class 7 is maintained in the occupancy state *in operation*. In order to control the effect of flow obstacles in the spill-over zone, an accelerated airflow should be maintained there – by using, for instance, an air velocity of at least 1.5 times the mean velocity of the unidirectional airflow maintained in the high-cleanliness area[12]. At such an air velocity, the pressure difference between grades A and B is still well below 1Pa.

Pressure differences are maintained between adjacent rooms if the transport of airborne contamination from one room to another – the so-called **cross-contamination** effect – is to be prevented. If a room is to be protected against airborne contamination from its surroundings, it is maintained at a **positive pressure** in comparison with its environment. If, on the other hand, contamination is to be contained within a room and propagation to the outside has to be prevented, the room has to be maintained at a **negative pressure**, i.e. in a **containment** condition.

5.5.4 Infiltration of false air through the building envelope

Overpressure can also be of help in impeding the infiltration of contaminated outside air into a building. Such infiltrations are, however, inevitable at high wind velocities and the resulting dynamic pressures – positive and negative – acting upon the building[13]. No building, after all, is completely airtight.

A simple remedy is the utilisation of the **house-in-house concept** where such infiltrations can be neutralised in the corridors surrounding the clean working areas. **Figure 16** presents the layout of a production facility for sterile medicinal products designed according to this principle.

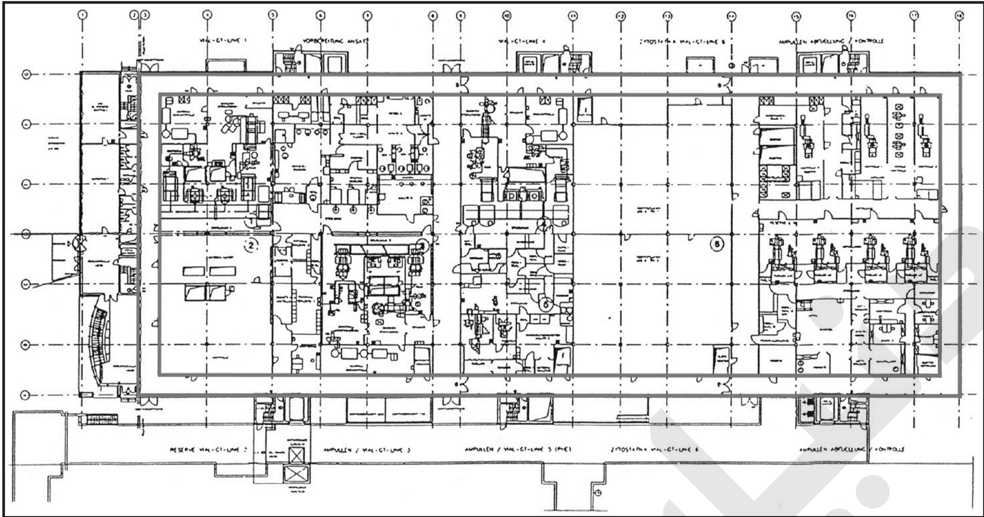


Figure 16: House-in-house concept for protecting clean facilities against infiltration of false air from the outside atmosphere.

5.6 Air handling concepts for GMP-compliant cleanrooms

5.6.1 GMP stipulations

The **Good Manufacturing Practice** guidelines (**GMP** guides, see also *Chapter 3*) elaborated by the regulatory authorities of the pharmaceutical industry are devoted to ensuring product safety – and product safety only – of the medicinal products sold in the market place. Only very general stipulations regarding pharmaceutical air handling systems are specified in the general GMP guidance as found, for example, in Part 1 of the GMP guideline[14] of the European Union (EU GMP guide) or the corresponding FDA guidance in 11 CFR 211[15], both discussed in *Chapter 3*. Specific stipulations for the air handling systems of pharmaceutical cleanrooms are given, for instance, in the chapter *Premises* of Annex 1 to the EU GMP guide – devoted to manufacture of sterile medicinal products – and in an analogous guideline[16] published by WHO. The principal requirements are:

- filtered supply air;
- maintenance of a positive pressure relative to surrounding areas of lower air cleanliness grade under all operating conditions;
- pressure differential of 10-15Pa (guidance value) between adjacent rooms of different air cleanliness grade (this author recommends 15Pa – see also[9]);
- particular attention to zones of highest risk;
- specific attention to requirements posed by pathogenic, highly toxic, radioactive or live viral or bacterial materials or products;
- warning system for indicating failure of the air handling system;
- pressure difference indicators where important in combination with regular data recording;
- sealing of false ceilings in order to inhibit the transfer of airborne contamination to the cleanroom from the space above it.

For non-sterile pharmaceuticals, no comparable determinations are found in the EU GMP guide or in FDA's 21 CFR 211. However, this topic is addressed very comprehensively in a GMP guideline[9] published by WHO specifically for this class of products.

Contrary to the European and American GMP authorities, WHO goes beyond the strict focus on

products and processes in its GMP guideline for products containing hazardous substances[17]: it widens its scope to cover also the protection of the facility's personnel and the environment.

5.6.2 Control parameters

The minimum **air cleanliness** requirements to be guaranteed by the HVAC system during sterile production are stipulated comprehensively both in the EU GMP guide and in the corresponding WHO guideline (see *Chapter 3* for details). If a high level of air cleanliness is required for manufacturing non-sterile pharmaceuticals, such requirements should be determined with the help of risk assessments.

The air handling system is also responsible for maintaining the specified **pressure differentials** between rooms and therefore essential for preventing cross-contamination.

Human comfort considerations will, as a rule, be decisive in the determination of the **room temperature**. Temperatures in the comfort range will ensure that the attentiveness of the personnel remains at its best. Sophisticated garmenting schemes as required, for instance, for operators present in grade B areas for interference into grade A areas, are normally quite inflexible from the thermal comfort point of view: a reasonable level of comfort is only assured if room temperatures are maintained within a narrow tolerance band (see VDI 2083 Part 5.1[18], Annex 1). Product or process will rarely require values outside the human comfort range. (VDI guidelines are published bilingually: left column in German, right column in English; the VDI 2083 series on cleanroom technology[19] can be highly recommended for worldwide use.)

Human comfort again will, as a rule, be the point of departure in determining the **relative humidity** of the room air. Only rarely product or process requirements will be the driving forces. One such case is tabletting, especially of effervescents: they are highly hygroscopic and therefore require maintenance of a very low air humidity level. Where human comfort is the decisive factor, values in the range 35-65% relative humidity are appropriate. Room air humidities below 35% are undesirable because they may cause drying of mucous tissues, also electrostatic charges could become a risk then. Supply air humidities above 80% may possibly favour the growth of micro-organisms – especially moulds – in fine dust, EPA and HEPA filter media.

The FDA *Guidance for Industry* regarding aseptic processing[20] requires a **minimum hourly air change** rate of 20 for the controlled areas surrounding aseptic cores. Similar requirements do not exist in the corresponding EU and WHO GMP guidelines.

5.6.3 Decentralised units for local air cleanliness control

An example of a decentralised unit for locally establishing a high air cleanliness level is the **clean work station**, also called **clean work bench**. Horizontal airflow (**Figure 17**) is appropriate if a single, isolated work process requires protection against contamination risks originating from the environment and/or the operator. On the other hand, vertical airflow is

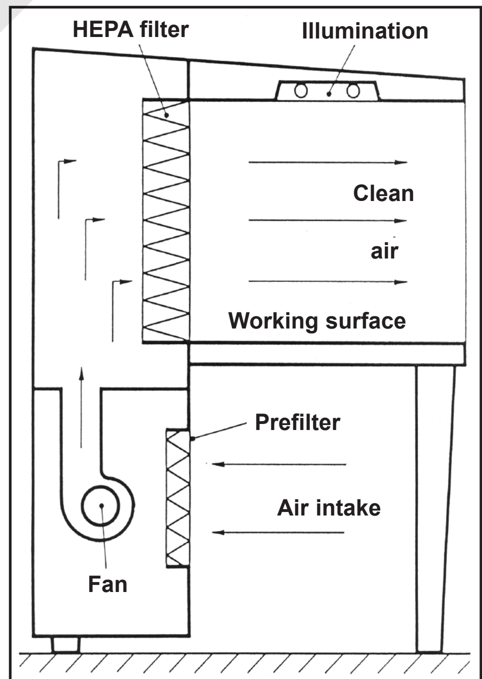


Figure 17: *Clean work station with horizontal unidirectional airflow.*

indicated if the environment and the operator have to be protected against the process. Particularly good containment and worker protection is provided by **biosafety work stations**: they are employed, for example, for handling highly contagious or genetically modified micro-organisms, or for manipulating highly active pharmaceutical ingredients as during the reconstitution of cytotoxics in hospital pharmacies (**Figure 18**).

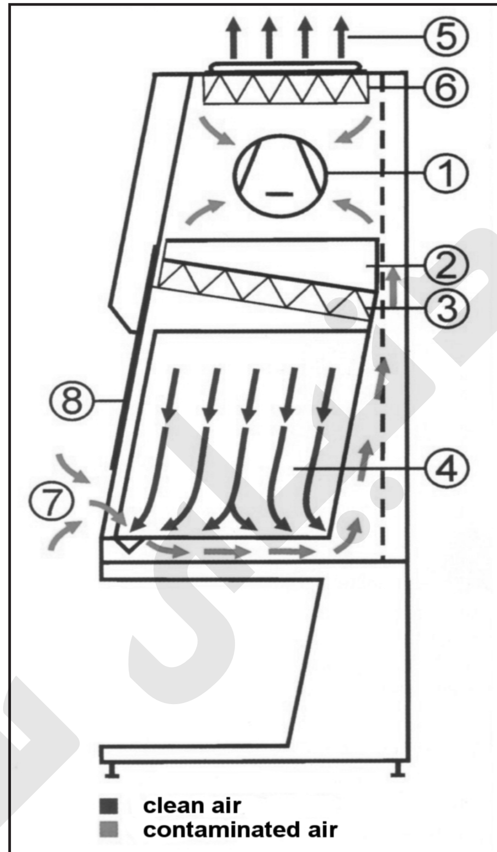


Figure 18: Biosafety work station for both product and personnel protection against hazardous substances. Copyright: Berner International GmbH.
KEY: 1 fan; 2 plenum chamber; 3 recirculated air filter; 4 unidirectional airflow; 5 extract air; 6 extract air filter; 7 induction air; 8 window.

Such clean work stations are autonomous **room air recirculation units**: they are positioned in the room just like any piece of furniture, draw their air supply from the room and need nothing but connection to the electricity system for becoming operational. Control of temperature and humidity is left to the HVAC system serving the room.

Isolators or Restricted Access Barrier Systems (RABS units, see *Chapter 11*) are also frequently built as room air recirculation units, i.e. as **dynamic** isolators or RABS units. Fan filter units (FFU's) as shown in **Figure 19** can also be employed for room air recirculation purposes. Installed into the room's ceiling void, they would then be fed with room air recirculated via extract air grilles incorporated into the false ceiling.

Apart from concepts of this kind, centralised air handling systems will be responsible also for ensuring the required air cleanliness level in cleanrooms.

5.6.4 Central air handling system concepts with turbulent airflow

For many contamination control tasks in the pharmaceutical industry, maintenance of turbulent mixing airflow, with terminal HEPA filters integrated into the supply air outlet elements, is perfectly sufficient for meeting the air cleanliness requirements.

Figure 20 presents the schematic flow diagram of an air handling system with **air recirculation**. Turbulent airflow is maintained in the working rooms. In order to minimise the risk of cross-contamination, only a sequence of rooms serving a similar purpose should be served by the same air handling unit.

The air handling unit presented here is subdivided into a supply air handling unit and an extract air handling unit. The **supply air handling unit** is responsible for establishing the required temperature and humidity levels as well as preliminary air filtration and, together with the **extract air unit**, for air circulation. Air handling units are assembled from a sequence of components:

– **dampers** for adjusting or controlling air flow rates to the required levels;

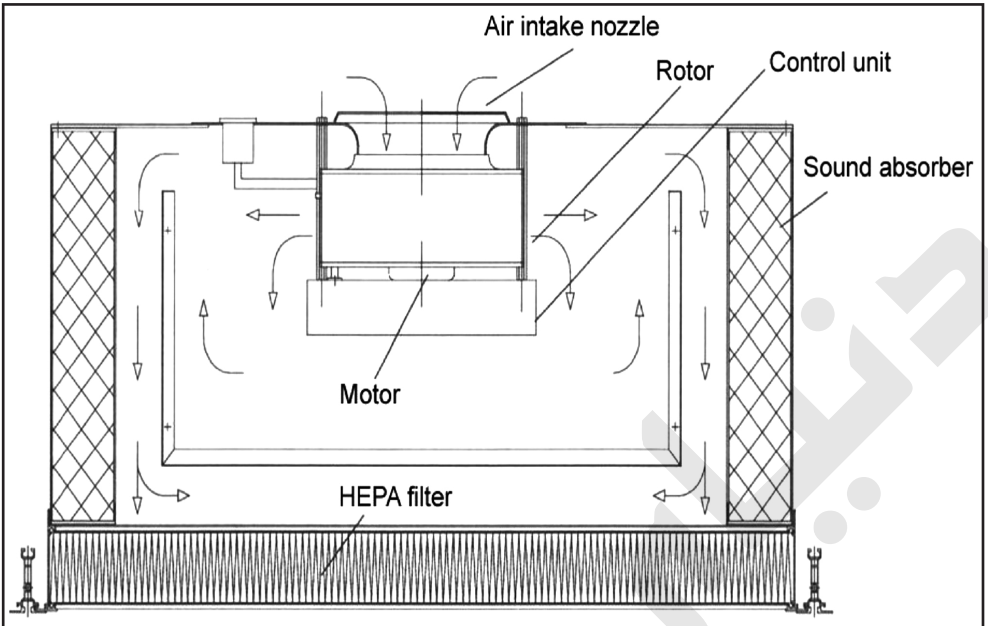


Figure 19: *Fan filter unit (FFU). From Renz[21].*

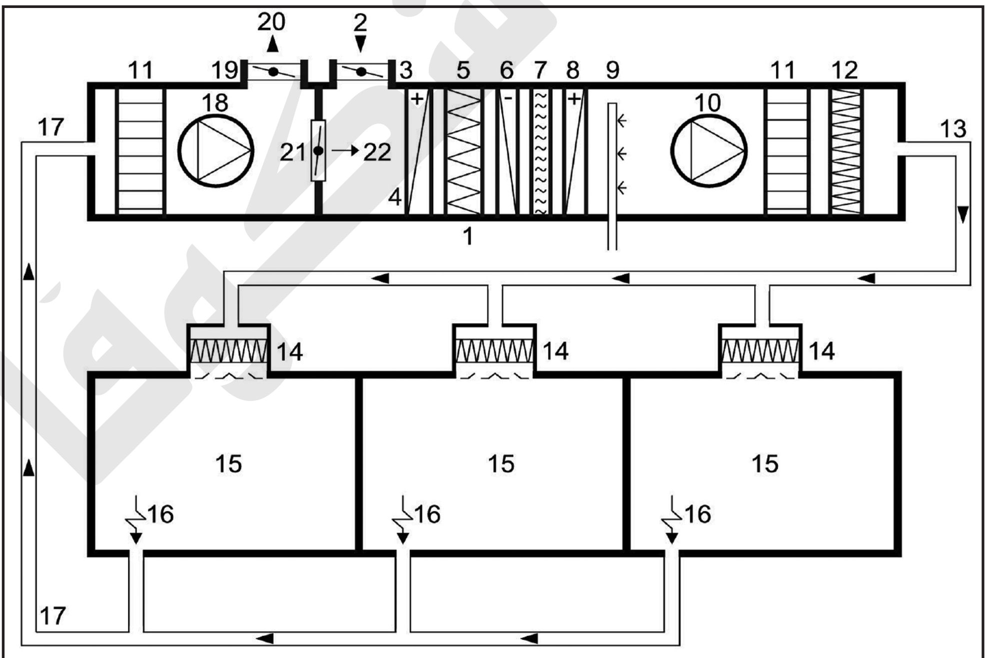


Figure 20: *Air circulation system with air recirculation.*

KEY: 1 air handling unit; 2 outside air; 3 outside air damper; 4 pre-heater; 5 first filter stage; 6 air cooler; 7 droplet separator; 8 air heater; 9 air humidifier; 10 supply air fan; 11 sound absorber; 12 second filter stage; 13 supply air duct; 14 integrated HEPA filter/air outlet; 15 rooms; 16 extract air capture; 17 extract air duct; 18 extract air fan; 19 extract air damper; 20 air exhaust to atmosphere; 21 recirculated air damper; 22 recirculated air.

– **ventilators**, also denominated **fans**, for maintaining the air in circulation;
– **air coolers** , employing chilled water as coolant, for reducing the air temperature as well as for condensing excessive water out from humid outside air;
– **droplet separators** (optional) for removing eventual droplets entrained into the supply air from the wet surfaces of the air coolers;
– **humidifiers** for increasing the relative humidity of excessively dry outside air if required;
– **sound absorbers** for reducing the noise generated by the ventilators to an acceptable level;
– **supply air filters** positioned as specified in *para. 5.4.1* plus **extract air filters** if required;
– **air heaters** operated with hot water or – exceptionally – electricity for increasing, if necessary, the air temperature; in cold climates, additional **pre-heaters** may be necessary if the first air filter stage requires protection against supercooled water droplets or snow borne by the outside air.

If an extremely low level is required for the relative room air humidity, conventional air coolers are no longer able fully to cope. A second dehumidification stage based upon the sorption principle, also called chemical dryers[9] must then be added.

From the air handling unit the conditioned air then proceeds through air ducts to the rooms where **filter air outlets** composed of terminal EPA or HEPA filters followed by an air outlet element for establishing the turbulent airflow are installed. As the pressure difference across the terminal HEPA filters is relatively high, the supply air ducts require a high level of air tightness; leakage rates

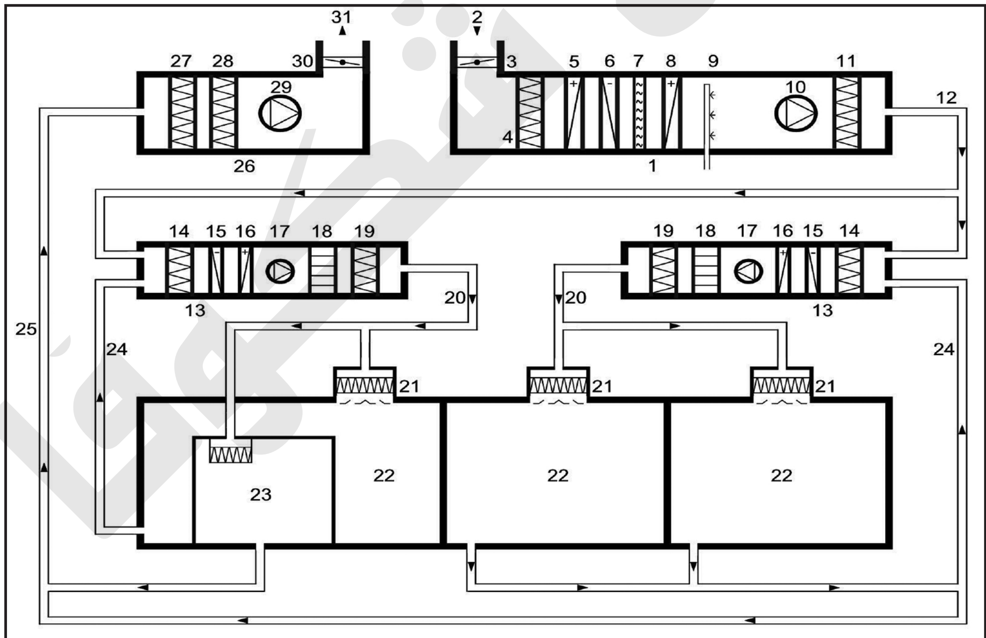


***Figure 21:** Air circulation system with sectorised air recirculation.*
KEY: 1 primary air handling unit; 2 outside air; 3 outside air damper; 4 first filter stage; 5 pre-heater; 6 air cooler; 7 droplet separator; 8 air heater; 9 air humidifier; 10 primary air fan; 11 second filter stage; 12 primary air duct; 13 secondary air handling unit with: 14 first filter stage (optional) – 15 re-cooler – 16 re-heater – 17 supply air fan – 18 sound absorber – 19 second filter stage; 20 supply air duct; 21 integrated HEPA filter/air outlet; 22 rooms; 23 process unit with HEPA filtered air supply; 24 recirculated air duct; 25 room and process extract air duct; 26 extract air handling unit; 27 first extract air filter stage or scrubber; 28 second extract air filter stage (if required); 29 extract air fan; 30 extract air damper; 31 air exhaust to atmosphere.

should meet at least the requirements of leakage class C according to EN 1507[22]. As air outlet element, WHO recommends[9] perforated plate or low induction swirl type diffusers.

Extract air grilles positioned close to the floor or – if particle dissemination in the room is low – close to the ceiling or integrated into it capture the extract air which is then carried via **extract air ducts** to the **extract air handling unit** from where it is partially exhausted into the outside atmosphere and partly recirculated into the supply air unit.

In facilities requiring a number of separate air handling circuits it makes sense (**Figure 21**) to dedicate a specific **primary air handling unit** to the conditioning of the outside air. This unit provides the necessary quantities of outside air for the **secondary air handling units**, where it is mixed with recirculated air. Each of these secondary units serves a specific facility sector composed of rooms which permit linking their air-conditioning together. As there is no recirculation of the outside air fraction, no airborne cross-contamination can take place between the zones served by different secondary air handling units. Thus, they may well serve facility sectors serving different purposes and differing in their contamination control requirements.

Figure 21 also illustrates how **process exhaust air** containing hazardous substances can be prevented from causing detrimental effects to the outside environment. It is exhausted into the atmosphere only after due purification: by air filtration in the case of an aerosol composed of dangerous particles or contagious micro-organisms, or by means of scrubbers for gaseous contaminants. If contamination of the extract air duct is to be prevented, special bag-in-bag-out filter housings equipped with HEPA filter cells must be installed as closely as feasible to the extract air capture. Such units (as described in ref.17) permit safe replacement of filter cells carrying hazardous particles.

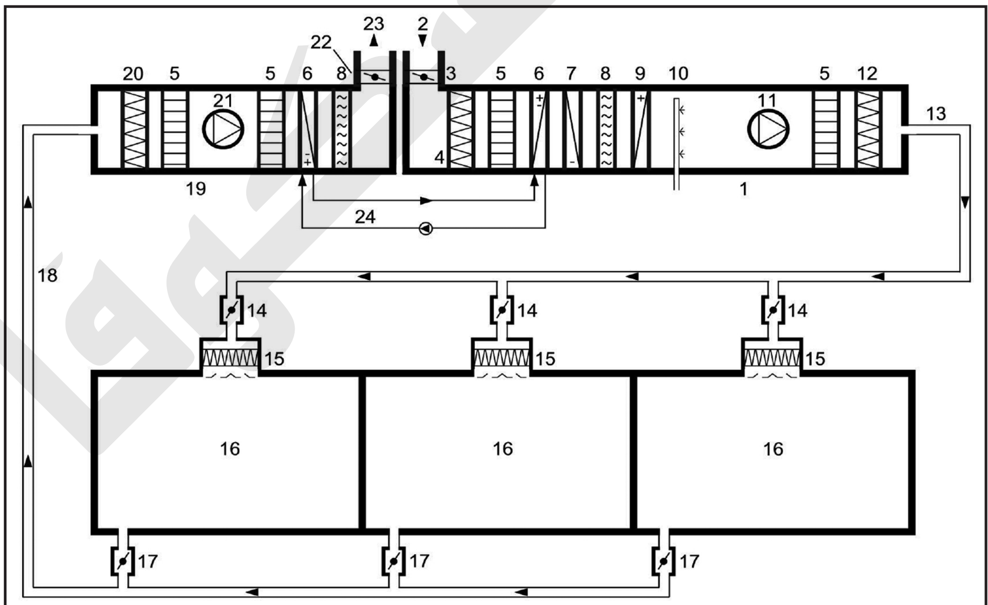


Figure 22: *Air circulation system operated with 100 % outside air.*
KEY: 1 supply air handling unit; 2 outside air; 3 outside air damper; 4 first filter stage; 5 sound absorber; 6 energy recovery heat exchangers; 7 air cooler; 8 droplet separator; 9 air heater; 10 air humidifier; 11 supply air fan; 12 second filter stage; 13 supply air duct; 14 constant air volume valve; 15 combined HEPA filter/air outlet; 16 rooms; 17 variable air volume valve; 18 extract air duct; 19 extract air handling unit; 20 extract air filter; 21 extract air fan; 22 extract air damper; 23 air exhaust to atmosphere; 24 water/glycol circuit for energy recovery.

Air recirculation is the most effective option from the energy conservation point of view. If, however, the cross-contamination risk is such that – even with HEPA filtration – air recirculation is deemed unacceptable, the air handling system will have to be operated with **100% outside air**. This is also required where volatile solvents or odorous substances are liberated into the room air during processing, as fibre filters are ineffective against them.

Figure 22 shows an air handling system operating with 100 % outside air. In order to minimise the heating and cooling requirements, energy exchange between outside and extract air by means of closed-loop heat exchangers should be foreseen, interlinked through a coolant circuit. Thus, during the winter season, the outside air is pre-heated by withdrawing energy from the extract air. On hot summer days, the outside air is pre-cooled by the extract air. Heat recovery by means of closed loop heat exchangers guarantees that no contamination can be transferred from the extract air to the supply air.

Figure 22 also illustrates how pressure differences between rooms can be established and maintained: by installing, for instance, constant air volume valves in the supply air ducts upstream of the terminal filters, and variable air volume valves in the extract air ducts.

5.6.5 Protection configurations with unidirectional airflow

Annex 1 of the EU GMP guide and the corresponding WHO guideline stipulate as guidance values an air velocity of 0.36 – 0.54m/s for unidirectional airflow during aseptic processing. Thus, a high airflow rate results in comparison with turbulent airflow. As a consequence, unidirectional airflow is expensive: it should be employed as sparingly as possible.

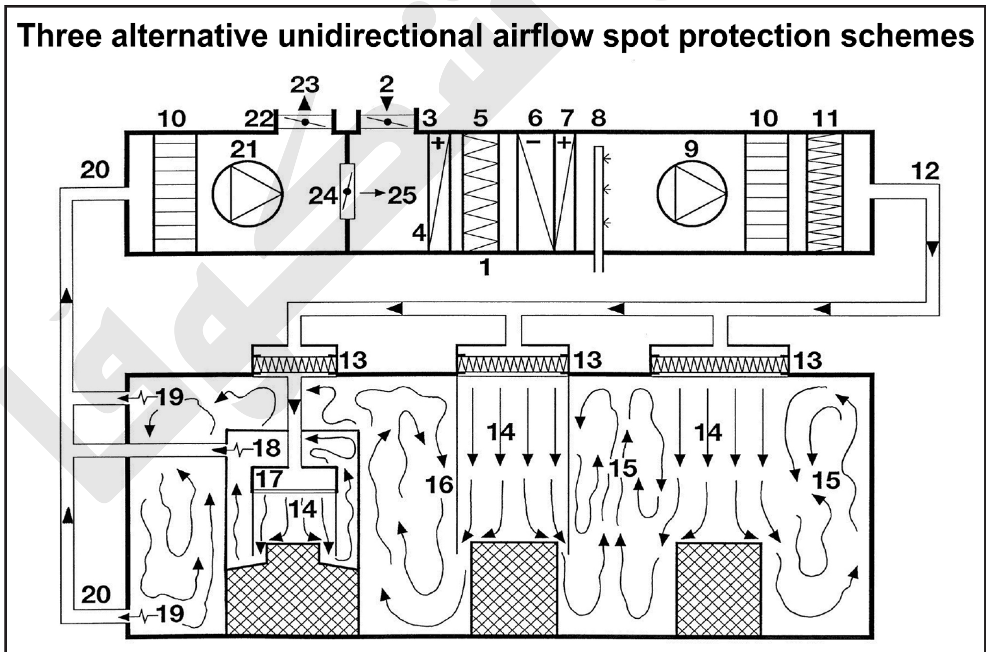


Figure 23: Air circulation system with three alternative unidirectional airflow spot protection schemes.
KEY: 1 air handling unit; 2 outside air intake; 3 outside air damper; 4 pre-heater; 5 first filter stage; 6 air cooler; 7 air heater; 8 air humidifier; 9 supply air fan; 10 sound attenuator; 11 second filter stage; 12 supply air duct; 13 HEPA filter as third filter stage; 14 work station, protected with unidirectional airflow; 15 room areas with turbulent airflow; 16 curtain or partition; 17 machine-integrated air distribution; 18 machine extract air; 19 extract air from room; 20 extract air duct; 21 extract air fan; 22 extract air damper; 23 air exhaust to atmosphere.

Figure 23 presents three conceptual alternatives of the so-called **spot protection** principle:

- free airflow around a work station;
- a work station shielded from its surroundings by means of curtains or partitions;
- a work station fully integrated into a machine, i.e. a situation comparable with an isolator as discussed in *Chapter 11*.

Wherever possible, partition elements should be foreseen between the critical process area and its surroundings: thus, clear separation of this area from its less exacting surroundings is achieved by means of an effective **aerodynamic barrier**[9]. Moreover, clear separation of worker and process results: the operator observes the process from the outside and only interferes in case of emergency.

Whether such guidance elements are foreseen or not: after providing protection with displacement airflow for the critical area, the air will spill over into the peripheral room areas. Whether this spill-over will be sufficient for maintaining the required air cleanliness level there, or whether additional ceiling air outlets will have to be foreseen, has to be decided case-by-case. **Computer-based airflow simulation** during the installation's design stage is a potent and cost-efficient tool for decision making; it is also very helpful for a preliminary assessment of complex flow situations expected in the vicinity of process equipment[23-26], thus avoiding unpleasant, costly and time-consuming surprises after start-up.

5.7 HVAC system design

5.7.1 Development of the basic HVAC system specification

As a first step towards design and calculation of the air handling system of a given investment object, a **basic specification** must be developed. Prior to its elaboration, fundamental determinations regarding the investment object such as the following must stand:

- description of the investment object, its objectives, the products to be manufactured in the facility and the markets to be served;
- exact geographical localisation of the building site plus identification of the exact positioning of the facility on the factory site;
- identification of the applicable GMP compendia;
- the technical standards to be obeyed for converting the GMP stipulations into technical hardware and software and for ensuring the desired performance and quality level of the installation;
- facility layout together with the air cleanliness zoning and pressurisation concept;
- basic architectural design and its integration into adjacent building sectors if applicable.

An example of a basic specification for the HVAC system of a facility devoted to aseptic filling of liquid dosage forms of parenterals into primary containers for the consumer market is given in **Appendix 5.A**.

Once the basic specification is agreed, the first draft of the **Qualification Master Plan** for the HVAC system should be elaborated, as a sub-module of the facility's overall **Validation Master Plan** (see *Chapter 8*).

5.7.2 Determining the supply airflow rate

Based upon these fundamentals, the architectural design departing from the sequence of the processing steps, material, personnel and waste flows, as well as the definition of the basic equipment data, a preliminary flow diagram of the air circulation system can now be drawn and subsequently improved in the general design context.

Point of departure for dimensioning the air circulation system is the determination of the cooling load it will have to dominate. Load calculations follow the proven procedures used in the air-conditioning field, taking the following contributory elements into consideration individually for each room:

- thermal transmission through the building shell under extreme summer and winter conditions;
- sun irradiation through windows;
- heat dissipation by process equipment;
- number of persons present and their state of activity;
- illumination.

Thermal transmission and the sun irradiation intensity will be considerably reduced if the facility layout follows the principles of the house-in-house concept as shown in *Figure 16*.

The airflow rate determined through the cooling load calculations may be insufficient for also meeting the required air cleanliness levels and/or for fulfilling formal GMP requirements. Therefore, additional aspects have to be taken into consideration:

- the extension of the areas to be protected with unidirectional airflow and the air velocity to be maintained there;
- the FDA requirement (see[20]) of not less than 20 hourly air changes in the controlled area associated with an aseptic core area;
- the EU and WHO GMP recommendation of a recovery time of 15-20 min in classified areas with turbulent airflow;
- eventual sources of intense particle dissemination and the extract airflow rate of eventual dust capturing devices;
- dissemination of micro-organisms by human beings and other sources.

If air recirculation is foreseen, the outside airflow rate must be sufficient:

- to compensate all leakage losses plus the air quantity exhausted to the outside atmosphere;
- to meet the outside air requirement of the personnel as determined in the national building regulations (author's recommendation: $50 m^3/h$ per person);
- to ensure odour control.

5.7.3 Estimating the airborne microorganism count

Intensive particle dissemination should be addressed by the steps proposed in *para. 5.3* as far as possible. In cleanrooms with turbulent airflow an estimate of the expected airborne particle concentration still remaining afterwards at the calculated airflow rate would be extremely valuable. However, so many uncertainties will remain that such an estimate would be quite arbitrary. On the other hand, the concentration of airborne micro-organisms to be expected in the cleanroom air under these circumstances can be estimated reasonably well by means of balance equations.

In a room with perfect turbulent mixing airflow the following balance equation applies:

$$C_s V^* + \sum C_i = C_r V^*$$

C_s = concentration of micro-organisms in the supply air [CFU/m^3];
C_r = concentration of micro-organisms in the room air [CFU/m^3];
$\sum C_i$ = sum of micro-organisms disseminated into room per unit of time [CFU/min];
V^* = airflow rate [m^3/min];
CFU = number of colony forming units.

The HEPA filtered supply air can be considered as free from micro-organisms:

$$C_s = 0 \text{ CFU/m}^3 \rightarrow C_s V^* = 0$$

Thus, the estimated concentration of micro-organisms in the room air will be:

$$C_r = \sum C_i / V^*$$

As dissemination source for micro-organisms, only the persons present in the room will normally require consideration. Their dissemination intensity is a function of the shedding characteristics of the garmenting scheme employed and can be estimated as follows (see *Reinmüller*[27]):

- normal laboratory and grade D garments (see *para. 5.2*): 500 CFU/min;
- grade C garments: 100 CFU/min;
- grade A and B garments: 25 CFU/min.

HVAC systems for clean areas and their components should obey a high standard of hygiene. Guidance for good hygienic design practice is compiled in the German guideline VDI 6022 Part 1[28].

5.8 Qualification, monitoring, verification for continued compliance

5.8.1 Qualification checks and tests

The basic determinations regarding qualification in general are given in *Chapter* 8. Regarding the qualification of the air handling system, the following ISO standards on cleanroom technology provide a sound and comprehensive fundament:

- **ISO 14644-4**[10] devotes its Annex C to a very comprehensive compilation of the requirements for the approval and qualification of cleanrooms. **Figure 24** presents the sequence of development, approval and qualification stages of an investment project and correlates them with the occupancy states of the clean area.
- **ISO 14644-3**[29] offers detailed guidance for the measurement of physical parameters for qualification and acceptance tests as well as for process monitoring. In addition, the minimum performance requirements for the test instruments are specified in detail.
- **ISO 14698-1**[30] provides comprehensive guidance regarding microbiological measurement procedures and **ISO 14698-2**[31] regarding the assessment and interpretation of biocontamination data.

Qualification should focus upon the system sectors with direct potential impact on product quality and safety, i.e. on the critical areas. Other parts of the HVAC system, such as refrigeration machinery supplying its air coolers with chilled water, are only of indirect impact and can be considered as non-critical elements. For these, **technical acceptance procedures** according to **good engineering practices** are sufficient. On drawings and functional diagrams the limits between sectors requiring qualification and those needing technical acceptance only, i.e. qualification limits, should be clearly identified.

A checklist identifying the checks and tests to be performed during the different qualification stages is given in **Appendix 5.B** to this *Chapter*.

5.8.2 Design review and design qualification

Before submitting the **basic design** of the HVAC system to tendering for the determination of the principal suppliers of equipment and the contractors for the installation work a **design review** should be performed. Its objective is to check whether the design is in agreement with the basic specification, the GMP stipulations and the applicable standards and guidelines, as well as to ensure that the set of documents is mature for the tendering phase. Prior to the design review, a risk analysis as described in *para. 5.8.3* should be performed for identifying – and subsequently correcting – all unacceptable design weaknesses. Core document for this review is a detailed flow diagram in which all elements of the installation, i.e. air handling units, air ducts, connections to dynamic pass-through hatches, dynamic isolators and RABS units, dampers and control valves, measurement sensors and other components are individually incorporated and identified.

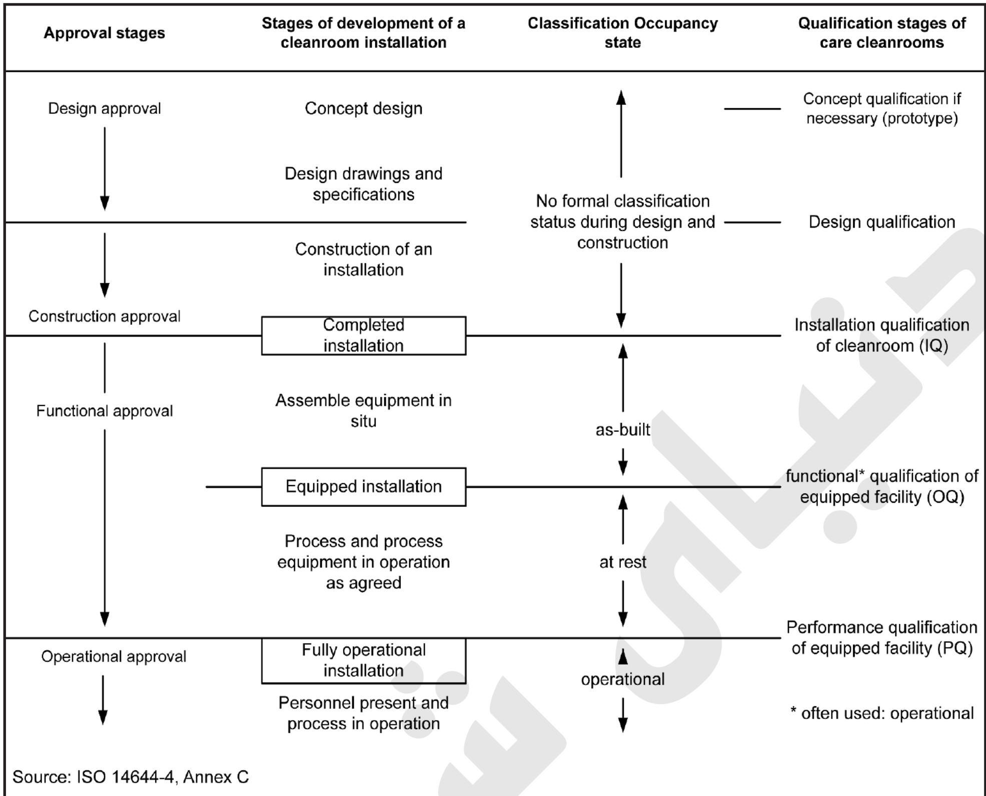


Figure 24: *Correlation of project development stage with the various cleanroom occupancy states and the corresponding qualification stages according to ISO 14644-4*[10]*.*

As result of tendering, suppliers of equipment, hardware and software providers as well as the contractors for the installation work have been selected and contracted, so that **detail design** and the preparation of the installation drawings and material lists etc. can proceed. This phase should be followed by an updating of the risk analysis as equipment-specific risks are only now capable of identification and correction. The objective of the subsequent **design qualification** (**DQ**) is to ensure that the detail design is in conformity with the prior basic design.

After successful completion of DQ, **change control**, a formal procedure to ensure that the qualification status of a system remains up-to-date and GMP compliant during its entire life cycle (see *Chapter 8*), should be triggered off, as from this point in time changes become the exception rather than the rule.

5.8.3 Installation and operational qualification

After completion of the installation services **installation qualification** (**OQ**) is per-formed. Core action is checking the installation for completeness and this is best performed with the help of the detailed flow diagram which has served as base for the detail design. For further details, see the checklist in *Appendix 5.B.*

After start-up, commissioning (see *Chapter 8* for details) and balancing of the airflow rates and subsequent to process equipment and furniture installation the HVAC system is ready for **operational qualification** (**OQ**). This means demonstration that the airflow rate for each room is

correct, that the air cleanliness levels and other control parameters agree with specifications and that detrimental effects caused by equipment (see also *para. 5.4.2* and *Figures. 13-14*) are under control. Some of the tests required are identical with those performed in conventional air-conditioning. Cleanroom-specific tests are:

– the **airborne particle count** essential for classification purposes; the number of sampling locations, sampling volumes and other stipulations are given in ISO 14644-1[1];
– the **installed filter system leakage test** for verification that terminal HEPA filters and their support structure are free from leaks;
– **airflow visualisation** for demonstrating by means of flow marking with smoke that particles are successfully swept away from critical zones as well as for analysing, for example, the airflow in the vicinity of equipment;
– the **recovery test** for demonstrating how quickly a room in which a high level of particulate contamination had been generated returns to its original state of air cleanliness.

All OQ tests are performed in the *at rest* occupancy state as defined in ISO 14644-1.

When all elements of the investment object have successfully passed OQ, it is ready for handing over from the contractor(s) to the facility proprietor. At this point in time, also the complete technical and qualification documentation is handed over. It must not only meet the expectations of the customer: it must also be in compliance with the requirements of the regulatory authorities. Their expectations regarding HVAC systems and other utilities are compiled in an aide-mémoire elaborated by the *Pharmaceutical Inspection Co-operation Scheme PIC/S* of the *Pharmaceutical Inspection Convention PIC*[32].

5.8.4 Performance qualification

The objective of **performance qualification** (**PQ**) is to demonstrate that the production system as an entity is fit for manufacturing the foreseen products reproducibly as specified. All PQ tests of the HVAC system are performed in the occupancy state *in operation*. Only OQ tests of parameters expected to be influenced by the presence of the personnel require repetition, e.g. air cleanliness classification. Microbiological tests, where required, are not meaningful prior to PQ.

5.8.5 Monitoring during production

Many advanced pharmaceutical production tasks – especially if microbiological risks are to be dominated – require proof that the stipulated pressure differentials and, where required, particle counts or air velocities and, possibly, other parameters have been met continuously and consistently during production.

This is but one of the many tasks of **process monitoring**, a task encompassing all measurement and documentation activities related to the manufacturing process. The objective is to obtain a comprehensive set of data in order to prove that the process has operated uninterruptedly within the specified limits. The contamination control parameters for which the air handling system is responsible are a fully integrated element within this general context of process monitoring and indispensable for the batch documentation.

Other control parameters such as airflow rate, temperature and relative humidity are, on the other hand, indispensable for the automatic control of the air circulation system.

Thus, data have to be fed, on the one hand, into the building control system, and on the other hand, into the process monitoring system. This last-mentioned system will have to satisfy comprehensive regulatory requirements which are laid down in Annex 11 *Computerised systems* to the EU GMP guide[33] and, in the context of FDA regulations, in 21 CFR 11[34]. The computerised

systems addressed in these guidance documents require validation and this should be performed according to the **GAMP 5** (**GAMP** = **G**ood **A**utomated **M**anufacturing **P**ractices) specifications[35].

Building automation systems, with their extensive ramifications throughout the entire building complex of a site, are as a rule not capable of being validated. This is another good reason for strictly separating them from the pharmaceutical data monitoring system.

The low voltage electricity supply for both process monitoring and automatic control purposes should be connected to an interruption-free electricity supply source, as even interruptions lasting less than a second can cause them to behave erratically.

In pharmaceutical air handling systems, room pressurisation, temperature and relative humidity are, as a rule, measured continuously. In grade A areas, also airborne particle counts and the air velocity are monitored continuously, or at least – as specified in Annex 1 to the EU GMP guideline and the corresponding WHO guidance – at such a frequency that all interventions, transient events and any system deterioration would be discovered. For grade B areas, the sampling frequency may be decreased, but should continue to be frequent. For grade C and D areas, however, periodical measurement of particle concentrations at longer intervals together with manual data recording is sufficient. Periodical measurements are also indicated for recording the pressure difference across HEPA filters. This pressure difference increases very slowly with time so that quarterly, half-yearly or even annual data collection with manual recording is sufficient.

Periodical measurements are again the rule for the sampling of airborne micro-organisms, except in grade A areas where frequent sampling is indicated.

Where continuous or frequent sampling is required in areas protected with unidirectional airflow, the sampling heads or sensors should be installed in locations close to the critical areas, at positions optimised during the OQ phase.

5.8.6 Operation and maintenance

In order to avoid the infiltration of false air into contamination controlled premises, the HVAC system of the facility must run continuously. In facilities with single-shift utilisation, however, the reduction of the airflow rate outside production hours, i.e. in the occupancy state *at rest*, is a potent energy cost saver: a reduction of the air flow rate to 50% of the nominal value will reduce energy costs by more than 80%! Of course, the predetermined pressure differences between rooms as well as the predetermined air cleanliness class in the *at rest* occupancy state as well as the specified airflow pattern must be maintained uninterruptedly during system operation with reduced airflow rate.

Maintenance of the HVAC system of a clean facility follows, in general terms, the procedures employed for other industrial air-conditioning systems. The only peculiarity are terminal HEPA filters: after filter replacement, an installed filter leakage test according to ISO 14644-3[29] is highly recommended.

5.8.7 Verification for proving continued compliance

Verification of the air handling system of cleanrooms for continued compliance above all with ISO 14644-1 (1) is covered in ISO 14644-2[36]. Two situations are distinguished:

– **periodical verification** which is normally performed annually;
– **extraordinary verification** after, for instance, a change of terminal HEPA filters or a significant stand-still of the HVAC system.

Cleanroom verification is performed in the *at rest* occupancy state and should address, according to ISO 14644-2, at least the following parameters:

– the **air cleanliness class**;
– **pressure differences** between rooms;
– the **air velocity** (for unidirectional airflow) or the **air flow rate** (for turbulent airflow).

Further tests may be specified according to requirements. In air handling systems serving facilities for aseptic manufacturing, FDA and WHO require the **installed filter leak test** of HEPA filters to be performed periodically. The interval between subsequent tests has been set by FDA as at least twice a year[20], whereas WHO permits an extension to once yearly[16]. Although surprisingly not mentioned in Annex 1 to the EU GMP guide, the installed filter leak test is recommended in PIC/S PI 032-2[37]. Indeed, it should be incorporated into the verification programmes of all systems employing terminal HEPA filters.

Some operators of facilities for aseptic manufacturing extend compliance verification exercises also to the microbial limits which must, of course, be assessed in the occupancy state *in operation*.

5.9 The risk perspective

5.9.1 HVAC system related risks

The objective of pharmaceutical HVAC systems is, above all, to generate benefits for product and personnel. However, if conceived and realised in an unprofessional way, it is capable of:

– distributing contaminants borne by the outside air efficiently air throughout a building;
– propagating airborne cross-contamination by particles of pharmaceutically active substances, processed in parallel within the facility, from room to room;
– offering in their humid elements (air coolers, humidifiers) the potential for the multiplication of micro-organisms and their subsequent entrainment into the supply air.

Dangerous micro-organisms carried in the outside air and known to be interacting with HVAC systems are[2]:

– *Clostridium tetani* causing tetanus infections, entrained into the air by wind or thermal currents from garden earth and agriculturally cultivated fields;
– *Legionella pneumophila* causing legionnaire’s disease, sometimes developing and multiplying in the water and the humid vicinity of cooling towers;
– *moulds* from decaying organic matter, a potential product contaminant particularly undesirable in facilities devoted to the filling of liquids.

Another species meriting attention in HVAC systems is *Pseudomonas aeruginosa* which is prone to multiply, for example, on the humid surfaces of air coolers.

5.9.2 Controlling the risks

Being aware of the risks is the first step towards controlling them. In doing so, the philosophy should be to combat each risk with at least two effective technical measures.

The biocontamination risk originating from the outside air is controlled:

– by positioning the outside air intakes at sufficient height above ground and flat roofs, at sufficient distance from exhaust air outlets and known biocontamination sources in the vicinity of the site, with due consideration of the predominant wind directions;
– by adequate outside air filtration.

Extract air should be ejected into the atmosphere at a level higher than that of outside air capture, and with a sufficient velocity to further its efficient mixing with the surrounding air.

The biocontamination risk inherent in the humid elements of air handling units is controlled (see also[28]):

– by efficient drainage of the water condensed out of the air stream;
– by positioning droplet separators downstream of air coolers;
– by air filtration downstream of all dissemination sources of micro-organisms.

Air humidifyers should be avoided where feasible in tropical climates; however, in dry climates and where winter temperatures are very low they are essential if excessively dry air is to be avoided. Preference should then be given to steam humidifyers operated preferentially with purified water.

These are just arbitrary examples. To systematically address the risks in the context of modern quality systems such as the ICH Q10[38] approach developed by ICH, the *International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use*, a comprehensive risk analysis is indicated.

5.9.3 The FMEA risk analysis procedure

Numerous risk assessment procedures exist; a review of the procedures applicable to the pharmaceutical industry and of their indicated application areas is compiled in the ICH Q9 guideline[39]. For the analysis of production facilities and their infrastructure such as the HVAC system as well as for the equipment installed therein the **Failure Mode and Effects Analysis** (**FMEA**) procedure according to EN 60812[40] is considered as particularly suitable. According to ICH Q9, this procedure methodically breaks down the analysis of complex processes into manageable steps. ICH Q9 classifies it as a powerful tool for summarising the important modes of failure, factors causing them and their likely effect and as a fit tool for design analysis.

In elaborating the FMEA analysis of a HVAC system, two problem dimensions are to be distinguished:

– the weaknesses of the production system to be controlled by the HVAC system or at least reduced in their gravity;
– the risks inherent in the HVAC system itself.

First step in performing a FMEA analysis is to compile a comprehensive list of all possible risk factors and, for each of them, to summarise its cause and its effect on process and product safety. For distinguishing the critical process risks from the remaining less critical ones, the FMEA procedure identifies three assessment criteria focussing on the risks from completely different angles:

– probability of occurrence O;
– relevance R;
– probability of detection D.

For quantification of each of these assessment criteria a numerical value scale is helpful, e.g. between 1 and 5, with 1 = negligible to 5 = very high risk with intermediary values signifying low, medium and high risk. Through multiplication of the assessment criteria a **Risk Assessment Number RAN** can be obtained, i.e.

$$RAN = O \times R \times D$$

which is a most helpful indicator for deciding whether actions to counteract the risk are necessary, desirable or unnecessary. For this, limit RAN values have to be agreed for distinguishing the three

Table 2: Example of a FMEA risk assessment plus subsequent action plan

Risk	Leak in terminal supply air filter of a HEPA filter ceiling for the establishment of unidirectional airflow
Cause	Damage during transport, carelessness during installation
Effect	Local increase of particle concentrations, air cleanliness class requirement possibly not met
Probability of occurrence	O = 4 (high)
Relevance	R = 4 (high)
Probability of detection	D = 4 (low)
Risk assessment number	RAN = 4 x 4 x 4 = 64 → Actions required
Actions	Visual inspection after installation, followed by installed filter leak test according to ISO 14644-3
Assessment of success of actions	O = 1 (very low); R = 4 (high); D = 1 (very high); → RAN = 1 x 4 x 1 = 4: no further action required.

(RAN ≤ 17: no action required; 18 < RAN ≤ 26: action desirable; RAN ≥ 27: action required)

action classes clearly from each other. An example of such an assessment is shown in **Table 2** which also presents an example for RAN action limits.

There can be no doubt as to the usefulness of FMEA assessments in the HVAC context. However, an uncritical attitude is not without its risks, as there are certain limits to this procedure which should be kept in mind. An interruption of the electricity supply is very rare or at least rare in most of the pharmaceutically strong nations (FMEA mark O = 1 or 2). It is discovered immediately (D = 1) and extremely relevant (R = 5). Although RAN = 5 or 10 results from this, it would be extremely unwise not to assess the pros and cons for connecting the HVAC system to an emergency power generator.

When should risk assessment and analysis, for instance with the FMEA procedure, be performed during project development? An answer is given in *para. 5.7.2*.

The entire risk assessment process, and the conclusions drawn subsequently, must be condensed into a transparent **risk report**. This report must clearly state the rationale for all decisions taken, including items where the decision was *no action*. It is the very core element of the quality risk documentation as all the risk-relevant information regarding a given project is concentrated into it. For this reason, inspectors from the regulatory authorities frequently use this compact document as point of departure for their inspections.

5.10 Energy saving options

Energy saving has so far frequently been neglected, or given a rather low level of attention in cleanroom technology. Such an attitude is no longer acceptable: for economical reasons and also as a contribution towards the battle against excessive global warming of the atmosphere. It is also to be expected that governments will increase pressure on industry to give energy saving the appropriate level of attention: comprehensive rules already exist, for instance, in the European Union[41], and follow-up standards have been developed for non-residential HVAC systems (e.g.[42]). The following deliberations will limit themselves to cleanroom-specific options (for details, see *Chapter 26*).

A guideline exclusively devoted to energy saving in cleanrooms is VDI 2083 Part 4.2[43], see also[44]. It is applicable both for the upgrading of existing as well as for design and realisation of new cleanroom facilities, and covers all application areas.

In pharmaceutical cleanroom systems, process safety must always predominate: **energy saving and optimisation in clean facilities must never and by no means interfere with process requirements. These must always be given absolute priority.**

During design of the cleanroom system, relevant questions to ask regarding the HVAC system are, for instance:

- What air cleanliness classes are required and how can the utilisation of unidirectional airflow be minimised?
- Do process requirements determine the temperature and relative humidity of the air? If not, human comfort considerations may prevail (in doing so, unnecessarily tight tolerance bands should be avoided).
- Are there any formal requirements, e.g. from legislation or GMP guidelines, limiting technical freedom regarding, for instance, air cleanliness classification, hourly air change rates, air velocities or pressurisation levels?
- Is it feasible to reduce air circulation outside production hours?
- What steps can be taken to reduce particle dissemination by the process into the room atmosphere?
- How can the requirements for outside air be minimised?
- What process extract air requirements have to be taken into consideration and are they capable of reduction?
- Does the process permit the utilisation of barrier technologies as described in *Chapter 10*?

Air recirculation is identified as the most efficient and least expensive way of recovering energy (both heat and humidity), with room-specific air recirculation as particularly advantageous, as it is distinguished by the additional merit of effectively avoiding cross-contamination between rooms. Wherever feasible, outside air treatment should be separated from the air recirculation subsystems of the individual process areas.

Reduced hourly air change rates outside production hours are strongly recommended for the air circulation system (see also *para. 5.7.6*).

Existing installations also merit critical attention. During the development stage, design uncertainties tend to be compensated by performance reserves, and instead of exact calculations, guidance values for the air change rate based upon experience are given preference. Thus, dissemination sources for particles and micro-organisms are frequently overestimated in cleanrooms using turbulent airflow, so that unnecessary low particle counts are found under operational conditions, i.e. an overkill had occurred. In situations of this kind, it may well be feasible to reduce system airflow rates considerably without prejudice to process safety.

5.11 Summary

In writing this chapter, the author's intention was to address the specific peculiarities of pharmaceutical HVAC systems. They serve, above all, safety objectives: contributing to ensure that pharmaceutical preparations are safe for the patient. Where hazardous substances are handled in the production facility, an additional objective is protection of personnel and the external environment against airborne hazardous particles and fumes. Essential elements for ensuring the required air cleanliness levels are air filtration, establishment of the desired airflow

patterns and pressure differentials or other means for preventing cross-contamination between rooms. Regarding the HVAC system concept, various basic options are illustrated. As essential feature of the design and realisation of pharmaceutical cleanrooms are comprehensive qualification exercises in consecutive steps in order to ensure their specified quality and performance characteristics, beginning in the design stage and ending when the facility is readied for production to start. Monitoring of critical data and periodic performance verification ensure continuous compliance of the HVAC system's performance during the facility's entire life cycle. Essential during system design is a consequent focus on the risk perspective. As additional dimension, energy saving presently comes to the forefront: this objective is worthy of promotion not only from the economic perspective, but also in consideration of the necessity to fight global warming of the atmosphere.

5.12 References

1. ISO 14644-1: Cleanrooms and associated controlled environments –- Part 1: Classification of air cleanliness. International Organization for Standardisation ISO, Geneva (May 1999); a revised edition has been circulated as ISO/DIS 14644-1:2010: Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness by particle concentration. Idem, ibid. (December 2010).
2. Schicht H.H.: HVAC requirements and design concepts for facilities manufacturing non-sterile dosage forms. In: Utilities used by the manufacturer of pharmaceuticals. Pharmaceutical Inspection Co-operation Scheme PIC/S, Pharmaceutical Inspection Convention PIC (ed.), Geneva (2002), p. 187-206.
3. Austin Ph.R.: People generated contamination. *Journal of the American Association of Contamination Control 1996;* **5** (1).
4. Nieth K.F.: Reinraumgerechtes Mitarbeiterverhalten (Cleanroom compatible personnel behaviour). *Reinraumtechnik* 1992; **6**: 34-37.
5. Förster B., Schroers D.: Luftfiltration (Air filtration). In: Gail L., Hortig H.-P. (ed.): Reinraumtechnik, 2. Aufl. (Cleanroom technology, 2nd edition). Springer-Verlag, Berlin, Heidelberg (2004), p. 103-131.
6. European Standard EN 779:2002: Particulate air filters for general ventilation – Determination of the filtration performance. European Committee for Standardization CEN, Brussels (June 2002); an updated version has since been circulated as Draft European Standard prEN 779:2009 with the same title. Idem, ibid. (May 2009).
7. EN 1822: High efficiency particulate air filters (EPA, HEPA and ULPA): Part 1 – Classification, performance testing and marking; Part 2 – Aerosol production, measuring equipment, particle counting statistics; Part 3 – Testing flat sheet filter media; Part 4 – Determining leakage of filter elements (scan method); Part 5 – Determining the efficiency of filter elements. Idem, ibid. (Nov. 2009).
8. WHO Technical Report Series no. 961: WHO Expert Committee on Specifications for Pharmaceutical Preparations, 45th report. World Health Organisation WHO, Geneva (May 2011).
9. WHO guidelines on good manufacturing practices for heating, ventilation and air-conditioning systems for non-sterile pharmaceutical dosage forms. Annex 5 to: (8), p. 215-260.
10. ISO 14644-4: Cleanrooms and associated controlled environments – Part 4: Design, construction and start-up. International Organisation for Standardisation ISO, Geneva (April 2001).
11. Rauschnabel J.: Zwischen Isolator und Sterilraum (Between isolator and sterile room) – Restricted Access Barrier System (RABS). *Steril Technik* 2006; **2** :16-20.
12. Ljungqvist B., Reinmüller Berit: Clean room design – Minimising contamination through proper design. Interpharm Press, Buffalo Grove IL/USA (1997).
13. Ziemer W., Förster B.: Partikeltransport durch undichte Fassaden. In: Reine Technologien – Aktuelle Fragen der Reinraumtechnik (Particle transport through leaking building envelopes; in: Clean Technologies – Current topics of cleanroom technology). VDI-Berichte 1238 (1996), p. 27-36.
14. EudraLex, the rules governing medicinal products in the European Union – Vol. 4: EU guidelines to Good Manufacturing Practice for medicinal products for human and veterinary use. European Commission, Brussels (frequently updated).

[15] 21 CFR 211: Current Good Manufacturing Practice for finished pharmaceuticals. Code of Federal Regulations, Food and Drugs, U.S. Government Printing Office, Washington DC/USA.

[16] WHO good manufacturing practices for sterile pharmaceutical products. Annex 6 to: (8), p. 261-284.

[17] WHO good manufacturing practices for pharmaceutical products containing hazardous substances. Annex 3 to: WHO Technical Report Series no. 957: WHO Expert Committee on Specifications for Pharmaceutical Preparations, 44th report. World Health Organisation WHO, Geneva (May 2010) p. 192-208.

[18] German guideline VDI 2083 Part 5.1: Cleanroom technology – Cleanroom operation: Annex I: Thermal comfort in cleanrooms – Recommended parameters for comfort. Beuth Verlag, Berlin (September 2007).

[19] VDI 2083: Cleanroom technology (actually 18 parts). Idem, ibid.

[20] Guidance for industry: Sterile drug products produced by aseptic processing – current Good Manufacturing Practice. U.S. Department of Health and Human Services, Food and Drug Administration (September 2004), p. 132-170

[21] Renz M.: Reinraumanlagen für Mikroelektronik und Pharma (Cleanroom systems for microelectronics and pharma). In: Gail L., Hortig H.-P. (ed.): Reinraumtechnik, 2. Aufl. (Cleanroom technology, 2nd ed.). Springer Verlag, Berlin, Heidelberg, New York (2004).

[22] EN 1507: Ventilation of buildings – Rectangular sheet metal air ducts – Requirements for strength and airtightness. European Committee for Standardization CEN, Brussels (July 2006).

[23] Gifford J. et al.: Computational fluid dynamics as a tool for designing quality into the pharmaceutical cleanroom. *Pharmaceutical Engineering* 2010 **30** 4, 54-60.

[24] Blackmore B.: Model formula. *Cleanroom Technology* 2007 **14** (2007) 5, 25-26.

[25] Manning A.: Airflow modelling in cleanroom design. *CleanRooms* 2005 **19** 5, 22-25.

[26] Burp St. et al.: Numerical simulation to assess airflow behaviour in buffers. *Pharmaceutical Engineering* 2003 **23** 3, 136-145.

[27] Reinmüller Berit: People as a contamination source – Clothing systems. In: Dispersion and risk assessment of airborne contaminants in pharmaceutical cleanrooms. Royal Institute of Technology, Building Services Engineering, Bulletin no. 56, Stockholm (August 2001), p. 54-77.

[28] VDI 6022 Part 1: Hygiene requirements for air handling systems and units. Beuth Verlag, Berlin (July 2011).

[29] ISO 14644-3: Cleanrooms and associated controlled environments – Part 3: Test methods. International Organization for Standardization ISO, Geneva (December 2005).

[30] ISO 14698-1: Cleanrooms and associated controlled environments – Biocontamination control – Part 1: General principles and methods. Idem, ibid. (September 2003).

[31] ISO 14698-2: Cleanrooms and associated controlled environments – Biocontamination control – Part 2: Evaluation and interpretation of biocontamination data. Idem, ibid. (September 2003).

[32] PIC/S PI 009-3: Aide memoire – Inspection of utilities. Pharmaceutical Inspection Co-operation Scheme PIC/S, Pharmaceutical Inspection Convention PIC, Geneva (25 September 2007).

[33] Computerised systems. Annex 11 to [14]. European Commission, Brussels (January 2011).

[34] 21 CFR 11: Guidance for industry – Electronic records; electronic signatures – Scope and application. U.S. Department of Health and Human Services, Food and Drug Administration, Rockville MD/USA (April 2007).

[35] GAMP 5: A risk-based approach to compliant GxP computerised systems. ISPE – The International Society for Pharmaceutical Engineering – in collaboration with FDA and other regulatory authorities. Tampa FL/USA (2008).

[36] ISO 14644-2: Cleanrooms and associated controlled environments – Part 2: Specifications for testing and monitoring to prove continued compliance with ISO 14644-1. International Organization for Standardization ISO, Geneva (September 2000); a revised edition has been circulated as ISO/DIS 14644-2:2010: Cleanrooms and associated controlled environments – Part 2 – Specifications for monitoring and periodic testing to prove continued compliance with ISO 14644-1. Idem, ibid. (December 2010).

[37] PIC/S PI 032-2: Recommendation – GMP Annex 1 Revision 2008, interpretation of most important changes for the manufacture of sterile medicinal products. Pharmaceutical Inspection Co-operation Scheme PIC/S, Pharmaceutical Inspection Convention PIC, Geneva (8 January 2010).

[38] ICH Q10: Pharmaceutical quality system. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH, Geneva (June 2008); see also [14], Part III.

[39] ICH Q9: Quality risk management. Idem, ibid. (January 2006); see also [14], Part III.

[40] EN 60812: Analytical techniques for assessing the functionality of systems: Failure Mode and Effects Analysis (FMEA) (IEC 60812:2006). European Committee for Standardization CEN, Brussels (November 2006).

[41] Directive 2002/91/EC on the energy performance of buildings. European Commission, Brussels (16 December 2002, revised 22 October 2008).

[42] EN 13779: Ventilation of non-residential buildings: Performance requirements for ventilation and room-conditioning systems. European Committee for Standardization CEN, Brussels (September 2007).

[43] VDI 2083 Part 4.2: Cleanroom technology – Energy efficiency. Beuth Verlag, Berlin (April 2011).

[44] Schicht H.H.: The VDI 2083 family of contamination control guidelines: An overview and a specific focus upon energy conservation. *Clean Air and Containment Review* 2011; **7** : 22-24.

Appendix 5.A:

Basic specification of the HVAC system for a facility for the aseptic manufacture of sterile liquid dosage forms

Introduction: The following is an exemplary basic specification for the HVAC system of a hypothetical facility aseptically manufacturing sterile liquid dosage forms (vials and pre-filled syringes), situated in Hyderabad/India. Products are to be manufactured for the local market and for export to neighbour countries.

In adapted form, this basic specification can also serve as orientation base for the HVAC systems of all other kinds of pharmaceutical facilities.

HVAC-relevant GMP guidance, standards and guidelines:
GMP guidance:

- WHO good manufacturing practices for sterile pharmaceutical products.
- Annex 11 (Computerised systems) to EU GMP guide (computerised systems only).
- PIC/S PI 009-3: Aide-mémoire: Inspection of utilities.
- GAMP 5 (computerised systems requiring GMP compliance only).

Standards, guidelines, company-internal directives:

- ISO 14644 series of cleanroom standards and related guidelines as specified.
- EN 779 and EN 1822 (air filters only).
- Applicable national standards and guidelines for building design and construction as well as for the building's infrastructure.
- Applicable company-internal directives and manuals.

Editions as valid at date of publication of this specification.

In case of conflicting stipulations, the following hierarchy applies:

– GMP guidance and PIC/S recommendations;
– ISO and EN standards;
– national standards;
– guidelines elaborated by professional societies.

HVAC system objective: The HVAC system supplies the air cleanliness controlled manufacturing area (zoned-in area) of the facility with conditioned air at the air cleanliness level as specified for the individual facility sectors. The zoned-in area is decoupled from the external walls of the building. Thus, the zoned-in area is protected against uncontrolled infiltration of outside air. A high reliability level of the HVAC system is stipulated.

Regarding the thermal conditions to be maintained, adequate comfort for the facility's occupants is the priority. There are no restrictions from the product and production point of view. No hazardous substances require consideration.

Economically justifyable energy saving measures should be pursued as long as they are GMP compliant. Air recirculation including room air recirculation can be foreseen where feasible. Advanced protection concepts such as isolators and RABS technology should be taken into consideration.

System concept: An outside air handling unit (AHU) supplies the sectorised secondary AHU's for the individual facility sectors with conditioned primary air. No air recirculation between the individual facility sectors, i.e. secondary air handling subsystems, should occur. Extract air is ejected into the atmosphere via a centralised extract AHU. Energy recovery between the outside and extract air to be foreseen if economically feasible.

Air cleanliness classification: According to air cleanliness zoning plan, (architectural drawing no. xxxx, revision yy dated zz.zz.20zz).

Outside air data (for load calculation purposes):

– Summer: 35°C/45% relative humidity (r.h.).
– Winter: 22°C/70% r.h.

(Such data vary widely depending upon location. For location in Skopje/Republic of Macedonia, they would read: summer 35°C/30% r.h.; winter: -15°C/87% r.h.)

Room air temperature:

– Grade A and B areas: 21°C ± 1°C.
– Grade C and D areas plus adjacent grey areas: 23°C ± 3°C.

Room air relative humidity: Range 35% r.h. (minimum) to 65% r.h. (maximum).

Pressure differentials: Airlock into zoned-in area: 5Pa; from there increase in 15Pa steps between subsequent air cleanliness grades (see also pressurisation plan (architectural drawing no. xxxx, revision yy dated zz.zz.20zz).

Air leakage losses to be compensated by roomwise modulation of the extract airflow rate (maximum leakage rates for doors, walls and false ceilings to be considered, for HVAC calculation purposes, as specified in the building design).

Pressure level in airlocks grey → grade D to be maintained by means of adjustable spill-over dampers in the doors leading into the grey area.

Airflow rates/airflow velocities:
Turbulent airflow: Initial determination by means of cooling load calculations, to be corrected for ensuring:

– Recovery time < 15 min in grade B areas.
– An airborne microorganism concentration not exceeding limits as specified in the WHO sterile guide; estimates to be based upon the specified number of persons per room, with calculatory dissemination rates as follows:

  Grade D area: 500 CFU/min (per person)

  Grade C area: 100 CFU/min (per person)

  Grade B area: 25 CFU/min (per person)

Unidirectional airflow: average air velocity 30cm below HEPA filter ceiling: 0.45m/s ± 20 %.
Air filtration:
Supply air:

- Classified rooms and corresponding airlocks: F 7 + F 9 + H 14.
- Adjacent unclassified, i.e. grey rooms: F 7 + F 9.

Extract air:

- Filter mats G 4 or better integrated into room extract air grilles;
- Extract air handling unit: F 7.

Noise level: The continuous room noise caused by the HVAC system not to exceed 55dB(A). If process machinery generates a considerably higher noise level, the HVAC system's contribution should remain at least 6dB(A) below the total noise level.

Air handling units: Conceived as monoblocs, quality level according to hospital requirements, inner surfaces easy to clean. Supply and return air fans duplicated, capacity 2 x 60% of the calculated airflow rate permitting maintenance or replacement of single fans with the system continuing in operational mode.

Supply and extract air ducts: External duct insulation stone wool mats or equivalent with outer aluminium cover. Supply air duct leakage rate not above $50m^3/h$ of air per $100m^2$ of duct surface at a constant test pressure level of 400Pa.

Component identification and marking: The air handling unit and all system components (in air handling unit or outside) require proper labelling. On air ducts and pipes the nature of air (supply air, extract air) and the airflow direction must be properly marked. Piping for chilled, hot and cooling water to be marked correspondingly.

System operation: Continuous throughout the year, except interruption once annually for maintenance purposes. Reduction of the airflow rate outside operational hours to not more than 50% of the nominal rate is desirable, as long as maintenance of the specified pressure differentials and room grades is ensured.

Process monitoring system: HVAC data relevant for product safety and batch documentations must be transmitted to the process monitoring system of the production process such as pressure differentials plus room temperatures and relative humidities as well as, where applicable, particle counts and airflow velocities. This system must be kept independent from the automatic control system of the HVAC system.

Automatic controls: The automatic control subsystem and the recording of technical data of the HVAC system will be integrated into the central building control system. No interference into the pharmaceutical process monitoring system must be possible.

Emergency operation: The automatic control subsystem of the HVAC system must be connected to an interruption-free electricity supply. An emergency power system is to be foreseen with capacity sufficient for enabling continuous operation of the HVAC system in the *at rest* occupancy state.

Qualification: Risk based, systematic and documented qualification of the HVAC system in compliance with WHO GMP requirements is required. A 5-step procedure is foreseen comprising design review, DQ, IQ, OQ and PQ. Details are to be specified in a qualification master plan and the subsequent qualification plans where the documentation requirements for all checks and tests are specified. Qualification limits separating the HVAC system elements subject to qualification from those subject to technical acceptance procedures only must be clearly identified on drawings and functional diagrams.

Change control: According to a GMP compatible procedure, with formal change control beginning after successful completion of DC.

Technical documentation: A complete technical documentation including actualised drawings and all supplier documentation including maintenance manuals is to be prepared.

Documentation management: Technical and qualification documentations must be well-structured and kept apart from each other. Responsible for management is the HVAC system designer. After successful termination of OQ, all documentations are handed over to the facility proprietor who will be responsible for all PQ activities and their documentation.

System verification for continued compliance: The HVAC system designer will prepare the documentation required for annual system verification, based upon the requirements specified in the HVAC system qualification master plan. The same quality standard as for the qualification documentations is required.

Appendix 5.B:

Qualification checklists

The same hypothetical example has been selected as base for qualification checklists to be found below. In the case of technically less ambitious investment objects certain items can be deleted from such checklists. Only GMP-relevant subject matter has been incorporated into the checklists, i.e. items submitted to technical acceptance procedures only are excluded.

In order to separate the two topics clearly from each other, qualification limits should be clearly marked on drawings and functional diagrams.

5.B.1 Design review

Items required:

- HVAC design review plan.
- Basic specification of general HVAC requirements.
- Detailed functional drawing, HVAC system, with identification of all air handling units, eventual connections to active isolators and/or RABS units, active material pass-through hatches, safety work stations, airflow control elements, sensors and incorporating all rooms to be served, with specification of room airflow rates, air cleanliness class, pressure levels etc.
- Technical description of HVAC system including operating concept and automatic control scheme.
- HVAC risk report.
- Cooling load calculations with resulting air flow rates/air change rates.
- Redundancies, e.g. double fans (eventually with list of additional, so far unapproved proposals).
- Provisional layout of machine room for air handling units and related equipment.
- Provisional layout for supply and extract air ducts including eventual connections to active isolators and/or RABS units, active material pass-through hatches, safety work stations, etc.
- Provisional layout of work areas with unidirectional airflow protection if integrated into the HVAC system.
- Specifications of air handling units (including reserve requirements).
- Specification of ductwork including maximum permitted leakage rates.
- Specification of components such as:
  - Constant and variable airflow control valves.

  - Filter housings and fan filter units (FFU's).
  - Air filters including HEPA filters.
  - Motorised and manually operated dampers including fire dampers.
  - Outside air dampers.
  - Supply air diffusers and extract air grilles.
  - Others as required.
- Proof of GMP conformity of the specifications for:
  - Room pressurisation scheme.
  - HVAC system concept.
  - Airflow rates (demonstration that air flow rates are sufficient for meeting recovery test requirements and airborne microorganism limits).
  - Airflow pattern requirements.
  - Alarm functions.
  - List of parameters to be incorporated into the pharma monitoring concept including number of sampling points for each parameter.
- Functional diagram and specification of the automatic control system, highlighting features for ensuring GMP compliance.
- Functional diagram and specification of the HVAC contribution to the pharma monitoring system.
- Design review report, prepared after successful termination of the design review, with confirmation that all errors and omissions identified during the design review have been rectified.

5.B.2 Design qualification (DQ)

If documents have not been modified since design review, a statement to this effect is sufficient. Documents required are:

- Design review report, HVAC system, including confirmation that errors and omissions identified during the design review have been corrected.
- DQ plan, HVAC system.
- Updated functional diagram of the HVAC system complete with qualification limits, with identification and positioning of control dampers, positioning of sensors, etc.
- Updated system description including operating concept and automatic control scheme (if unchanged, a statement to this effect is sufficient).
- Updated HVAC system risk report, with due consideration of the risks resulting from the selected hardware.
- Confirmation by HVAC system installation contractor of correctness of cooling load calculations.
- Detailed layout of machine room for air handling units and associated equipment.
- Supply and extract air ducts:
  - Detailed arrangement drawings.
  - Specifications and drawings regarding thermal insulation where required.
  - Details of duct connections to active isolators and/or RABS units, dynamic material pass-through hatches, safety work stations, locations of sensors plus aerosol injection points for the installed filter leakage tests together with installation drawings of their incorporation into the ducts.

– Updated layout plans and installation drawings for work areas with unidirectional airflow if integrated into the HVAC system.
– Air handling units: complete documentation with installation drawings, component lists and technical data.
– Supplier documentation with technical data and component lists for.
  • Constant and variable airflow control valves.
  • Filter housings and fan filter units (FFU's).
  • Air filters including HEPA filters.
  • Motorised and manually operated dampers including fire dampers.
  • Outside air dampers.
  • Supply air diffusers and extract air grilles.
  • Others.
– Specification for air duct cleaning after installation and before start-up.
– Specification for testing air handling units and ducts for airtightness.
– Updated functional diagram of the automatic control system, plus material specifications and wiring diagrams.
– Updated functional diagram and specification of the HVAC contribution to the pharma monitoring system plus material specifications and wiring diagrams.
– Updated functional diagram and specification of the automatic control system including where applicable, its integration into the building management system of the facility.
– Calibration plans for measuring instruments.
– Logbook for HVAC system change control (if not supplied by future user).
– Change proposal, approval and assessment form (if not supplied by future user).
– Competence and training certificates for:
  • Project and qualification manager, HVAC system.
  • Supervisor of HVAC system installation.
– DQ report, prepared after successful termination of DQ, with confirmation that all errors and omissions identified during DQ have been rectified.

5.B.3 Installation qualification (IQ)

For HVAC equipment, normally no factory acceptance tests (FAT's) are required. IQ documentation should comprise:

– DQ report, HVAC system, including confirmation that errors and omissions identified during DQ have been rectified.
– IQ plan, HVAC system.
– Updated functional diagram of the HVAC system, corresponding with *as built* status.
– Revision drawings of the HVAC system corresponding with the *as built* status.
– All components installed and functional (checked by means of functional diagram and component lists):
  • Constant and variable airflow control valves.
  • Filter housings and fan filter units (FFU's).
  • Air filters including HEPA filters including valid factory test certificates (HEPA filters only).
  • Motorised and manually operated dampers including fire dampers.
  • Outside air dampers.

    - Supply air diffusers and extract air grilles.
    - Others.
- Labelling of air handling units and components correct and complete.
- Copy of *as built* functional diagram of HVAC system attached to air handling units in prominent position.
- Air duct insulation, where required, in place and undamaged.
- Air nature marking (supply air, extract air) and flow direction identification on air ducts in place and clearly visible.
- HVAC system piping for process fluids correspondingly marked (nature, i.e. technical compressed air, chilled, hot or cooling water plus flow direction).
- Leakage test of air handling units and air ducts successfully performed and documented.
- Air handling units and air ducts correctly cleaned.
- Operation and maintenance manuals in existence, complete and approved.
- Maintenance plan complete and approved.
- Spare part list in existence, complete and approved.
- Initial spare parts stock purchased and in storage.
- Standard operational procedures (SOP's) for OQ measurements complete and approved, e.g. for:
    - Installed filter leak test (HEPA filters only).
    - Pressure difference.
    - Room air classification.
    - Airflow rates.
    - Air velocities (areas with unidirectional airflow only).
    - Airflow visualisation.
    - Recovery time.
    - Room air temperature and relative humidity.
    - Noise level.
- Interfaces to other utility installations without conflicts and without pending items.
- Correct availability of media required for HVAC system operation (chilled water, cooling water, warm water, technical compressed air etc.).
- Pharma monitoring system: statement regarding completeness, correct installation and functionality of HVAC contribution.
- Automatic control system: statement regarding completeness, correct installation and functionality plus, if applicable, correct connection to the facility's building automation system.
- Sensors and measurement instruments correctly installed, labelled and calibrated according to calibration plan; calibration labels in place complete with date of next calibration.
- Calibration certificates in accordance with specification and complete.
- Training manuals for operational personnel prepared and complete.
- Change control logbook and change control forms correctly filled in, complete and up-to-date up to IQ status.
- Changes requiring qualification correctly executed and qualified up to IQ status.
- Training and corresponding certificates complete for:
    - Commissioning personnel.
    - Qualification manager, HVAC system.

  • Qualification measurement team.
- Maintenance personnel successfully trained.
- GMP conformity declarations.
- IQ report, prepared after successful termination of IQ, with confirmation that all errors and omissions identified during the IQ stage have been rectified.

5.B.4 Operational qualification (OQ)

In order not to impair the commissioning of the process equipment, certain OQ steps can already be performed during or immediately after the IQ stage. This is indicated above all for the balancing of the room airflow rates and for the installed filter system leak tests for the terminal HEPA filters. Replacing damaged HEPA filter cells at a later point in time might prejudice the process equipment OQ.

OQ documentation should comprise:

- IQ report, HVAC system, including confirmation that errors and omissions identified during IQ have been rectified.
- OQ plan, HVAC system.
- Reserve capacity of air handling units tested for compliance with specification.
- Room specific airflow rates/air change rates correctly balanced and in compliance with specification.
- Qualification measurements in the *at rest* occupancy state successfully performed and documented:
  - Pressure differences across HEPA filters.
  - Installed filter leak test (HEPA filters only).
  - Room pressurisation during normal and, where specified, reduced operation.
  - Room air cleanliness classification *at rest* during normal and, where specified, reduced operation.
  - Airflow rates during normal and, where specified, reduced operation.
  - Air velocities (areas with unidirectional airflow only).
  - Airflow visualisation (areas with unidirectional airflow only).
  - Recovery time where required.
  - Room air temperature and relative humidity (for specified outside air summer and winter conditions).
  - Noise level in specified working rooms.
- Worst case situations for HVAC system completely and successfully checked.
- Simulation of an electrical blackout: Specified HVAC emergency operation successfully tested.
- Test certificate confirming the correct functioning of HVAC part of the pharma monitoring system including alarm and emergency functions.
- Test certificate confirming the correct functioning of automatic control system plus, if applicable, correct connection to the facility's building automation system including alarm and emergency functions.
- Change control logbook and change control forms correctly filled in, complete and up-to-date up to OQ status.
- Changes requiring qualification correctly executed and qualified up to OQ status.
- OQ report stating that OQ of the HVAC system is complete, with confirmation that all errors

and omissions identified during OQ have been rectified.

5.B.5 Performance qualification (PQ)

Regarding the HVAC system, only those parameters require PQ testing where the presence of personnel will influence the test results. Other tests can be repeated where found necessary or desirable. Below only those tests where changes of results are expected have been listed:

- PQ plan, HVAC system.
- Room air temperature and relative humidity per room at full heat load, with full operation of process equipment and the presence of personnel as specified for the occupancy state *in operation*, for summer and winter conditions.
- Room air cleanliness classification for the occupancy state *in operation*.
- Airborne microbial count with the specified number of personnel present.
- Airflow pattern visualisation at critical locations, e.g.
  - Unloading area of autoclaves into room areas with unidirectional airflow.
  - Transfer belts penetrating walls.
- Change control logbook and change control forms correctly filled in, complete and up-to-date up to PQ status.
- PQ report stating that PQ of the HVAC system is complete, with confirmation that all errors and omissions identified during PQ have been rectified.

Cleanrooms in hospitals

Alexander E Fedotov, Managing Director, Company "Invar-project", Russia

6.1 Introduction

It is well known that staying in hospital can be dangerous for your health. One reason for this is hospital-acquired infections, including those caused by micro-organisms that have adapted to traditional hygienic measures and become resistant to antibiotics. It is sad to say, but it is a fact that hospital infections kill many people and this is additionally costly for the economy. A healthy person operated on in hospital may become infected by a pathogen. Some national statistics, presented below, indicate the extent of such hospital-acquired infections.

United Kingdom

In the UK, 5,000 people die a year from hospital infections. This costs more than £1 billion per year and exceeds the cost of preventing a fatal road traffic accident. It is estimated that approximately 8% of hospital patients were infected in 2006[1].

France

Every year 60,000 to 100,000 people become infected during their stay in hospital or 6 to10% of the total number of patients. Every year from 5,000 to 10,000 people die from hospital infections. These numbers are similar to victims of traffic accidents[2].

Russia

About half the post-operative deaths are caused by infection (Prof. Yu Biryukov from Moscow Medical Academy).

6.2 Hospital infections: history of protection

The problem of hospital infections is very old and it remains a current concern despite many efforts to solve it. The history of the fight against hospital infection can be divided into three periods:

a) Pre-antiseptic period

Until the middle of nineteenth century up to the half the patients subjected to amputation died of infection. Operations conducted in small hospitals, at home or in field conditions were less dangerous. This points to the fact that a high concentration of patients in one place leads to cross-contamination and spread of infection. Fresh air and absence of other people dramatically improved the situation. The early hospitals were very dangerous places.

b) Antiseptic era

British surgeon, Joeseph Lister, devised an antisepsis technology that included soaking instruments, sutures and sponges in carbolic acid. These measures lowered mortality after amputation from 40% to 15% during the years 1864 to 1866. This significant breakthrough marked the beginning of a new epoch of antisepsis in surgery. The core change with this era was a wide acceptance of ***hygienic*** principles for routine hospital practice. Lister also tried to use carbolic spray for air disinfection but this was without success.

c) Clean air and aseptic principles

The next step in reducing cross-contamination in hospitals required proper 'cleaning' of the air. The

benefit of the supply of fresh air for patients was known long ago. In the nineteenth century it was understood that microbial contamination of the air caused infection. Lister was a forward thinking surgeon and knew the importance of clean air in the fight against infection. However, proper technologies of air treatment were not developed and the problem of air cleaning could not be solved at that time. Lister's attempts to use carbolic spray were unsuccessful because the rather large droplets of acid could not cover a significant number of viable particles (micro-organisms). Macro-technology could not solve the problem. The solution was found later thanks to microcontamination research.

The next development occurred in the ***middle of the nineteenth century*** when the rate of hospital infections was influenced by ***new factors***:

- New kinds of operations were developed which changed surgical practice (such as hip joint and knee implants; cardiac surgery etc); these operations took a long time to complete (4-8 hours) and the size of wounds were large; this meant that the ***risk of infections*** during operation became much higher;
- Surgical applications expanded to an industrial scale, serving many patients in big hospitals; thus the danger of ***cross-contamination*** between people (patients and hospital staff) became much greater;
- Antibiotics provided a breakthrough in protecting patients but at the same time some antibiotic resistant micro-organisms colonised hospitals and patients not carrying such micro-organisms had a high ***risk of becoming infected by cross-contamination during their stay at hospital.*** MRSA (Methicillin-resistant *Staphylococcus aureus*) and other bacteria became of real concern within hospitals. Related issues were 'sick building syndrome', especially within older buildings colonised by fungi such as ***aspergillus***, which compounded the problem. A stay in hospital arguably became even more dangerous than during Lister's era.

These new factors led to the development of aseptic technology: air filtration, cleanrooms, laminar flow and clean air devices. The principle of ***aseptic technology*** is not to kill bacteria already present but to retain these bacteria and prevent their penetrating into the cleanroom or clean zone.

British surgeon Sir John Charnley started to use laminar flow in the early 1960s to protect the patients during artificial hip joint replacement. His experiments with clean air gave positive results: infections were reduced after such operations from 9% to 1.3%[3]. Based on this statistic it seemed that the problem was nearly solved. But this was not so! Clean air technology is still not applied in all hospitals in all countries. The risk of contracting an infection in hospital still remains very high. Furthermore, there is still a lack of clear and well understood criteria for air cleanliness in hospitals and anti-infection measures in general.

6.3 Criteria for protection against infections

The treatment of patients in hospital can be divided into two different processes: treatment with medicines and operations (that can also entail medicines).

These two processes set different risks for the patient. If a patient is infected it does not matter to him where the infection originated. The patient expects the risk to be low but the real risk for the two processes is very different (**Table 1**).

To achieve the necessary sterility assurance in manufacturing sterile products, GMP guidelines set requirements for air cleanliness in different zones, both for particles and micro-organisms. The risk during operations is much higher yet this is not controlled by proper mandatory regulations. This is a key contradiction in the protection against hospital infection.

Is 1% infection rate acceptable? In comparison with GMP and pharmacopoeial requirements for

Table 1: Comparison of the various risks of infection caused by different processes

Source of contamination	*Risk of contamination*	*Normative document*
Manufacturing of medicinal products:		
– terminally sterilised	10^{-6} (SAL – Sterility Assurance Limit)	European Pharmacopoeia (EuPh)
– aseptic manufacturing (open process)	approximately 10^{-4}	GMP EC Annex 1
– aseptic manufacturing with a closed process (barrier technology)	10^{-6}	Result of experiment in the USA
Operation theatres:		No requirements
– without laminar flow	approximately 10% (or 10^{-1})	
– with laminar flow	approximately 1% (or 10^{-2})	

manufacturing of sterile medicinal products this risk is too high. What does this 1% mean for a patient? If one falls into this 1% then it becomes 100% for him and all statistics can be binned. So safety dictates one clear rule: to reduce risk as much as possible by all means that can be used at the present state of technology.

From this a number of questions arise:

- Is there any correlation between the number of particles and micro-organisms in the air?
- Is it necessary to control particle concentration in hospitals?
- Whether unidirectional/laminar flow is necessary at all?
- What is the more important, hygiene or clean air?

In considering these questions, it is sometimes easy to forget that the fight against infection is very complex. Micro-organisms are viable entities that try to survive and mutate which sets new problems that require the efforts of different specialists.

6.4 Particles and micro-organisms in the air

How many micro-organisms are in the air compared with particles? NASA textbook NHB 5340[4] has the answer to this question:

– One in 1,000 particles with sizes ≥0.5µm in clean zones ***5 ISO carries a micro-organism;***

– One in ***40 000 particles*** with sizes ≥0.5µm in cleanrooms ***8 ISO carries a micro-organism.***

Deposition on 1m^2 surfaces per 1 hour can be described as follows:

– 80 micro-organisms for class 5 ISO and

– 2,000 micro-organisms for class 8 ISO.

For non-classified (dirty) rooms the number will be much greater.

The following is an approximate estimation but it describes the whole picture.
2,000 micro-organisms can be deposited on 1m^2 of surface in clean room class 8 ISO. It gives an understanding as to how many particles can enter an open wound. If the size of the wound is 20x20cm = 0.04m^2, then during 6 hours 480 micro-organisms can enter the wound in a class 8 ISO room. For a class 5 ISO room, a similar calculation gives only 20 micro-organisms. It is not ideal, but it shows the effect of laminar flow.

Correlation between viable and non-viable particles

Why do we try to find this correlation? We try because:

– air cleanliness for particles is described by well known and accepted standards
– specifying the cleanliness class of a room or zone gives clear guidance how to design, construct and test them
– particle counting is a rapid process and gives real time results compared with testing microbial contamination.

Most micro-organisms have sizes of from 0.5 to 20μm. These sizes fall into the range of particle sizes from 0.1 to 100μm. It is also known that micro-organisms do not travel by themselves in the air. They are deposited on particles. So there are two kinds of particles in the air: viable and non-viable particles. And both are retained by filters, especially HEPA filters.

This means that air filtration reduces the number of both viable and non-viable particles. The cleaner the air, the fewer the numbers of viable and non-viable particles present. So a correlation between them obviously exists, but it cannot be described by a strict mathematical formula. It is not ***deterministic*** but a more complex ***stochastic*** relationship that can only be described in terms of probability. Correlation can be estimated with a variation factor and by other parameters. It means that correlation cannot be described by a simple linear equation, but can be shown by an area with flexible borders and using terms of probability for analysis.

6.5 Sources of microbial contamination

To find a satisfactory method of anti-bacterial protection it is necessary to use a systematic approach and take all important factors into consideration (**Table 2**).

Table 2: Sources of hospital infections

Source of contamination	Route of contamination			
	Direct contact		Cross-contact	
	Presence of direct contact	Measures for protection	Carrier of contamination	Measures for protection
Hospital personal and other patients: – hands – mouth – nose – others	–	–	Particles in air	Masks Air filtration
	+	Hygiene	Surfaces Hands	Personal Cleanliness Hygiene
Patient himself	+	Hygiene	Surfaces Hands	Cleanliness Hygiene
Food for patients	+	Hygiene Clean food	?	?
Medicinal products and medical devices	+	GMP	–	–
Materials	+/–	Cleanliness	Particles in air Hands	Air filtration Hygiene
Surfaces	+/–	Cleanliness	Particles in air Hands	Air filtration Hygiene
Indoor air	+/–	Air filtration	Particles in air	Air filtration

Cross-contamination is especially dangerous. This route of spreading contamination is not obvious but it may be the reason why hygiene measures do not always give sufficient effect, say, for protection against antibiotic resistant micro-organisms.

6.6 Protective measures

Hygiene

This means *cleanliness* of *hands, bodies, pure food and beverages, garments* etc. This protects patient against *direct contamination.* This is a necessary and effective measure. But it is not enough.

Face masks

What does a face mask really do?

Humans distribute *particles and droplets* from the mouth and nose. When *breathing and speaking* this pollution spreads from human for distances of 2-4m in the direction where the human *looks and speaks*. Coughing and sniffing distributes pollutions for greater distances. A mask reduces air velocity and reduces these distances.

Surfaces

Surfaces attract contamination. Viable and non-viable micro-organisms can be deposited on surfaces. A clean surface quickly becomes contaminated if the air is contaminated. Often and thorough cleaning of surfaces reduces contamination in the air and helps to fight against infection. Cleaning of surfaces requires intensive manual work. But it does not remove particles from the air.

Air filtration and cleanrooms

Air filtration is the most effective way to eliminate airborne particles and micro-organisms (**Figures 1** and **2**). In conjunction with proper construction and operating procedures, it may provide the necessary level of air cleanliness. Together with hygiene precautions, it can provide good protection against infection.

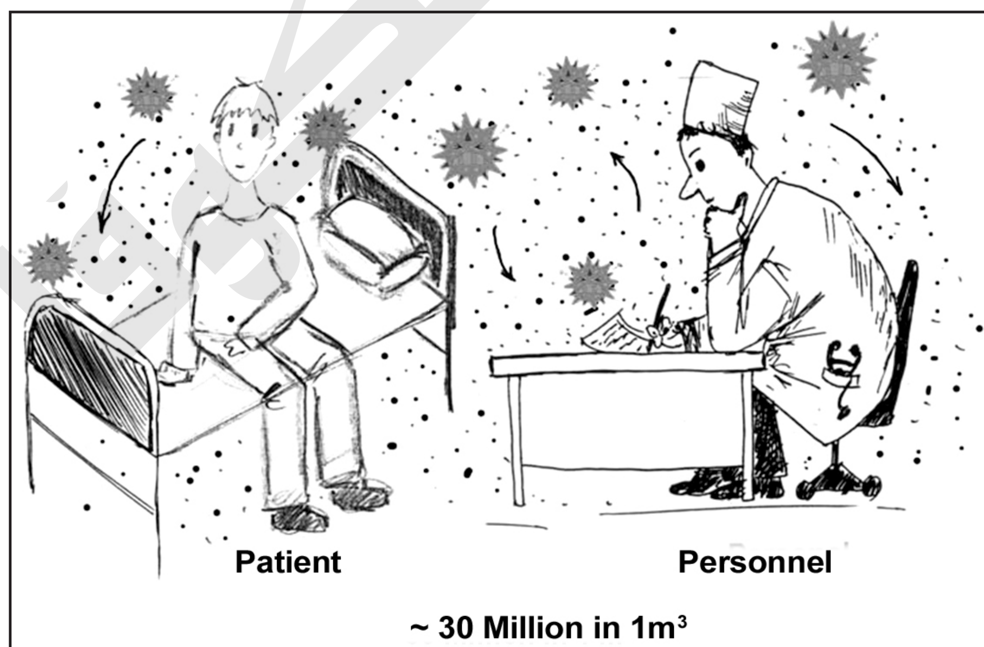


Figure 1: *Hospital room without filters.*

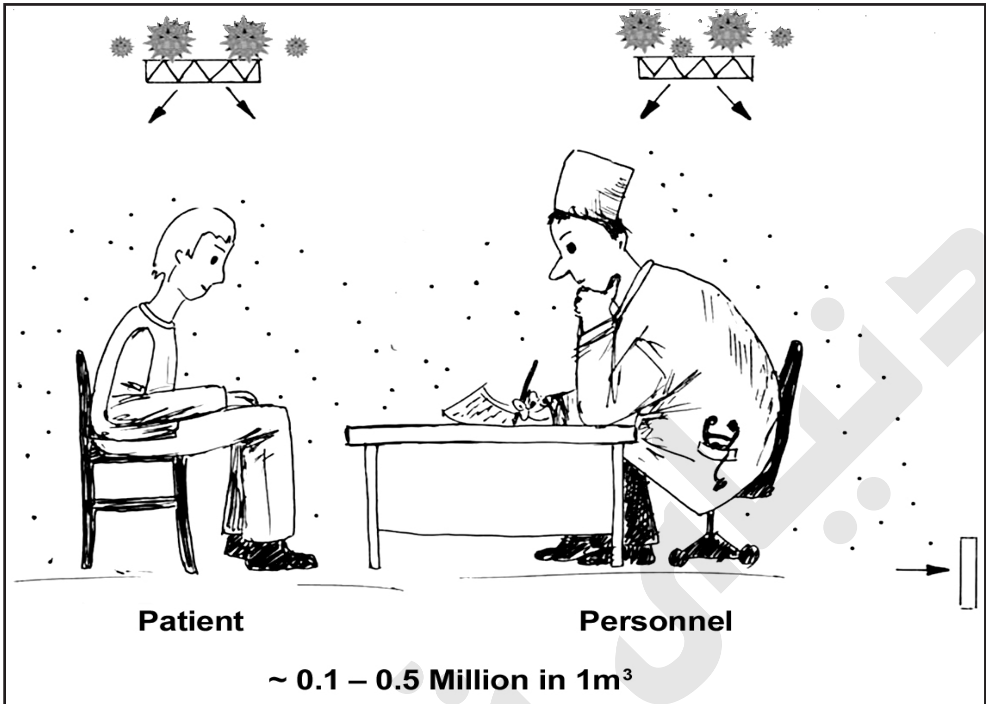


Figure 2: *Hospital room with filters.*

The concentration of both viable and non-viable particles can be reduced by air filtration, and by intensive air exchange in the room using other methods of cleanroom technology (unidirectional air flow, smooth surfaces, proper cleaning, etc.).

6.7 Standards and norms for air cleanliness in hospitals

The technique of cleanrooms has been used in western hospitals since the early 1960s.
The technology of cleanliness in the 1970s and 1980s was based on ventilation and air conditioning systems and application of high-efficiency filters became an integral part of European and American hospitals. At the same time, the first standards for air cleanliness in hospitals were adopted in Germany, France and Switzerland. Now the second generation of standards, based on current levels of knowledge, is being adopted.

Switzerland

In 1987, the Swiss Institute of Public Health and Hospitals (SKI – Schweizerisches Institut für Gesundheits- und Krankenhauswesen) adopted the "Hospital Air Preparation Systems: Construction, Operation and Maintenance" manual[5], which distinguished three groups of rooms (**Table 3**).

Table 3: Three room groups as defined in the Swiss manual

Room group	Microbial contamination in air (CFU/m³)	Supply air flow rate, m³/h
I	<10	10 000
II	<50	2000 – 3000
III	<200	2000 – 3000

Table 4: Maximum permissible concentrations of particles and micro-organisms in the French standard

Designation of Zone	Maximum particle concentration/m^3 ≥0.5 microns	Class ISO
Zone 4	<3 500	5
Zone 3	<350 000	7
Zone 2	<3 500 000	8
Zone 1	Not specified	

Class by micro-organism	CFU/m^3
B 100	100
B 10	10
B 5	5
B 1	≤1

In 2003, the Swiss Society of Heating and Air-Conditioning Engineers adopted SWKI 99-3 “Heating, Ventilation and Air-Conditioning Systems in Hospitals” (designing, construction and operation manual[6]).

Its essential feature is that there is ***no standardisation of air cleanliness by microbial contamination (CFU)*** to assess the work of a ventilation and air-conditioning system.

The assessment criterion is the concentration of particles (not micro-organisms) in the air. The manual establishes clear requirements for air treatment of operation rooms and gives an original technique of assessment of efficiency of measures for maintaining cleanliness using an aerosol generator.

Germany

In 1989, Germany adopted the standard DIN 1946, part 4 “Cleanroom technology. Systems of maintaining the air cleanliness in hospitals” – DIN 1946, Raumlufttechik. Raumlufttechishe Anlagen in Krankenhäusern, Dezember, 1989. It was revised recently.

The Society of German Engineers (VDI) has prepared a project of the standard VDI 2167 “Hospital equipment – heating, ventilation and air-conditioning”. This is identical to the Swiss manual SWKI 99-3 but contains editing corrections.

France

The standard AFNOR NFX 90-351 concerning the air cleanliness in hospitals was adopted in France in 1987 and revised in 2003[7].

The standard establishes maximum permissible concentrations of particles and micro-organisms in the air. The particle concentration is determined as: ≥0.5 microns (**Table 4**). The French standard also specifies classes for particle decontamination (10 min. time).

The standards listed itemise the requirements for operation rooms, establishes the number of filtration stages, filter types, sizes of laminar zones, etc.

Development of the national standard of Russia

Analysis of the experience of our foreign colleagues has emphasized some key questions, some

of which having caused heated debate when discussing the Russian standard. The main issues were as follows:

– Classification of hospital rooms
– At what occupancy state is it necessary to test hospital cleanrooms to estimate the job of the cleanroom design/construction company: only "at rest" or both "at rest" and "in operation"?
– What should be counted: only particles or both particles and micro-organisms to estimate quality of cleanroom, not pollution from patients and personnel.
– What particle size should be used for cleanroom classification?

These and other problems were solved and the Russian national standard GOST R 52539-2006, "Air cleanliness in hospitals. General requirements"[8] was approved in 2006. Further material in this chapter is based on this standard.

6.8 Classification of hospital rooms

The Russian standard GOST R 52539, sets classification and requirements for different rooms and zones in hospitals.

Former standards basically concerned operation rooms. Some standards concern isolation wards and other rooms. But there is no detailed classification for rooms of all purposes included in the ISO cleanliness classification.

All hospital premises are divided into five groups, depending on the risk to patients:

Group 1 – Operating rooms with aseptic technique and unidirectional airflow with extensive wound and long-duration surgery.

Group 2 – Intensive care units, with unidirectional airflow, for patients after bone marrow transplantation, patients with immunodeficiency, etc.

Group 3 – Operating rooms without unidirectional airflow or with unidirectional airflow but less sectional area than for group 1.

Group 4 – Rooms with no special protective measures required for the patient in question, personnel and other patients.

Group 5 – Rooms for infected patients (isolation wards).

Classification of hospital rooms into groups is shown in **Table 5**. The list of operations and diseases given in the Table is not exhaustive.

Requirements for group 5 rooms are complementary to the operating standards for the rooms intended for infected patients.

Other rooms, subject to their functions, can be referred to any of the groups described by mutual agreement of the customer and executer (designer).

Criterion for assessment of air cleanliness

What should be taken as a basis for air cleanliness assessment, particles, micro-organisms or both?

Development of standards by this criterion has its own logic. At first, air cleanliness in hospitals was assessed only by the concentration of micro-organisms. Then, concentration of particles also began to be applied. In 1987, the NFX 90-351 standard of France introduced the control of air cleanliness by both particles and micro-organisms. Using a laser particle counter allows the concentration of particles to be defined in real time, while incubating micro-organisms in nutrient medium takes several days.

Table 5: Classification of rooms in hospitals according to the Russian standard

Group of rooms	Function	Characteristics
1	**Operating rooms with aseptic technique and unidirectional airflow** to perform: – grafting and transplantation of organs and tissues – implantation of foreign bodies (replacing coxofemoral, knee and other kinds of joints, hernia repair with mesh, etc.) – reconstructive or reparative surgery carried out on the heart, large blood vessels, urinary system, etc. – reconstructive or reparative surgery applying microsurgical technique – combined surgery for different tumour sites – open thoraco-abdominal operations – neurosurgical operations – surgery on extensive operative areas and/or of long duration that requires the instruments and materials to be open for a long time – surgery after pre-operative chemo- and/or radiotherapy for patients with low immunological status and multiple organ failure – surgery of multisystem trauma, and others	Introduction of sterile and clean foreign bodies including implants into the human body. Long-duration surgery. Extensive wound (operative field). Operations performed on debilitated or immunocompromised patients
2	**Intensive care units, with unidirectional airflow,** for the patients: – after bone marrow transplantation – with extensive burns – undergoing high-dose chemo- and radiotherapy – after extensive surgical operations – with lowered or no immunity	Patient's immunodeficiency, microbial allergy, weakness, long-term stay in the intensive care unit
3	**Operating rooms without unidirectional airflow, or with unidirectional airflow but with less cross-sectional area than for group 1,** to perform: – endoscopic operations – endovascular operations – other treatment and diagnostic manipulations with a small operative area – haemodialysis, plasmapheresis, etc. – caesarean operation – sampling the umbilical blood, bone marrow, adipose tissue, etc., followed by isolating stem cells Rooms with the higher demands for cleanliness without unidirectional airflow, including: – wards for patients who have had organ transplantation – wards for burn patients – preoperating and other rooms preceding operating rooms – dressing rooms – maternity units – post-anaesthesia rooms – reanimation wards – neonatological departments* – room to store sterile materials – postoperative wards (for the patients admitted from the intensive care units inclusively) – wards for debilitated or seriously ill patients getting non-surgical, general treatment	Risk of infecting the patient is less than for group 1 rooms, but it is necessary to provide patients and materials with protection against airborne infection

Table 5: ***Continued***

Group of rooms	Function	Characteristics
4	**Rooms with no special protective measures required for an individual patient, personnel and other patients**: – wards for patients, except for groups 2, 3 and 5 rooms – rooms for endoscopic diagnostics (gastro-duodenoscopy, colonoscopy, bronchoscopy, retrograde cholangiopancreatography, etc.) – admission department – rehabilitation wards	—
5	**Rooms for infected patients (isolation wards**): – wards for patients with suspected infections, including droplet infections – dressing rooms for patients with pyogenic infection. Operating rooms for patients with pyogenic infection, patients with anaerobic infection and others.**	The priority is protection of all personnel and other patients. Air of these rooms is prohibited from entering the adjacent rooms.

* If required, special conditions in completely isolated zones (devices), to nurse premature infants, for example, can be provided.

** Zones with unidirectional airflow with cross-section 3.0 – 4.0m² should be provided

The following question arises: ***what, in fact, is to be tested at the qualification stage of cleanrooms and ventilation systems?***

The quality of the contractors' work and the correctness of the design solutions are controlled. These factors are unequivocally assessed by particle concentration on which the number of micro-organisms depends.

Surely, microbiological sedimentation depends on the cleanliness of walls, equipment, personnel and so forth. However, these factors concern actual work and operation, but not the assessment of engineering systems.

In this connection, in Switzerland (SWKI 99-3) and Germany (VDI 2167) the following stipulation was made: ***the air is to be tested only by particles.***

The concentration of micro-organisms remains a function of the hospital epidemiological service and is directed at the actual control of cleanliness.

Cleanroom occupancy states

ISO 14644-1 distinguishes three occupancy states of cleanrooms:

- In an *as-built* occupancy state, the performance of some technical requirements is checked. Concentration of pollutants is not generally regulated
- In an *at-rest* occupancy state, the room is complete with the equipment, but there is no personnel and technological process (for hospitals, there are no medical staff or patients)
- In an *operational* occupancy state, all the processes stipulated by the purpose of the room are carried out.

GMP EC Guide stipulates the control of concentration of air-borne particles both in the *at-rest* occupancy state and the *operational* occupancy state, while the control of the number of micro-organisms is applicable to the *operational* occupancy state only. There is some logic here.

Pollutants discharged from equipment and personnel in the manufacture of medicinal products can be controlled to provide their conformity to the regulations by means of technical and organisational measures.

In a hospital, there is a non-controlled element – the patient. It is impossible to dress patients and medical staff in class 5 ISO overalls and completely enclose the surface of their bodies. Since the sources of pollution in hospital rooms in an operational occupancy state cannot be controlled, there is no sense in establishing norms and certificating the rooms in an operational occupancy state, at least concerning particles.

Developers of previous standards understood that. In the GOST standard, we also included the control of rooms in the *at-rest* occupancy state only.

Particles size

Initially, the concentration of ≥0.5 micron particles was controlled in cleanrooms. Then, proceeding from specific activities, requirements were developed for the concentration of particles ≥0.1 microns and ≥0.3 microns (microelectronics), ≥5.0 microns (manufacture of medicinal products in addition to particles ≥0.5 microns), etc.

Table 6: Basic requirements for air cleanliness in rooms in the at-rest state

Room group		Maximum permissible number of particles in 1m^3 of air (particle size ≥0.5 microns)	Cleanliness class according to ISO 14644-1	Maximum permissible CFU number in 1m^3 of air
1	Zone of the operating table	3 520	5 ISO	5
	Zone around the operating table	35 200	6 ISO	20
2	Zone of the patient's bed	3 520	5 ISO	5
	Zone around the patient's bed	35 200	6 ISO	20
3*		3 520 000	8 ISO	100
4		Not standardised	–	500
5*		3 520 000	8 ISO	100

* Air cleanliness requirements of a zone with unidirectional airflow correspond to those in the zone of the operating table (group 1).

The analysis indicates that there is no sense in following the GMP pattern “0.5 and 5.0 microns” in hospitals. It is sufficient to control particles of ≥0.5 microns.

Requirements for air cleanliness in rooms at-rest state are shown in **Table 6**.

Air cleanliness for each group of rooms is preset by the maximum permissible concentration of particles ≥0.5 microns (in accordance with ISO 14644-1), and also by maximum permissible CFU concentration in the air for a room at-rest, i.e. with no patients or personnel present.

Values of concentrations of particles in air for rooms of groups 3 and 5, except for zones with unidirectional airflow, are reference values and are used if it is required to confirm a cleanliness class of room under the agreement between the customer and the contractor.

Classification of particles corresponds to ISO 14644-1 standard.

Table 7: Type of airflow and classes of filters

Room group		Class of room (zone) cleanliness	Type of airflow	Air exchange rate	Classes of filters
1	Zone of the operating table	5 ISO	U	Not specified	F7+F9+H14
	Zone around the operating table	6 ISO	N	30-40	F7+F9+H13
2	Zone of the patient's bed	5 ISO	U	Not specified	F7+F9+H14
	Zone around the patient's bed	6 ISO	N	30-40	F7+F9+H13
3		8 ISO	N	12 – 20	F7+F9
4*		–	N	1 – 3	F7+F9
5		8 ISO	N	12 – 20	F7+F9

* In rooms of group 4, natural ventilation is the most common. For forced ventilation, it is recommended to apply filters of the classes mounted in rooms of groups 3 and 5, but to provide a lower air exchange rate.

Notes:
1. U – unidirectional airflow, N – non-unidirectional airflow.
2. In order to extend the service life of filters of class F7, it is advisable to provide a preliminary stage of filtration by means of filters of classes G3 (G4).
3. Air exchange rates shown in the table are reference ones and reflect only the requirements for air cleanliness. When defining the air exchange rate, it is necessary to consider other factors influencing air cleanliness (removal of excess heat and moisture, exhaust of harmful substances, etc.) as well. When calculating the air exchange rate, the work of local air-cleaning devices and installations creating unidirectional airflow is to be taken into account.

Requirements for air exchange rate and classes of filters for different room groups are shown in **Table 7**.

Classification of filters is in accordance with EN 799 and EN 1822.

Velocity of unidirectional airflow

It is impossible to apply the GMP norm of 0.45m/s ±20% in medicine. This would lead to discomfort and dehydration of the surface of the wound, could injure the wound, etc. Therefore, for the zones with unidirectional airflow (operation rooms, intensive care units) a velocity from 0.24m/s to 0.3m/s is recommended.

Size of zones with unidirectional airflow

Swiss, German and Russian standards stipulate the sizes of ceiling diffuser creating the unidirectional airflow as 3 x3m, without "solid" surfaces inside. Exceptions are admitted for some less important operations.

Ventilation and air-conditioning solutions

These solutions correspond to Western standards; they are economic and effective. Some changes and simplifications without loss of sense have been made. For example, filters H14 (instead of H13) cost the same but are much more effective, are applied as final filters in operation rooms and intensive care units.

Local air cleaning devices

Local (independent) air cleaners are an effective means of maintaining air cleanliness (except for group 1 and 2 rooms). They do not demand high expenses; they allow making flexible decision-making and can be used in large numbers, especially in old hospitals.

Table 8: Controlled air parameters for every group of rooms

Room group		Name of parameter							
		L ($N_{ac.}$)	$V_{air.}$	C_n	N_{CFU}	ΔP	v_B	t°	φ
1	Within zones with uni-directional air flow	–	+	+	+	–	–	+	–
	Outside zones with uni-directional air flow	+	–	+	+	+*	+*	+	+
2	Within zones with uni-directional air flow	–	+	+	+	–	–	+	–
	Outside zones with uni-directional air flow	+	–	+	+	+	–	+	+
3		+	–**	+	+	+*	+*	+	+
4		–***	–	–	+	–	–	+	+
5		–	–**	+	+	+	–	+	+

* The following parameters are controlled: differential pressure or airflow direction and velocity (leakage) in an under-door slot when the door is closed.

** Parameters are controlled in accordance with airflow velocity requirements for rooms of such groups if they have zones with unidirectional air flow.

*** Air flow rate in rooms of group 4 is only controlled in case of forced ventilation.

Names of parameters, used in the table:

L – air flow rate, m^3/h;

$N_{ac.}$ – air changes per hour, h^{-1};

$V_{air.}$ – velocity of air in zones with unidirectional air flow, m/s;

C_n – particle concentration in the air in the room, particles/m^3;

N_{CFU} – micro-organisms concentration, CFU/m^3;

ΔP –pressure differential between rooms, Pa;

v_B – displacement airflow velocity at the border of rooms with different cleanliness classes, m/s;

t° – air temperature, ºC;

φ – relative air humidity, %

There is a wide range of air cleaners on the market. Not all are effective; some are harmful (they discharge ozone). The basic danger is a wrong choice of air cleaner.

Test methods

GOST R 52539-2006 gives a systematised programme of testing hospital cleanrooms by all necessary parameters (**Table 8**).

The given parameters should be tested at qualification stage. During routine operation, the concentration of particles and micro-organisms in the air should be tested.

Adapted from articles earlier published in European Journal of Parenteral & Pharmaceutical Sciences 2008; ***2****: 31-35 and in Renhets Teknik, the Official Cleanroom Magazine for R3-Nordic and presented at the ICCCS conference in Tokyo, 2010, the R3-Nordic conferences in Gothenburg, 2009, Espoo, 2010 and others.*

6.9 References

[1] James R. Superbugs: media type or a threat to healthcare systems? – Presentation at Cleanroom Europe Conference in Stuttgart, 24 March 2009.

[2] Dorchies F. France: standard on air cleanliness in hospitals – *Cleanroom Technology*, April 2005.

[3] Cleanroom design. Edited by White W, published by John Wiley and sons, 1992.

[4] NASA NHB 5340 – "Biological Handbook for Engineers", Marshall Space Flight Center – Oct. 1976.

[5] Richtlinien für Bau, Betrieb and Überwachung von raumlufttechnischen Anlagen in Spitälern. Ausgabe 1987. SKI, Band 35, Switzerland.

[6] Richtlinie 99-3. Heizungs-, Lüftungs- und Klimaankagen in Spitalbauten (Planung, Bau, Betreib). SWKI 400/5/2003, Switzerland.

[7] NF S 90-351, June 2003. Health care establishments – Cleanrooms and associated controlled environments – Requirements for the control of airborne contamination.

[8] GOST R 52539-2006 Air cleanliness in hospitals. General requirements.

Commissioning and qualification of cleanrooms

Kevin Beauchamp, Crowthorne Hi-Tec Services, Farnborough, UK
Miroslav Tonovski, KTM Proekt, Skopje, Macedonia

7.1 Introduction

This chapter outlines a practical approach for the qualification of cleanrooms and clean air devices. The aim of the chapter is to present the various tests which need to be undertaken for validation from the practical perspective of the engineer and to allow the cleanroom user (or contractor) to follow the required steps, based on regulatory expectations, for the qualification of a cleanroom. The chapter primarily does so by presenting a series of test sheets which can be adapted by cleanroom operators or engineers to allow for a step-by-step approach to cleanroom qualification. This chapter does not expand on the theory of cleanroom qualification (this is covered elsewhere in this book); however it helps to simplify the complexities of cleanroom validation by presenting the different tests required in a logical and straightforward manner.

7.2 Planning

The planning for validation is critical as it is said “to Fail to plan is to Plan to fail”, therefore the better the plan the smoother the exercise.

Validation Master Plan (VMP)

The Validation Master Plan (VMP) is a document that has never been mandatory, but is always one of the first documents a regulator asks to view. This is an extremely important document because in constructing it, many serious commitments and decisions have to be made. Programme conceptions have to be connected to the User Requirements Specifications (URS), and these specifications have to be mated to the VP or VMP.

From these plans the Design Qualification (DQ), Installation Qualification (IQ), the Operational Qualification (OQ), and the Performance Qualifications(PQ) have to be authorised, authored, approved for content, and issued for execution. The completed documentation has to be reviewed and accepted as complete by persons authorised to execute this role. All of these functions must be detailed in the VMP, when the project concept demands that a VMP is required or the Validation Plan (VP) where it does not.

Responsibilities have to be declared, people have to be nominated, and everyone involved is duly served with a copy that carries the full authority of the company. The prospective progress of the project is there for everyone to compare to the actual progress. The VMP is a document that documents the way the company will operate, who has control over the various aspects of the validation activities, and how production, quality control and personnel management will be directed.

Factory Acceptance Testing (FAT)

Factory Acceptance Testing (FAT) is undertaken by executing a suite of documented tests on a completed system or item of equipment. Each individual test, known as a case, exercises a particular operating condition of the user's environment or features of the system, and will result in a pass or fail. The test environment is usually designed to be identical, or as close as possible, to the anticipated user's environment, including extremes (such as temperature and humidity). These

test cases must each be accompanied by test case input data or a formal description of the operational activities (or both) to be performed—intended to thoroughly exercise the specific case—and a formal description of the expected results.

7.3 The Qualification

It comprises the verification of the design and its compliance with the required standards, procedure recommendations and guidelines.

Design Qualification(DQ)

The Design Qualification (DQ) is a document (protocol) which compares the User Requirement Specification (URS) and the Proposed Design, this is usually executed in conjunction with input from the end users (Client) and the design company, It is to confirm that what will be produced or tendered will meet the URS and design intentions and provides formal procedures/protocols for determining compliance.

Commissioning cleanrooms

Commissioning process starts when the installation of the cleanroom is completed. That is a process usually performed between the contractor and the client or its representative as a witness, where all parties are evaluating using approved tables which shows how much of the design is fulfilled concerning the requirements, performance, operation and maintenance.

Some of tests to be performed during the commissioning period are:

- Airflows and air-changes
- Differential pressures
- Duct leakage tests,
- Interlocks
- Temperature and relative humidity
- Unit performance

It is mandatory that all cleanrooms to be qualified by a personnel familiar with all requirements and procedures for certain tests to be performed. It is compulsory for the personnel involved in the qualification tests to present unbiased reports with a summary of the test results and, where appropriate, with conclusions and recommendations.

Different Qualification Tests within one cleanroom are performed during its lifetime. These consist of:

Installation Qualification

The Installation Qualification (IQ) ensures that the installation of the equipment is accurate, where possible, by comparing the existing system against the design and the manufacturer's specifications and it is providing a complete documentary record of that installation.

The IQ ensures that the components marked on the Piping and Instrumentation Diagrams (P&ID's) are installed as described; that Heating Ventilation and Air Conditioning (HVAC) instrumentation measuring devices are calibrated; and that the installation of the Wall, Ceiling and Lighting systems (that is the cleanroom in general) is according to the specification.

The IQ Protocol must contain the following information:

- Information on equipment services
- Identification of mechanical and electrical components
- Equipment information and documentation
- Manufacturers/suppliers calibration records

- Drawing information
- Verification of correct location

Operational Qualification
The objective of the Operational Qualification (OQ) is to check those operational aspects of the HVAC system that are deemed critical to its satisfactory performance and compare the data obtained with approved specifications and current Good Manufacturing Practice (cGMP). Prior to this, it should be confirmed that all critical instruments have been calibrated for performing the OQ test.

When assisting the end user during the validation of predetermined specifications with the OQ, a contractor must determine whether the system corresponds to the manufacturer's description and installation requirements. In doing so, the reproducibility of the operating characteristics can be evaluated thus verifying that adequate control of the air handling system can be routinely exercised. The tests detailed within the OQ Protocol will have been specifically requested by the client.

ISO 14644-3:2005[5] provides the procedures for the OQ tests and details how they are to be performed (**Table 1**).

Table 1: Showing the procedures for Qualification tests

Test	Procedure described within the ISO 14644-3: 2005 can be found in:
Airflows and Air-change rate Test	Section B.4
Differential Pressure Test	Section B.5
Temperature and Relative Humidity Test	Section B.8 and Section B.9
Filter Leakage Test	Section B.6
Airflow Visualisation Test	Section B.7
Recovery Test	Section B.12
Particle Counting Test	Section B.1

There are also other tests described within the standard ISO 14644-3:2005[5] such as Electrostatic and Ion Generator Test, Particle Deposition Test and Containment Leak Test which are not covered in this chapter.

Performance Qualification
The Performance Qualification (PQ) is performed once the OQ has been completed within the time specified between the client and the contract tester. For the PQ not all of the tests undertaken in the OQ need to be performed: only those tests deemed critical and which can show that the system can run continuously. With the PQ tests it is essential to demonstrate that the HVAC system is capable of maintaining parameters such airflows and air-changes, differential pressure, temperature and relative humidity within the acceptance criteria, i.e. demonstrate that the process or equipment performs as intended in a consistent manner over time.

Re-Qualification
Re-qualification tests refer to the period of time during which it is obligatory to conduct the tests defined in the ISO 14644-2[4] for cleanrooms in order to prove there is continued compliance between the cleanroom performance and the requirements set in the standards. For re-qualification a number of tests are required:

a) The Particle Counting Test is required to confirm if the classification of the cleanroom remains

within the required ISO classification. The frequency of re-classification is:

- ≤ISO 5 on every 6 months
- >ISO 5 on every 12 months

b) The Airflow Volume or Airflow Velocity and Differential Pressure Tests are tests which must be performed during the period of 12 months.

c) Other optional tests recommended to be conducted within a period of time of 24 months are:

- Filter leakage test (except when a new filter is installed than the test should be performed immediately)
- Airflow visualisation (required for some unidirectional airflow devices within cleanrooms)
- Recovery test
- Containment leakage test

The frequency intervals for performing tests are the following:

- If the test must be performed on every 6 months the average interval must not exceed 183 days and the test not to be performed for more than 190 days.
- If the test must be performed on every 12 months the average interval must not exceed 366 days and the test not to be performed for more than 400 days.
- If the test must be performed on every 24 months the average interval must not exceed 731 days and the test not to be performed for more than 800 days.

7.4 Tests required for the commissioning and qualification of cleanrooms

This section of the chapter examines the tests required for commissioning, qualifying and re-qualifying cleanrooms.

7.4.1 HVAC system component installation check

This test is part of commissioning and/or IQ process. Here "as built" drawings have to be challenged with the installation. Components have to be checked for any visible damage and any visible impurities. **Table 2** is an example of a table showing the latest revision of the ductwork distribution drawing(s) and HVAC schematics to be completed.

Table 2: Example of table which registers basic drawings data required for this test

Drawing Nº:	Description	Revision	Date	Confirm provided	
				Initials	Date
XXXXX	XXXXXX	XX			

Components Verification

Using a copy of the above drawing, it has to be verified that the "Components" in the selected section of the distribution ductwork and installed clean room elements are correct (suitably located, installed securely and free from damage) by using a highlighter pen (for instance Yellow). For not verified sections has to be marked (for instance Blue highlighter). For not correct information, amend the details on the drawing, correct by using a highlighter pen (for instance Pink) and initial the correction so that the drawing contains the final as built details. When this is completed the drawing has to be identified by writing title. Drawings at the end should be signed or initialled with the date.

Components are defined as:

- Constant Volume Control Boxes
- Ductwork
- Volume Control Dampers & Motorised Dampers
- Sockets, switches, lights
- Terminal diffusers, grilles
- Panels, doors

- Sensors (Pressure sensors, Pressure switches, Temperature and Humidity Sensors etc.)
- Diffusers and Grilles
- Filters (EU4, EU9, H14, U15)
- Air handling units, coils, humidifiers, dehumidifiers, inverters
- Pumps, valves, pipes, instruments
- Sockets, switches, lights etc.

Another installation check is where the checker within the **SECTION for CONFIRMATION** should write next to every item **YES** or **NO** then put the initials and the date when the activity has been performed (**Table 3**). Checkers with the reviewers and approvers of this section may add initialled and dated comments or notes if necessary.

Table 3: Commissioning table for confirmation of the installed equipment

AHU XXXXXX SUPPLY		
ITEM	**SECTION for CONFORMITY WRITE Clearly YES or NO**	**INITIALS/ DATE**
Correct Installation of Sand Trap Louvre		
Fresh Air Damper Positioned and Locked		
Filter Section EU4 (filters installed and out of damage)		
DPS for filter section EU4 installed, connected and out of damage		
....	....	....
Comments:		
Completed by:	Date:	
Reviewed by:	Date:	

7.4.2 Airlock Test

For performing this test, interlock systems within the Clients Facility must be identified and checked. Test starts with using the latest revision of the Air Lock Signalisation layout, where is confirmed that Interlocking systems have been installed in the required location, supplied with power (or compressed air where applicable, usually in fire-rated areas), cables (or pipes where compressed air) are properly connected and tested, and the semaphors are installed together with the emergency button.

Objective is to determine if the airlock doors are operating according specifications hence ant the acceptance criteria will be to identify if the airlock system operates as per manufacturer and design specifications.

Some of the interlock tests are to:

- Verify that room interlocked doors cannot be opened simultaneously.
- On approach to door, button should be pressed or swipe card (where applicable) to gain access
- LED lights will change from red to green.
- Enter into room/airlock sensors (where applicable) will detect presence of personnel and once the person has passed through doors, closer will activate to close doors. (applicable only where doors are semi-automatic or automatic)

7.4.3 Fan commissioning test

This test is typical for the period of commissioning after system is balanced (**Table 4**). The purpose of this test is to give identity to the installed fan section of the air handling unit (motor, fan, pulleys, belt, power, etc.). This test also has target to confirm the spare capacity of the motor/fan.

Table 4: Table for AHU fan section

HVAC SYSTEM: XXXXXXX SUPPLY			
FAN DETAILS		**MOTOR DETAILS**	
Manufacturer		Manufacturer	
Model		Serial No./Prod. Code	
Product No./(Ref. No.)		Current (A)	
Speed		Power kW	
Power. (KW)		Voltage (V)	
Pulley Size		Speed	
Other:		Pulley Size	
		Belt Type	
FINAL DUTIES			
Fan Static Pressure (Pa)	Inlet:	Outlet:	
Motor Running Current (Amps)			
Motor Running Hertz (Hz)			
Comments:			
Completed By:	Date:	Accepted By:	Date:

7.4.4 Ductwork leakage testing according to DW/143

Duct leakage tests confirm that what has been designed, then installed on site has minimum losses of air to save energy, achieve airflows, temperature, etc**.**

There are three classes of ductwork concerning pressurisation (**Table 5**):

- Class A: up to 500Pa positive and maximum negative 500Pa
- Class B: up to 1000Pa positive and maximum negative 750Pa
- Class C: up to 2000Pa positive and maximum negative 750Pa

In relation to the ductwork, the classification system is tested as follows (**Table 6**):

- If the ductwork is classified as **high pressure** ducts – all ductwork is tested

Table 5: Ductwork classification and air leakage limits

Duct pressure class	Static pressure limit (Pa)		Max. Air velocity (m/s)	Air leakage limits l/sec/m^2 of duct surface area
	Positive	Negative		
1	2	3	4	5
Low pressure Class A	500	500	10	$0.027 \times p^{0.65}$
Medium pressure Class B	1000	750	20	$0.009 \times p^{0.65}$
High pressure Class C	2000	750	40	$0.003 \times p^{0.6}$

- Where **medium pressure** class, 10% of random selected ducts will be tested
- **Low pressure** ducts are untested, and only tested if there is agreement between the customer and the installers

Table 6: Air leakage rates (recommended mean tests parameters are highlighted in bold)

Static Pressure differential (Pa)	Maximum leakage of ductwork (litres/sec/m^2)		
	Class A	Class B	Class C
100	0.54	0.18	
200	0.84	0.28	
300	1.10	0.37	
400	1.32	0.44	
500	1.53	0.51	
600		0.58	0.19
700		0.64	0.21
800		0.69	0.23
900		0.75	0.25
1000		0.80	0.27
1100			0.29
1200			0.30
1300			0.32
1400			0.33
1500			0.35
1600			0.36
1700			0.38
1800			0.39
1900			0.40
2000			0.42

Procedure

Procedure starts with defining the area that needs to be tested, and marking at the drawings. Fan has to be provided with enough capacity to achieve the required pressure which is defined for testing. With flexible connections then is connect to the ductwork. All open ends on the ductwork needs to be blanked. Manometer Gauge has to be provided at the test rig to read initial pressure. Flow instrument needs to be provided for reading the leakage. Test to be maintained for approximately 15 minutes. All details from the test should be recorded and signed (**Table 7**).

Other ways how to find leaks:

1. By looking any visual damages on the ducts.
2. By listening: when leak appear noise is significant at that place.
3. By feeling: running your hand over the area (helps if the hand is wet), and this is more recommended for supply ducts (when positive pressure is tested).
4. Soap and water: it is visual test, where target is to find bubbles at the duct surface.
5. By generating smoke inside ductwork. Helps until ductwork is not insulated.

Example of a completed test:

Table 7: Example of a completed test (*Note: if the ductwork is 500mm x 500mm where periphery is 2,000mm or 2m and the length is 35m, by using the table described within DW/143, the surface area will be 70m^2.

Test Nº	XXXXXX
Name of Job: XXXXXX	
Building Reference:	XXXXXX, Building West
Part 1 – Physical details	
a. Section of ductwork to be tested	Supply ductwork Zone XXX, 2nd floor
b. Drawing Number	XXXXX / XX
c. Pressure classification	Class C
d. Test static pressure	1200Pa
e. Leakage factor	0.30 litres/ sec/m^2.... ***see table 6 for 1200Pa***
f. Surface area of duct under test	70m^2 . ***periphery of the ductwork(m) x length(m)****
g. Maximum permitted leakage	21 litres/second ***(e x f)***
Part 2 – Test particulars	
h. Duct static pressure reading	1200 ***(from the manometer on test rig)***
i. Manufacturer of the device	XXXXXXX
j. Serial number of the device	XXXXXXX
k. Range of the device	0-100 litres/sec
l. Reading of the measuring device	16 litres/sec ***(from rig)***
m. Duration of the test (normally 15min)	15min
Acceptance criteria achieved write clearly Yes or No	YES
Comments:	
Tests Completed by	Date
Test Witnessed by	Date

7.4.5 Airflows and air-changes

Some of the most important tests conducted in the cleanrooms are the examination of airflows and air change rates. The purpose of these tests is to measure the supply/extract airflow rate in the cleanrooms and clean zones and to confirm the design specification for the air changes per hour.

Effective airflows are essential in order to reach the desired cleanliness levels, as well as the required temperature and relative humidity levels within cleanrooms. Within cleanrooms the air is normally operating at a turbulent flow (this is where air enters the room with non-uniform velocity). With clean air devices, the object is to have unidirectional airflow.

Each cleanroom grade has a set number of air changes per hour. Air changes are provided in order to dilute any particles present to an acceptable concentration. Any contamination produced in the cleanroom is theoretically removed within the required time appropriate to the room grade. This is important because particles would otherwise build up in enclosed spaces if there is no ventilation.

Examining air-changes

The air-changes per hour are expressed through a mathematical formula which examines the airflow (m^3/h) supplied to a given room volume (m^3).

To clarify:

- The airflow enters in the Air Handling Unit (AHU) with a certain velocity (m/s) which directs this airflow through the fan and the ductwork to the terminal diffusers in the cleanroom.
- The airflow velocity (m/s) x the floor area of the ductwork or the terminal filter (m^2) = airflows (m^3/s).
- To calculate the airflow in m^3/h one should simply multiply the value of m^3/s x 3600.
- The volume of the room is given in m^3 as a result of multiplying the room floor area (m^2) by the room height (m).

Hence, Air changes per hour are calculated with the following formula:

$$\textbf{Air changes per hour (1/h)} = \frac{\text{Airflows } (m^3/h)}{\text{Volume } (m^3)}$$

Tables are available which show the values of air changes per hour (from-to) that are needed in order to attain a certain cleanliness level in a given industry. It is worth mentioning that the designer should pay attention to the following issues, regardless of their partial accuracy:

- The purpose of the given room
- The filtration level
- The number of operators
- The heat gains from the equipment, etc.

Examining airflow

The most appropriate method for measuring airflows is at the air inlet:

a) First select the correct size hood to fully cover the filter or terminal outlet/inlet.
b) Select the volume program on the processor program.
c) After covering the terminal outlet hold the hood on the downstream face of the supply diffuser and measure air volume in m^3/hr.
d) Read the indicated volume directly from the instrument and record it. That volume presents actual air per terminal ref diffuser.

To calculate Room Total Air, a summary of Actual Air Supply for all terminal diffusers within the cleanroom, follow the following formula:

$$\sum \textbf{\textit{Room TOTAL Air}} = \textit{Terminal Ref 1 + Terminal Ref 2 + Terminal Ref n.}$$

Then calculate:

$$\textbf{Air changes per hour (1/h)} = \frac{\sum \textit{Room TOTAL Air } (m^3/h)}{\text{Room volume } (m^3)}$$

For example, see **Table 8**:

Table 8: Airflow and air-changes test table

Room Nº:	Air Handling Unit:				ISO Class:
Room Name:					
TERMINAL REF	ACTUAL AIR (m^3/h)	DESIGN AIR (m^3/h)	ROOM VOLUME (m^3)	DESIGN ROOM ac/h	ACTUAL ROOM ac/h
1					
2					
.....					
n					
TOTAL					
Acceptance criteria achieved, write clearly **YES** or **NO**					
Completed by:	Date:	Accepted by:		Date:	

The acceptance criteria are a matter of consent between the customer and the supplier. The object of the test is to verify if the specified number of air changes per hour have been achieved and if the airflows for the room have been met (this is usually within the limits of ±10-15%)(**Table 9**).

Table 9: Table with recommended tolerances limits of measured airflow, Reference BSBTC-01 Appendix M

System type	Performance effect	Terminal	Branch	Total air flow
Mechanical ventilltion, comfort cooling	Low	+20% of design value	+10% of design value	+10% of design value
Process air conditioning, Escape route pressurisation	Medium	+15% of design value	+8% of design value	+10% of design value
Close control air conditioning	High	+10% of design value	+5% of design value	+5% of design value
Notes: Where the supply flow tends towards a tolerance limit, the associated extract flow should be regulated towards the same limit. Tolerance shown for terminal are those accumulated during terminal and sub-branch regulation.				

It must be noted that the flow hood recordings are not always accurate and that is why many manufacturers provide a table of the *k* (correction) factor, which is directly influenced by the airflow rate. The calculation of the *k* factor should be taken into consideration whilst performing measurements. This is affected by the air passing through, the dimension of the diffuser, the shape of the diffuser, whether a terminal filter is installed, etc.

Example:

Cleanroom with area of 87.4m^2 and height of 2.8m is designed to achieve 14ac/h. At the ceiling are 6 terminal filters which bring conditioned and filtered air from one central Air Handling Unit located at the building plant room. Each terminal that is designed to achieve the required classification and microclimate conditions is estimated to supply 600m^3/h of air (**Table 10**).

Room volume: Floor area x Room height = 87.4 x 2.8 = 244.72m^3

Table 10: Table with calculation of the percentage of the Actual Air entering within the cleanroom

Terminal Ref.	Design Air (m^3/h)	Actual Air (m^3/h)	Percentage of Design (%)
1	600	605	100.83
2	600	627	104.50
3	600	573	95.50
4	600	597	99.50
5	600	612	102.00
6	600	622	103.67
TOTAL	3600	3636	101.00

Note: Percentage of design (%) calculation: Actual Air/Design Air x 100

$$\textbf{Actual Air changes/hour} = \frac{\sum \textit{Room TOTAL Actual Air}\ (\mathrm{m^3/h})}{\text{Room Volume}\ (\mathrm{m^3})} = \frac{3636}{244.72}$$

Actual Air Changes/hour = 14.85(1/h)(see **Table 11**).

Table 11: Calculation for Actual Air flow and Actual room air-changes

Room Nº:	Air Handling Unit:				ISO Class: 8
Room Name:					
TERMINAL REF	ACTUAL AIR (m^3/h)	DESIGN AIR (m^3/h)	ROOM VOLUME (m^3)	DESIGN ROOM ac/h	ACTUAL ROOM ac/h
1	605	600	244.72	14	14.85
2	627	600			
3	573	600			
4	597	600			
5	612	600			
6	622	600			
TOTAL	3636	3600			
Acceptance criteria achieved, write clearly **YES** or **NO**					*YES*
Completed by: xxxxxxxxx	Date: xxxxxx	Accepted by: xxxxxxxxx		Date: xxxxxx	

Supply airflow rate calculated from filter face velocity

To meet ISO 14644 measurements of the airflow velocity should be at approximately 150mm to 300mm from the filter face (it should be noted that for the FDA Guide to Aseptic Processing the location should be risk assessed by the use and to meet EU GMP, the measurement should be taken at the 'working height'). In order to comply with these different standards additional measurements may need to be taken).

According ISO 14644-3:2005 the number of measuring points should be sufficient to determine the supply airflow rate in cleanrooms and clean zones. This should be the square root of 10 times the area in square meters. However, not less than 4 readings should be taken. At least one point per filter should be measured.

More positions for measuring are better because in that way is determining the uniformity of the air in front of the filter. For example, see **Figure 1** and **Table 12**.

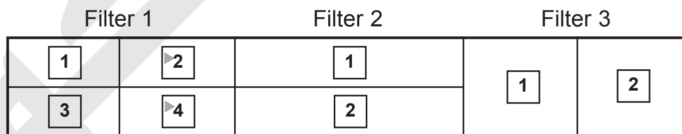


Figure 1: *Map of measuring points for measurements filter face velocity*

Table 12: Filter face velocity table

Room Nº:		Room Name: Cleanroom LAF		ISO CLASS	
Filter Serial Nº:			**Filter ID:**		
Filter Size: (mm) x (mm)			Filter Area = m^2		
Point	1	2	3	....	n
Velocity m/s	xx	xx	xx	xx	xx
Vavg =	$\frac{\text{Point 1+Point 2+Point n}}{\text{N}}$		= (m/s)		
$\dot{V}$1 =	Vavg x Filter Area x 3600		= (m^3/h)		
∑ ***TOTAL Air*** = $\dot{V}$*1* (m^3/h) + $\dot{V}$*2* (m^3/h) + $\dot{V}$*n* (m^3/h)					
*Acceptance criteria achieved write clearly **Yes** or **No***					
Completed by:	*Date:*	*Witnessed by:*		*Date:*	

Equipment used for airflow measurements:

- Flow hood – instrument capable of measuring total supply or extract air volume (**Figure 2**), the instrument may have various combinations of frame sizes and hoods and can therefore cover a large number of grilles or filter housing sizes. The instrument works on the Wilson grid principle of air measurement, and involves the collecting hoods gathering the full volume of air and passing it over the Wilson grid. The pressure readings taken across the grid are fed into the meter and this produces a direct readout of the volume.

- Vane anemometer and thermal anemometer (**Figure 3**).

- Pitot tube is a pressure measurement probe used to measure fluid velocity, hence fluid flow (**Figure 4**). Pitot tube is a tube within a tube and has two ports named total port (total pressure) and static port (static pressure). The difference between total and static pressure is dynamic pressure ($P_t – P_s = P_d$) from which the velocity can easily be calculated ($P_d = pv^2/2$), i.e. velocity v = sir ((2 P_d/p)).

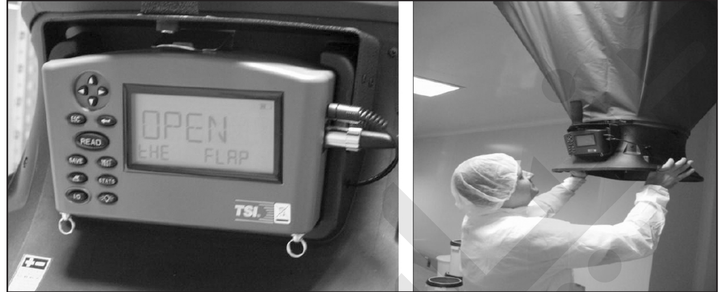


Figure 2: *Micromanometer attached to the flow hood (left), measuring supply terminal with the flow hood within cleanroom (right).*

Figure 3: *Vane anemometer (left), Thermal anemometer (right).*

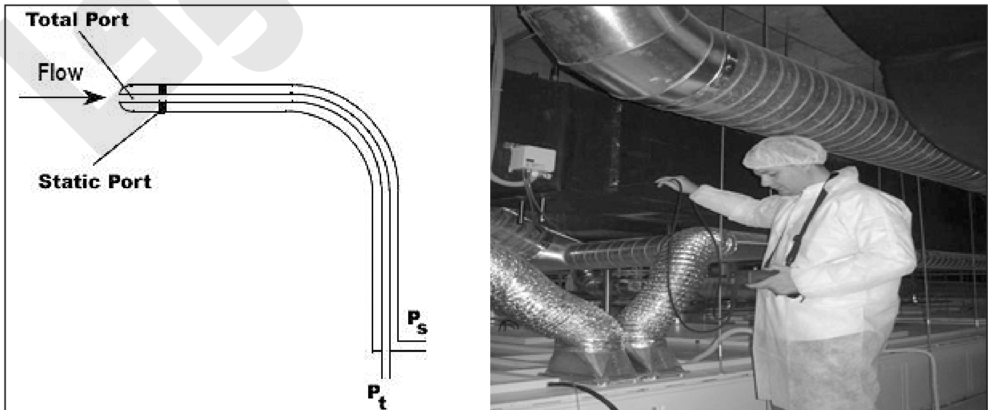


Figure 4: *Sketch of the Pitot tube (left); measuring supply air at the main branch ductwork with Pitot tube (right).*

Duct airflow Pitot traverse commissioning test

Method for rectangular duct (**Table 13**).

For Duct airflow, Pitot traverse test velocity measurement locations are determined by using Log – Tchebycheff rule, creating matrices of measuring points. The air velocities within the rectangular duct are measured using a Pitot tube and using the measuring points to obtain an average velocity which is converted to the air volume from a knowledge of the cross sectional area of the duct.

Table 13: Measuring positions in rectangular duct as proportion of a duct size (mm)

Points per traverse	Duct proportion
5 points	0.074, 0.288, 0.5, 0.712, 0.926
6 points	0.061, 0.235, 0.437, 0.563, 0.765, 0.939
7 points	0.053, 0.203, 0.366, 0.5, 0.634, 0.797, 0.947

Method for round duct (**Table 14**).

If the duct is round, then the measuring is performing by establishing areas of equal volume across the duct, and if possible, but not always practical, where all points are taken on 3 diameters by dividing the area into 6 parts of 60°.

Table 14: Measuring positions in round duct as proportion of a duct diameter (mm)

Number of areas across duct	Distance from the wall of the duct
3 areas	0.032, 0.135, 0.321, 0.679, 0.856, 0.968
4 areas	0.021, 0.117, 0.184, 0.345, 0.655, 0.816, 0.883, 0.979
5 areas	0.019, 0.076, 0.153, 0.217, 0.361, 0.639, 0.783, 0.847, 0.924, 0.981

Example:

Supply Duct Size: 800mm x 600mm.

Duct traverse of 30 points (5 x 6), where at the side of 600mm will be measured 5 points from the wall of the duct, i.e. side with length of 800mm will be measured 6 points from the wall of the duct.

Using **Table 13** *and also see* **Table 15** on the following page*:*

	Distance from the wall of the duct in millimetres					
5 points	0.074 x 600	0.288 x 600	0.5 x 600	0.712 x 600	0.926 x 600	
6 points	0.061 x 800	0.235 x 800	0.437 x 800	0.563 x 800	0.765 x 800	0.939 x 800

It is essential for measuring the velocity using Pitot tube or any other probe within the rectangular and round ducts that the measuring location must be at the place where the air will be less turbulent, i.e. on the straight part of the duct where it can be proved that the minimum requirement for the location is to be 1-2 lengths or diameters beyond the measurement site and at least 5-6 lengths or diameters after any disturbance of the ductwork such as elbows, t-parts, damper, etc.

Also, measuring within the ductwork is only satisfactory as long as reasonably high numbers of measurements are taken.

7.4.6 Differential pressures

In order to maintain air quality in a cleanroom, the pressure of a given room must be greater than a room of a lower grade. This is to ensure that air does not pass from "dirtier" adjacent areas into the higher grade cleanroom. Thus the differential pressure is one of the main characteristics to be

Table 15: Example table where the results from the measurements should be written

HVAC SYSTEM: **xxxxxx**	TRAVERSE LOCATION: **xxxxxxx**
DESIGN AIRFLOW VOLUME: **xxxx**	DUCT SIZE: **800mm x 600mm**
TRAVERSE READINGS	

H = 600

1	6	11	16	21	26
2	7	12	17	22	27
3	8	13	18	23	28
4	9	14	19	24	29
5	10	15	20	25	30

W = 800

∑ =

ID	m^3/h	ID	m^3/h
1	Xx	16	Xxx
2	Xx	17	Xx
3	Xxx	18	Xx
4	Xx	19	Xxx
5	Xxx	20	Xxx
6	Xx	21	Xxx
7	Xx	22	Xxx
8	X	23	Xxx
9	Xx	24	X
10	X	25	Xx
11	Xx	26	X
12	X	27	Xxx
13	Xx	28	Xx
14	X	29	X
15	xxx	30	xx

Average Air Flow (m^3/h):	
Percentage of Design (%):	
Static Pressure (Pa):	
Comments:	
Completed by:	Date:
Checked by:	Date:

considered in every cleanroom. Usually cleanrooms are exposed to a higher pressure than the pressure of their surroundings. Very often a differential pressure is required between different cleanrooms (a cascade of differential pressures). This will depend upon the different cleanliness levels required.

This test is conducted in order to prove that the air system is capable of maintaining differential pressures between cleanrooms and their surroundings in a given period of time. During the measurements of the differential pressure, all doors which belong to the inspected area must be closed.

Cleanroom differential pressures are normally set at between 5Pa and 25Pa as this allows doors to be opened and overcomes problems for cleanroom operators in relation to the high pressure difference, which arises due to air leakage. Air leakage arises due to ‘gaps’. Such gaps are mainly due to the uneven floors and the impossibility of a door gasket to entirely cover an uneven surface. Furthermore, if certain parts of the cleanroom have not been siliconised, such irregularities can increases the ‘air flow’ noise and reduce the comfort for the operators working inside the cleanroom.

The differential pressure check is conducted after any air balancing of the cleanrooms has been completed. Some rooms/areas are more or less pressurised than the others and by comparing the differences, differential pressure is determined. In order to attain the required room pressure, it is necessary to determine the volume of airflow entering the cleanroom and the volume of air leaving the cleanroom. For instance, in a perfectly sealed room the most simple explanation is when, for example, a cleanroom has $1000m^3/h$ air entering the room, measured with an instrument fixed on the distributive element and $1000m^3/h$ air leaving the room, also measured at the distribute element at the exit. With this example, the cleanroom is neither positively nor negatively pressurised. In contrast, where the supply air volume in the cleanroom is higher than the air volume which is extracted from the cleanroom, that room is positive pressurised. Conversely, where a room has a negative pressure this means the supply air volume in the cleanroom is lower than the extracted air volume. In reality, such ideal conditions are not always achieved because the extracted air (treated or untreated) from the room does not always pass through diffusers and grilles and often leaks through the gaps which are existing within one cleanroom. In short, all cleanrooms leak to a certain extent. What matters is the size of the leak and can the required pressure differential be maintained?

A leak can be calculated as follows:

$$\dot{V} = \sqrt{\frac{\Delta p \ x \ 2}{\rho}} x \ A \ x \ \mu$$

Where:

- $\dot{V}$ = volume flow in m/s
- Δp = pressure drop in Pa
- P = specific gravity in kg/m
- A = leakage area in m^2
- μ = discharge coefficient 0.72 (dependent on geometry)

Example:
To calculate leakage volume flow between two cleanrooms belong to primary and secondary packaging where given leakage area and pressure difference between the two cleanrooms:
Supply Air Volume flow (primary packaging) = $3636m^3/h$
Leakage area = $0.02m^2$
Pressure difference between cleanrooms = 15Pa

To be calculated:
Leakage volume flow V_{leak} (m^3/h)
Exhaust air volume (m^3/h).

$$\dot{V} = \sqrt{\frac{15 \ x \ 2}{1.2}} x \ 0.02 \ x \ 0.72$$

Leakage volume flow $V_{leak} = 0.072m^3/s$ i.e. $259m^3/h$
Exhaust air volume = Supply air volume flow – Leakage volume flow = 3636 – 259 = $3377m^3/h$

The instruments used for determining the differential pressures are the electronic micromanometer, the inclined manometer, or other differential pressure gauges.

Cleanrooms should be equipped with differential pressure gauges which enable continuous monitoring of the differential pressures and provide an opportunity to the persons in charge of monitoring the performances of the cleanroom to react in case of a drop in pressure.

Most cleanrooms are fitted with magnahelic gauges which serve for measurement of the differential pressures between two rooms. A magnahelic gauge has two ports: one port is located within the cleanroom with higher pressure (plus (+)) and one located in the cleanroom which is designed to have lower pressure (minus (–)). The difference between these two cleanrooms gives the differential pressure. Depending on the accuracy of the magnahelic gauge, which is based on its scale, the pressure differences can vary considerably. When the range is wider the instrument is less sensitive.

The digital manometers for differential pressures are significantly better than magnahelic gauges as they can be more easily fitted, be located in areas which allow easier maintenance and because they can provide continuous monitoring. Such devices can be set in the cleanrooms, on the technical floor above each room or at any other suitable place. Normally these manometers are connected to a BMS (Building Management System) where the cleanroom user has a clear display of the overall state of the system and the differential pressure influence between the rooms.

Both instruments should be maintained and calibrated at annual intervals.

Before commencing pressure measurement, the following should be checked to ensure that:

- All HVAC systems of the cleanroom facility are in continuous operation.
- The supply air volume balancing is within specification before commencing the measurement of differential pressure between rooms or between rooms and the outside area.
- All doors in the facility are closed and no traffic is allowed through the facility during the test.

Once this is checked, the measurement continues. The service engineer should:

- Adjust the manometer to give a reading of zero in accordance with the manufacturer's instructions.
- Connect one end of the tubes to the higher pressure side of the manometer and place the other end of the tube in the area of higher pressure such that it is free from obstruction and directed away from any airflow. Alternatively, a reading can be taken within the room with higher pressure whilst leaving the unbiased (+) port on the manometer.
- Next, connect one end of the second tube to the lower pressure input side of the manometer and place the other end of the tube in the area of lower pressure such that it is free from obstruction and directed away from any airflow. If the tubing passes through a surface ensure that any gaps surrounding the tube are sealed.
- The measurement should be continued until the pressure difference between the last enclosure and surrounding ancillary environment and against the external environment is measured.

The table for recording results should contain all the information regarding the room being tested (the occupancy state, class, specified differential pressure, the equipment used for this purpose and information about the calibration certificate of the instrument used during the measurement).

As an example, **Tables 16, 17** and **Figure 5**, on the following pages, show the cleanness level according to ISO 14644-1 and also the pressure of the determined room pressure for some cleanrooms. They also show that a differential pressure exists between the room pressure and the ambient pressure. The pressure of the room M169 is 65Pa and it is maintained through automatic controlled dampers, that are part of the ductwork installation.

The following differential pressure between the cleanrooms is considered as acceptance criteria:

- The acceptance criteria will be ≥5Pa between areas of the same ISO Class according to ISO 14644-1[3].
- The acceptance criteria will be ≥15Pa between areas with different ISO Class according to ISO 14644-1[3].

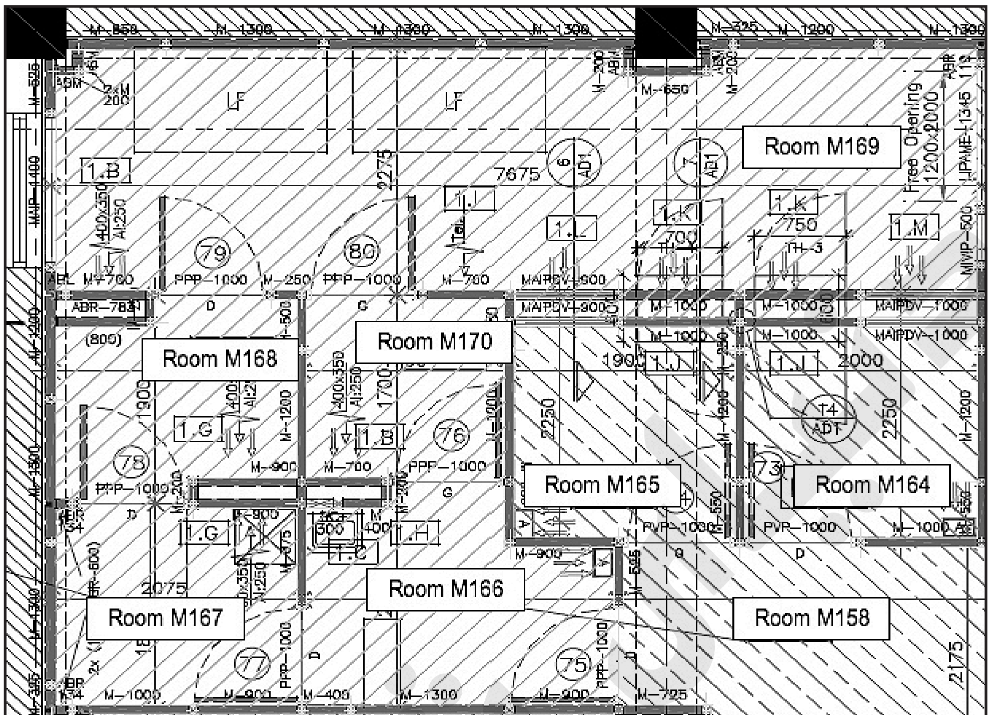


Figure 5: *Map of a facility showing the differential pressure cascade between cleanrooms.*

Table 16: Rooms from Figure 5 displayed with ISO classifications and specified room pressures

Room	Class according ISO 14644-1	Specified Pressure (Pa)
M158	ISO CLASS 8	15 (Pa)
M164	ISO CLASS 8	15 (Pa)
M165	ISO CLASS 8	15 (Pa)
M166	ISO CLASS 7	30 (Pa)
M167	ISO CLASS 5	45 (Pa)
M168	ISO CLASS 5	55 (Pa)
M169	ISO CLASS 5	65 (Pa)
M170	ISO CLASS 5	50 (Pa)

Differential pressure table:

Table 17: The rooms from Table 2 are displayed with specified and actual differential pressures

Pressure from (room number)	ISO Class 14644-1	Pressure to (room number)	ISO Class 14644-1	Specification dp[Pa]	Actual dp [Pa]	PASS/FAIL
M169	ISO Class 5	M168	ISO Class 5	≥5Pa	7	Pass
M169	ISO Class 5	M170	ISO Class 5	≥5Pa	10	Pass
M168	ISO Class 5	M167	ISO Class 5	≥5Pa	9	Pass
M170	ISO Class 5	M166	ISO Class 7	≥15Pa	16	Pass
M166	ISO Class 7	M158	ISO Class 8	≥15Pa	15	Pass

Very often within cleanrooms differential pressures are out of the specification. Certain checks must be done such as:

- Check if the doors are properly closed
- Check that the gasket under the door is properly closed, ie. was the room properly sealed before this discrepancy happened
- Check if something is blocking any supply or extract point within the room
- Check the neighbouring rooms if there is anything unusual with the pressures
- Check and compare with the previous measurements the supply airflow for the cleanroom
- Check and compare with the previous measurements the supply airflow for the air handling unit
- Check that the dampers on the extract are in the same position as during the commissioning time
- Check the frequency of the unit
- Check the set point of the velocity sensors, or the differential pressure sensor located in the supply duct
- Check the pressure drop through the filters on the air handling unit and terminal filters, if any

7.4.7 Temperature and humidity test

The purpose of this test is to demonstrate the capability of the installation's air-handling system to maintain the air temperature and humidity level within the control limits and over the time period specified in the user requirement specification. ISO 14644-3 has adapted the test methods for temperature and humidity tests from IEST-RP-CC006.3:2004, *Testing Cleanrooms, IEST.*

Temperature scales

The temperature scale must first be defined. For most temperature measurements the Celsius scale (°C) is used. Within the US the Fahrenheit scale is used. A third scale is named in honour of the Scottish physicist, Lord Kelvin (Kelvins scale) who first defined the scale, whose symbol is K.

(0° K is equal to – 273.15 °C which represents absolute zero, or – 459.68 °F).

General temperature test

This test is conducted when the room is in *as built* occupancy state. This test is started after the air-conditioning system has been operated and the conditions have been stabilised. The temperature should be measured at a minimum of one location for each temperature controlled zone. Each sensor should be placed at the designated location at operational height (usually 900mm). If there are several rooms in which this parameter should be measured, sufficient time is allowed for the sensor to stabilise and then the temperature reading at each location should be recorded.

Comprehensive temperature test

This test is usually conducted in *at rest* or *operational occupancy state* and is recommended in areas having strict environmental specifications (for EU GMP this is a minimum of Grade A and Grade B clean areas). As in the general method, the air-conditioning system should be operated and the conditions within the cleanroom stabilised. The work zone should be divided into a grid of equal testing areas. For this test there should be at least two locations. The temperature probe should be positioned at the operational height and at a distance of no less than 300 mm from any part of the installed cleanroom.

The measurement time for both the general temperature test and the comprehensive temperature test is at least five minutes, with one value recorded at least every minute.

Humidity is the term for the amount of water vapour in the air and may be defined as absolute humidity, relative humidity and specific humidity. For cleanrooms relative humidity is normally considered, which is expressed as a percentage and calculated with the following equation:

$$\phi = \frac{p_{(H2O)}}{P^{*}_{(H2O)}} x 100$$

Where:

- $p_{(H2O)}$ is the partial pressure of water vapour in gas mixture
- $P^{*}_{(H2O)}$ is the saturation vapour pressure of water at the temperature of the gas mixture
- ϕ is the relative humidity expressed as a percentage

Relative humidity is not meaningful without data for the air temperature, as it is directly linked to the temperature i.e. is amount of water vapour in the air at a specific temperature compared to the maximum water vapour that the air is able to hold without it condensing.

The humidity test should be performed in conjunction with the temperature test. Before test commences, the HVAC system must be balanced according the user requirements, and be fully operational. One location point is enough for each humidity control zone which has to take at least 5 min per location, which should allow sufficient time for the sensor to get stabilised.

The acceptance criteria for this test should be those specified by the cleanroom user. An example test table form is shown in **Table 18**.

Table 18: Table to insert the values from the measurements

Room Number	Design Temperature (ºC)	Actual Temperature (ºC)	Pass/Fail	Design Relative Humidity (%)	Actual Relative Humidity (%)	Pass/Fail
xxxxxx	22±3	*22*	Pass	30 – 65	*56*	*Pass*
Completed by:		Date:		Accepted by:		Date:

The minimum measurement test resolution for the apparatus stated within the ISO 14644-3 should be 1/5 of the allowable temperature/humidity range for the difference between the set point and the permissible range of variation allowed from that set point. Instruments used for measuring temperature and humidity should have an accuracy appropriate to the measurements as stated in ISO 7726 (**Table 19**).

Typical test sensors are:

Table 19: List of instruments for measuring temperature and humidity

Temperature	Humidity
Thermometers	Dielectric thin film capacitor humidity sensor
Resistance temperature devices	Dew point sensor
Thermistores	Psychrometer

7.4.8 Filter leakage test

Principle

This test is conducted in order to confirm that the filter system is properly installed and that leaks have not developed during use. The test verifies the absence of leakage, relevant to

the cleanliness performance of the installation. The test is performed by introducing an aerosol challenge upstream of the filters and scanning immediately downstream of the filters and support frame. The test is a leak test of the complete filter installation comprising the filter media, frame, and gasket. This test will be applied to cleanrooms and clean air devices in “as built” or in “at rest” occupational states, and will be undertaken also when commissioning new cleanrooms, installations which require retesting, or after the final filters have been replaced.

High efficiency filters

Currently there are two types of high efficiency filters known as HEPA (High Efficiency Particulate Air) or ULPA (Ultra Low Penetration Air). Velocity measured on the downstream for these filters should be around 0.45m/s and the pressure fall from 120Pa to 220Pa. The filters are designed to control the number of particles entering a clean area by filtration. HEPA filters function through a combination of three important aspects. First, there are one or more outer filters that work like sieves to stop the larger particles of dirt, dust and hair. Inside those filters, there is a concertina – a mat of very dense fibres – which traps smaller particles. The inner part of the HEPA filter catch particles as they pass through in the moving air stream. There are different grades of HEPA filters based on their efficiency ratings, these are shown in **Table 20**.

Table 20: Classification of HEPA filters according to EN 1822[1]

Filter Class	Overall Value Efficiency (%)	Overall Value Penetration (%)	Leak test efficiency (%)	Leak test penetration (%)
H10	85	15	–	–
H11	95	5	–	–
H12	99.5	0.5	–	–
H13	99.95	0.05	99.75	0.25
H14	99.995	0.005	99.975	0.025
U15	99.9995	0.0005	99.9975	0.0025
U16	99.99995	0.00005	99.99975	0.00025
U17	99.999995	0.000005	99.9999	0.0001

The type of filter purchased depends upon the requirement of the facility. Most pharmaceutical manufacturing facilities use H13 or H14 HEPA filters.

When specifying filters special attention should be given to the gasket, of which there are different types (**Figures 6, 7**).

- The neoprene rubber gasket is between 6 and 8mm thick to suit the filter frame. The gasket is pressed to the box and serves to prevent a contaminated air leak. In most cases this method is successful but in case of a small deficiency in the box design or if the gasket is too tight or not tight enough and if its quality is not high, the leaking of contaminated air will be easily registered.
- The gel gasket provides ideal packing and guarantees impermeability, ie. preventing contamination of the cleanroom with unfiltered air through a filter frame. The frame is a channel filled with gel while the box designed for this purpose and the part connected to the frame have the shape of a knife which when in contact enables the gel to entirely fill the box and which in turn does not allow any space for unfiltered air leak.

Figure 6: *Filter gaskets, neoprene rubber gasket (left), and gel gasket (right).*

Necessary equipment for conducting tests

In order to start the test, the following equipment is required:

Injection port

An injection port serves for injecting an aerosol which is then mixed with the air that should flow into the high efficiency filters (**Figure 7**).

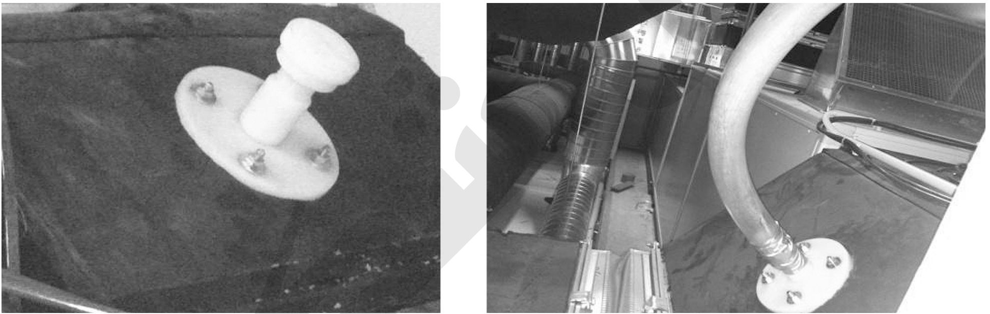


Figure 7: *Injection ports.*

Sampling port

Each filter to be tested needs to have a sampling port (**Figure 8**). This port is for confirming the upper concentration of aerosol. The port is installed within the cleanroom, except for terminal filters, where the port is located in the cleanroom area and it is connected with the upper side of the filter box, but in case the high efficiency filters are installed depend on the area can be used more than one port to confirm that concentration of aerosol is equally spread before filter.

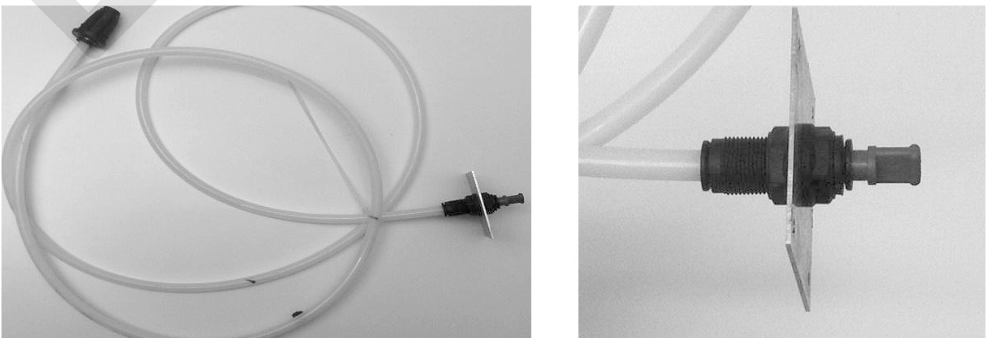


Figure 8: *Sampling ports.*

Thermal generator with positive injection pump

Thermal generators (**Figure 9**) use inert gas such as carbon dioxide (CO_2 at 7 bar) and oil (Shell EL Ondina Oil at 316°C) infused into a heated handling unit where the oil evaporates. When leaving the generator, the oil condenses as a fine aerosol composed of particles of 0.1-1µm. There are different manufacturers of thermal generators who can produce from 1g/min up to 20g/min of aerosol depending on the need.

Figure 9: *Connected positive injection pump and thermal aerosol generator.*

Aerosol photometer

An aerosol photometer (**Figure 10**) is an instrument without which it is impossible to measure the integrity of the High Efficiency Filters. The aerosol photometer has to be fixed in a way to be able to measure the concentration of aerosol in front of and behind the filter. A sample of the air is taken at the filter downstream with a fish-tail probe through which the air is distributed through silicon tubes to the instrument in the part where the light is concentrated. When particles pass through this part, they reflect the light and this light passes through lenses and through a photomultiplier where the light is converted into an electric signal. The result is the higher the number of particles the stronger the signal. The photometer usually measures the concentration of aerosol from 0.0001µg/l to 100µg/l which means that this instrument reads not the number of particles but their weight.

Figure 10: *Aerosol photometer.*

Particle counter

A particle counter can be used during the test for filter integrity. This method is very sensitive because the particle counters take an air sample in a given time interval which means that depending on the filter dimensions it might happen the counting to stop which causes the process to continue, adjusting the counter, from the place where the counting occurred the last time.

This method has several weaknesses because aside from the limited time interval, the concentration of aerosol in front of the filter might be much higher than the range of the instrument which might cause contamination of the instrument itself.

The test for filter integrity with a particle counter is given in Annex B6 in ISO 14644-3 and will not be discussed further in this chapter.

Methods

There are several methods for testing the filter integrity which are incorporated as a standard in ISO 14644-3. These are the generally accepted methods for testing filter integrity. Before testing, the following requirements must be met:

- The filters have to be installed and balancing the airflow has to be finalised
- There must be free access to the filter during the test
- There must be no visible damage of the filter (see **Figure 11**).

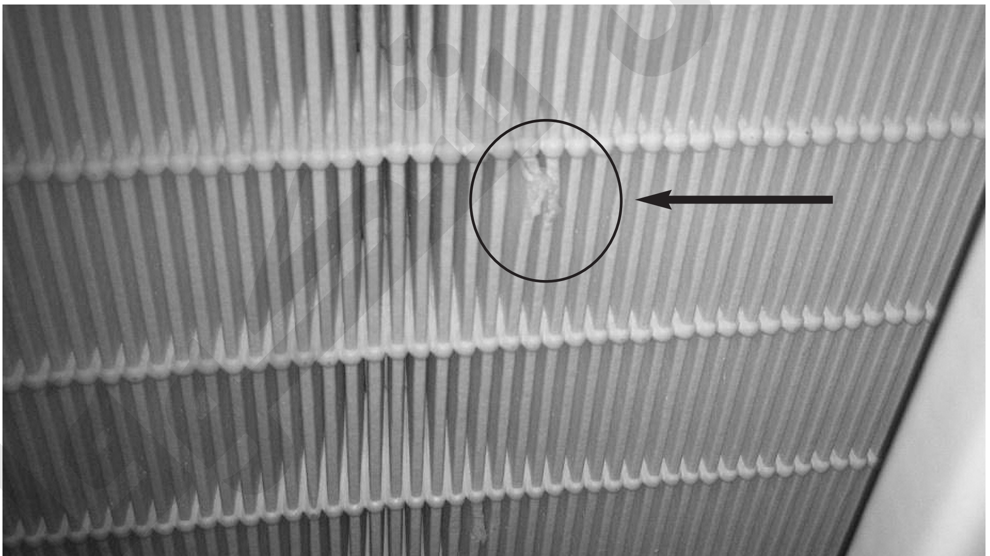


Figure 11: *Damaged filter media on H14.*

After the particle integration has started, it needs to be proved that the concentration of particles in front of the filter, confirmed at several points, is within the limits of ±15%. This is required because the inequality of the concentration of particles sometimes causes irregularities which are explained in more detail below. Furthermore, ISO 14644-3[5] advises that it is best practice to keep the concentration of particles around ±15% from the starting concentration during testing.

The integration of an aerosol is usually done in a plant room where the units for air distribution are situated because this enables sufficient time for the aerosol to mix with the remaining air which is then brought to the cleanroom.

However, adjusting the concentration of particles in front of the filter is considered a priority during the filter integrity test. For this, ISO 14644-3[5] recommends a concentration between 10 and 100µg/l. The standard also indicates that a concentration below 20µg/l can reduce the sensitivity of the photometer, while a concentration above 80µg/l might be too high for certain types of photometer and will cause instrument damage as well as providing an incorrect result.

After initiating the concentration of particles of aerosol and after measuring the concentration in front of the filter, the concentration can be converted into percentage with a total value of 100% for reference purposes.

The next step is scanning the filter medium and the filter box, where the aerosol leakage is calculated as follows:

$$\textbf{Leakage (\%)} = \frac{Y}{X} \times 100$$

Where:

Y = the biggest leakage measured

X = measured value of the average concentration which represents reference particle concentration in front of the filter

There are two alternative measures: photometer and the Discrete Particle Counter (DPC) method.

To comply with 14644-3: for correlation with the acceptance criterion of the photometer method the maximum allowable penetration could be adopted to 0.01% for filters with an integral penetration of 0.05 and 0.005%.

For the Discrete Particle Counter, ref. ISO 14644-3:2005[5], Section B.6 method standard leak penetration P_L is chosen by agreement between the customer and the supplier, or can be based according to **Table 21** and is a function of K and P_S.

$$P_L = K \times P_S$$

Table 21: Factor K in function of P_s

Maximum allowable penetration	$\leq 5 \times 10^{-4}$	$\leq 5 \times 10^{-5}$	$\leq 5 \times 10^{-6}$	$\leq 5 \times 10^{-7}$	$\leq 5 \times 10^{-8}$
Factor K	10	10	30	100	300

Where:
P_S = the maximum allowable integral MPPS (Most Penetrating Particle Size) penetration of the filter to be tested as specified by the manufacturer.
K = ????????????????????
The usual method for filter scanning is with a photometer or a particle counter; the part being scanned is the gasket of the filter with the filter box and the filter medium (**Figure 12**). It is recommended to start the test by scanning the gasket because that is the place where a leakage is most likely to occur and because this will indicate if leakage comes from the medium or is due to an inefficient gasket. Once the scanning of the frame/the part that serves as gasket is complete, the scanning of the filter medium follows.

When scanning a filter medium, the probe should be at 5-30mm distance and the scanning done by overlapping the movements with a velocity defined by the ISO 14644-3 standard.

The size of the probe should be calculated considering the sample flow rate of the measuring instrument and the filter exit airflow velocity. The sampling probe is usually of a square or rectangular configuration. The probe dimension is expressed in centimetres (cm):

$$D_p = \frac{q_{va}}{U \times W_p} \quad \text{or} \quad \frac{cm^3/s}{cm/s \times cm}$$

Where:

- Dp is probe dimension parallel to the scan direction
- q_{va} is the actual sample flow rate of the measuring instrument
- is the filter exit airflow velocity
- W_p is the probe dimension perpendicular to the scan direction

It should be noted that: $1.2U \geq U_s \geq 0.8U$

Where U_s is the air velocity at the probe inlet:

$$U_s = \frac{q_{va}}{D_p \times W_p} \quad \text{or} \quad \frac{cm^3/s}{cm \times cm}$$

The scanning velocity with a probe is very important because a fast velocity of the movement of the probe might omit to register a leakage while a movement which is too slow might register an insignificant or inconstant leakage.

To determine the scanning velocity of the probe being used for the filter scanning:

$$\textbf{Probe scan rate } \mathbf{S_r} = \frac{\mathbf{15}}{\mathbf{W_p}} \text{ (cm/s)}$$

Where W_p is the probe dimension perpendicular to the scan direction.

The method prescribes that even if a leakage is registered the scanning still continues; the place where the leakage occurred is marked and the scanning continues throughout the filter surface. After the scanning has finished, places where a leakage has been marked are re-examined by slowly passing the probe over that marked places several times with the probe in order to confirm the maximum leakage and if that leakage is constant.

During this test one of the problems that might occur is if during the scanning of the filter medium there is a significant leakage of the gasket, causing a dispersal of a large concentration of particles through the filter medium. In such circumstances it will be difficult for the examiner to find the actual location where the filter leaks. A baffle plate is used for this purpose which is fixed at the filter frame and the testing continues.

When the testing is finished, a report followed by an opinion about the results of the test should be prepared (**Figure 13**).

Figure 12: *Measuring an air filter for leaks using a fish-tail probe.*

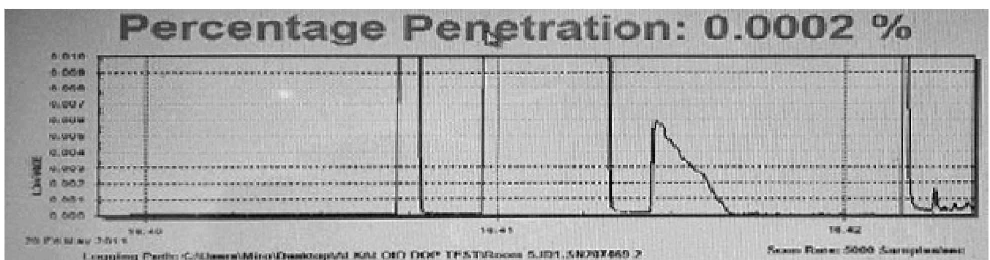


Figure 13: *Example of record from photometer software for filter leakage test.*

Leakage repairs

The ISO 14644-3 standard allows repairs of any part of the filter to be made provided that this is acceptable to the client. Procedures used for filter repairs may be made using procedures agreed between the customer and supplier. After a repair, a filter needs to be retested for leaks using the defined method.

Overview of the test report

The results of the test for filter integrity should include:

- Information on the location where the filter has been tested
- Filter related information (serial number, dimension, class, type, manufacturer)
- Information about the equipment used (serial number, calibration certificate)
- Results of the measurements (the picture shows a software overview of the test)
- Comments (after the measurement, an opinion is given if the filter passed or did not pass the test)
- Signature of the examiner and the person present as a witness during the testing

Three examples of filter leak testing are provided below. In each example:

- The testing method is in accordance with ISO 14644-3:2005
- The air balancing was completed and the air flow is identical in each of the three examples (0.5m/s)

Example 1

What is characteristic for this example is that the air **sampling port (SP)** is at the immediate proximity to the place where the aerosol is injected. **Figure 14** shows that the concentration of aerosol has a maximal value at the beginning when the aerosol is injected but there are parts where the concentration is low.

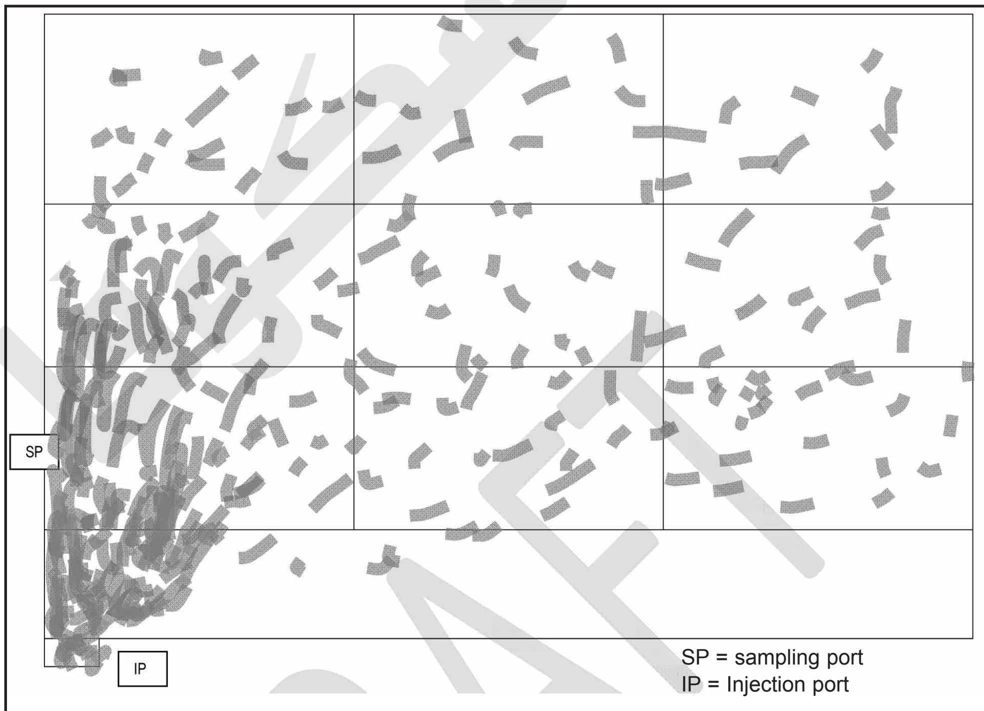


Figure 14: *Concentration of aerosol after the aerosol has been injected, showing areas where the concentration is low.*

When taking a sample of air through the SP, the concentration is 40μg/l and considered as reference and within the limits set by the standard. During the measurement the maximal concentration registered is 0.0034% of the filter medium and 0.0022% of the filter gasket. In both cases the concentration is lower than 0.01% which shows that the filter meets the requirements set by the standard and that the result is filter PASS.

Example 2

What distinguishes this example from the previous one is that the air **sampling port (SP)** is embedded crosswise compared to its previous position, although again only one port is required as the reference. The input for aerosol injection remains at the same place so once more the maximal value of the concentration of aerosol is largest at the beginning where the aerosol in injected. By taking a sample of air from the SP the aerosol generator can be adjusted in order to generate a similar concentration to Example 1, which this time is 38μg/l, only 2μg/l less than with Example 1. This time 8 leakages are registered: 7 of the filter medium and 1 of the filter gasket (as indicated in **Figure 15**). This produces the following results:

1=0.0142%	2=0.0118%	3*=0.0510%	4=0.020%
5=0.0183%	6=0.0155%	7=0.0192%	8=0.0212%

* Leakage of filter gasket.

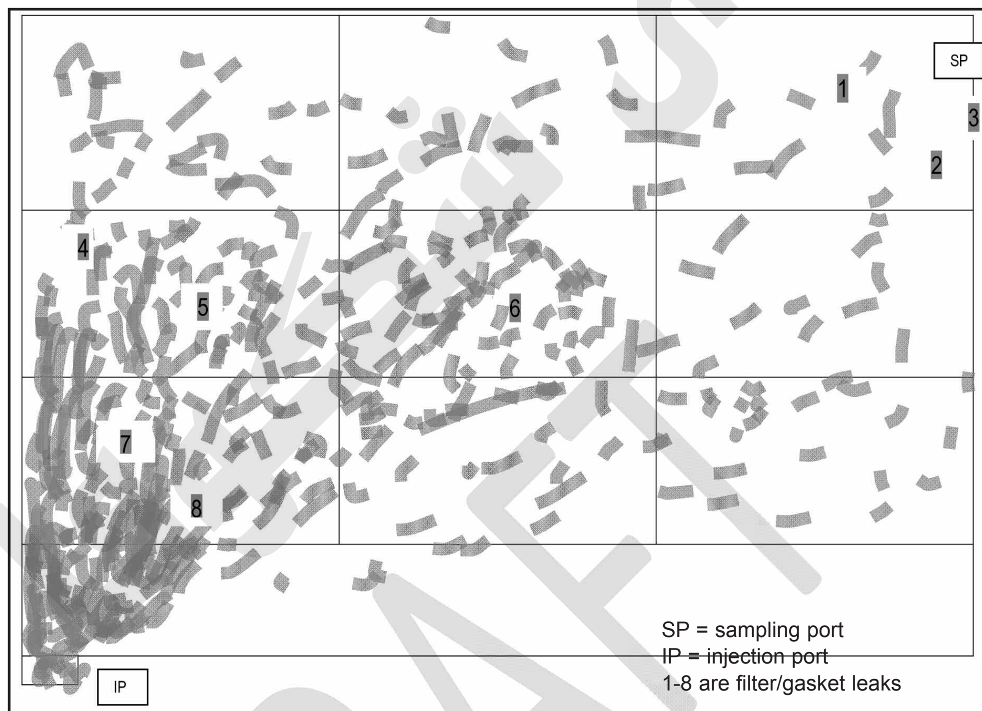


Figure 15: *Concentration of aerosol after the aerosol has been injected, showing several areas where leaks appear due poor dilution of aerosol with the air.*

Example 3

In this example, ten air **sampling ports (SP)** are integrated. A second modification is that the aerosol does not go directly to the filter medium but first passes through a sparge pipe which is integrated in a way to spread over the ceiling width, which in turn enables the aerosol to be well mixed with the air.

The procedure here starts with a check of the upper concentration by taking a sample of the air for each SP from 1 to 10 (see **Figure 16**). The results are as follows:

SP1=36µg/l	SP2=34µg/l	SP3=36µg/l	SP4=37µg/l	SP5=38µg/l
SP6=37µg/l	SP7=34µg/l	SP8=35µg/l	SP9=34µg/l	SP10=36µg/l

In this case, all samples taken from the upper concentration are within the limits with an acceptable variation of ±15%. These results are obtained via an additionally integrated perforated tube which enables the aerosol to be well mixed and dispersed all over the filter surface.

The lowest concentration measured at SP2, a value of 34µg/l, is considered as the reference concentration. The lowest concentration is taken in order to set a more sensitive reading for the instrument.

With Example 3, once the scanning was complete, three leakages were noted: two of the filter medium and one of the filter gasket, both of which are located at a same place as during the second testing. The other leakages registered during the second testing are not noted in this test. The results were as follows:

1*=0.0134% – medium 2*=0.0120% – medium 3*=0.0568% – gasket

Figure 17 is an example of how the report of these tests should be presented. Such a report needs to be prepared for each filter tested.

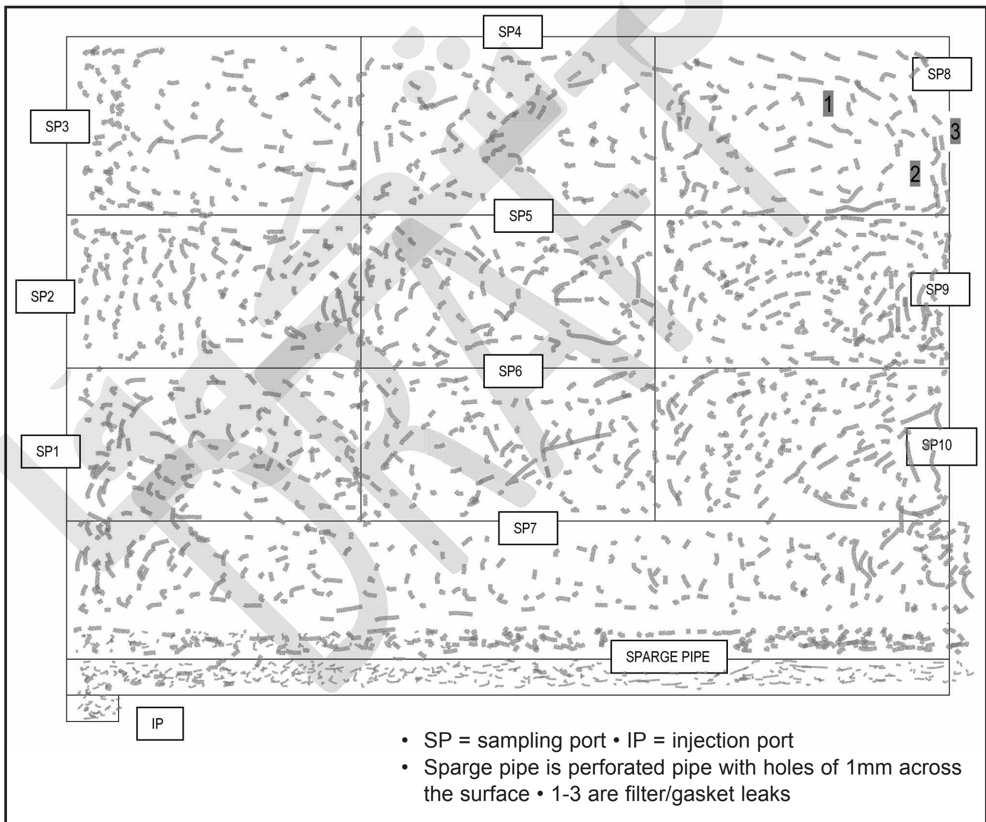
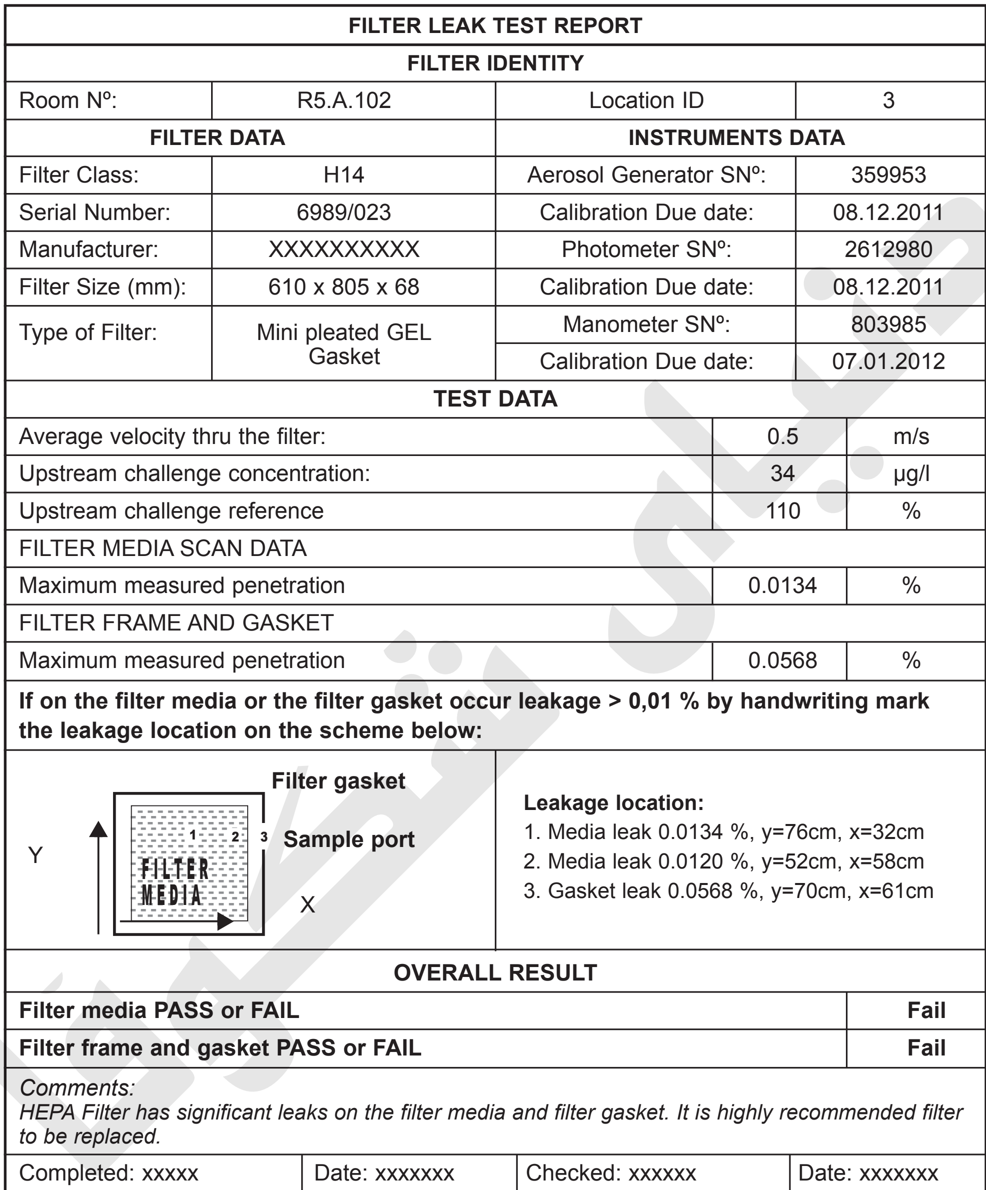


Figure 16: *Concentration of aerosol of well diluted air and aerosol by using sparge pipe and more sampling ports for each filter.*

Figure 17: Example of a test report for filter testing



FILTER LEAK TEST REPORT			
FILTER IDENTITY			
Room Nº:	R5.A.102	Location ID	3
FILTER DATA		**INSTRUMENTS DATA**	
Filter Class:	H14	Aerosol Generator SNº:	359953
Serial Number:	6989/023	Calibration Due date:	08.12.2011
Manufacturer:	XXXXXXXXXX	Photometer SNº:	2612980
Filter Size (mm):	610 x 805 x 68	Calibration Due date:	08.12.2011
Type of Filter:	Mini pleated GEL Gasket	Manometer SNº:	803985
		Calibration Due date:	07.01.2012

TEST DATA		
Average velocity thru the filter:	0.5	m/s
Upstream challenge concentration:	34	µg/l
Upstream challenge reference	110	%
FILTER MEDIA SCAN DATA		
Maximum measured penetration	0.0134	%
FILTER FRAME AND GASKET		
Maximum measured penetration	0.0568	%

If on the filter media or the filter gasket occur leakage > 0,01 % by handwriting mark the leakage location on the scheme below:

Leakage location:
1. Media leak 0.0134 %, y=76cm, x=32cm
2. Media leak 0.0120 %, y=52cm, x=58cm
3. Gasket leak 0.0568 %, y=70cm, x=61cm

OVERALL RESULT	
Filter media PASS or FAIL	**Fail**
Filter frame and gasket PASS or FAIL	**Fail**

Comments:
HEPA Filter has significant leaks on the filter media and filter gasket. It is highly recommended filter to be replaced.

Completed: xxxxx	Date: xxxxxxx	Checked: xxxxxx	Date: xxxxxxx

Conclusion

In most cases the high efficiency filter integrity tests are conducted according to the first or the second example. This is due to the unpreparedness of the designer or of the contractor to make ready the test area. Moreover, many examiners when confronted with a similar situation base their work on assumptions and previous experience so the test again is conducted following the first or the second example.

With regard to the first example, because the location of the air **sampling port (SP)** taken as the reference is very close to the place where the aerosol is being injected (which is not equally spread

throughout the surface), the real leakages might not be registered. The situation is different in the second example due to the location of the air **sampling port (SP)** and because of the unequal distribution of aerosol concentration throughout the filter surface, which results in leakages of four filters. This is because the concentration of aerosol is much higher in these filters than the one considered as reference. Both examples could potentially lead to damaged filters not being replaced and the air-cleanliness of the cleanroom being affected.

The third example is an example of best practise. It demonstrates that the concentration of aerosol, by integrating a perforated tube, is within the limits of ±15% throughout the filter surface. When a test is conducted in this way, both the examiner and the client can be reassured that the results obtained are accurate and that the damage is minor. The damage of the tested filter which did not meet the criteria set as a limit is probably due to the damage during transport or following installation. Considering the leakages of the medium and of the filter gasket which occur, it would be recommended not to repair the filter but to replace the filter with a new one. The new filter should also be tested following this method.

Other tests, such as the particle counting test, should not follow until the leak test gives acceptable results. If such a problem occurs during the cleanroom qualification or filter replacement, not only will other tests will be delayed, but the previous tests may need to be repeated in order to demonstrate consistency of the results.

7.4.9 Airflow visualisation test

The objective of this method is to confirm the airflow direction and its uniformity in a given room served by the Air Handling Unit (AHU) system. Before this test is started, it needs to be proven that the AHU system is balanced. The air velocity, the differential pressure and the temperature are parameters which influence the airflow within a cleanroom.

The following four methods for conducting this test are defined in the ISO 14644-3:2005 Section. B7:

Tracer thread method

To complete this test, streamers or threads or even tapes of music cassettes are used because of their large surface and lightweight. This method is good for confirming the airflow direction but is not used for other purposes because of the weight of the streamers which prevents them following the airflow.

Tracer injection method

This method is conducted by generating fog which shows the airflow direction throughout the area. When applying this method one should take care to isolate all smoke detection and fire alarm circuits within the suite including adjacent areas. Generating white visible fog at the critical locations (a critical location is defined as any area where product or material is exposed to the working environment), also includes the immediate boundary outside the critical area. The fog should demonstrate that there is no entrainment of air from areas outside of the critical area. The use of fog in cleanroom areas leads to increased contamination which is not permissible in certain industries. In such cases which are containment free, a water vapour fog may be used as an alternative. The problem with using this vapour fog is that it evaporates rapidly and becomes invisible to the human eye. In the first two cases a video camera should be used to record the airflow.

Airflow visualisation method by image processing techniques

This method is used to demonstrate quantitatively the airflow velocity distribution in the installation. This technique is based on tracer particle image processing techniques using computers.

Airflow visualisation method by the measuring of velocity distribution

When using this method, thermal or ultrasonic anemometers are used and the velocity at several locations determined for the testing is measured. What is characteristic for the airflow in a non-

regulated area is that airflow deviations occur, i.e. deviations in regard to its direction and volume at different locations and at a different time. This is due to the position of the distributive elements, the equipment, the current provoked by the convection of the operators and the equipment, the position of the lights, the windows, the walls, etc. Uncontrolled airflow in a given area is a complex issue which can be solved only by statistical methods.

When conducting this test it is important to ensure that an operator is not blocking the airflow being tested as this will cause disruption.

7.4.10 Particle Counting Test

The most important of all tests is the Particle Counting Test which is used for proving that the cleanroom functions are in conformity with the requirements and that it fulfils the set standards in terms of meeting its required classification.

A prerequisite for starting the Particle Counting Test is:

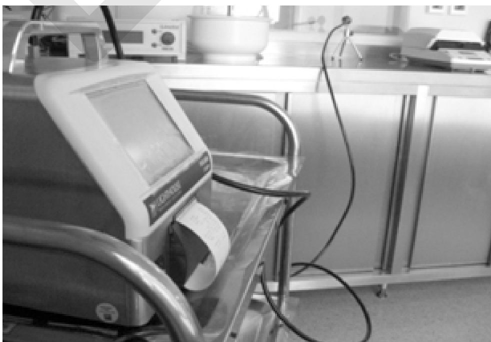
- The air system must be balanced and meet the required number of air changes per hour;
- The differential pressures between the cleanrooms and their surroundings must be confirmed;
- The filter integrity test must have been completed finished;
- The room being tested must be clean and ready for use.

After the prerequisites for starting the Particle Counting Test are fulfilled, the conditions in which the testing will be done are to be defined.

The measurements can be conducted in one of the following three occupancy states defined according to ISO 14644-1:

- As built — This is a state where the installation in the cleanrooms is set, connected and in function but the cleanrooms are not equipped and there is no staff.
- At rest — This is a state where the installation in the cleanrooms is set, connected with the equipment and in function according the requirements foreseen but there is no staff.
- Operational — This is a state where the installation in the cleanrooms is set, connected with the equipment and in function according the requirements foreseen with appointed staff.

Note: *As built* usually is a testing for just built cleanrooms; it is conducted only once because if the room is tested in *as built* state, every following test of the room should be in *at rest* or in *operational state*. Of these the operational state is the most challenging; it is normally a regulatory expectation that cleanrooms are tested in the operational state.



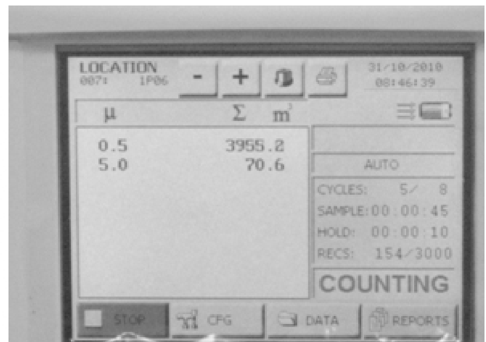


Figure 18: *Typical particle counter and display screen.*

Particle counters are used for the test (**Figure 18**). These instruments count airborne particles of different sizes by drawing in a known sample of air. Normally one cubic metre of air is sampled.

The particle counter is a basic and irreplaceable tool for determining a cleanrooms class. There are different types of particle counter from different manufacturers with a range from 0.1µm up to 25µm and a capacity from 2l/min up to 56l/min.

Methods

The particle count tests are conducted following the ISO 14644-1. This test is composed of three parts:

- Qualification test
- Test for determination of ultrafine particles (optional test)
- Test for determination of macro-particles (optional test)

Qualification test (description)

The ISO 14644-1 standard defines the cleanroom class based on the air particle concentration of a predetermined size and then requires that the room is measured for airborne particulates and for the measured particles to be compared against pre-set limits as outlined in the standard.

To qualify a cleanroom, it is necessary to take a certain number of samples of the air in the given room. The number of sample locations depends on the room's dimension; the bigger the room, the more air samples are taken.

Number of sample locations

ISO 14644-1 provides a formula for calculating the minimum number of sample locations:

$$N_L = \sqrt{A}$$

Where:

N_L = minimum number sample locations (a round number)

A = surface of the cleanroom (m^2)

Once the number of particle count locations is determined, samples should be taken at locations equidistant apart within the cleanroom. The ISO standard requires the air samples to be taken from locations at operational height.

Sample volume: the minimum volume of air to be taken as a sample should be determined for each location. ISO 14644-1 requires the volume of the air sample to be sufficient to count 20 particles from the biggest particle defined for a given class.

The following formula is used to calculate the minimum volume to be taken as a sample:

$$V_s = \frac{20}{C} \times 1000$$

Where:

V_s = minimum volume of a sample taken from a particular location (expressed in litres)

C = number of particles/m? for the biggest particle associated to the class being examined

20 = reflects the number of particles to be counted from the determined class

The minimum volume of each location should be 2 litres and, the minimum time for taking a sample being 1 minute. For measuring ISO class 5/Grade A environments, it is recommended that one cubic metre of air is sampled at each location.

If only one location is required, a minimum of three air samples should be taken from the room being tested.

Obtaining results
Results are recorded from each location and for each particle size being examined.

In case two or more samples are taken from one location, the average concentration of particles on that location for a given size should be determined. The calculation is done according to the following equation:

$$\bar{x}_i = \frac{\mathrm{X}_{i,1} + \mathrm{X}_{i,2} + \cdots + \mathrm{X}_{i,n}}{\mathrm{n}}$$

Where:

$\bar{x}_i$	=	average value of the particle concentration in a given location, which represents any location
$\mathrm{X}_{i,1} - \mathrm{X}_{i,2}$	=	concentration of particles from individual samples
n	=	number of samples taken from the given location

Necessity of calculating an Upper Confidence Limit of 95% – 95% UCL

This procedure is applied only if the number of samples to be taken is greater than 1 and less than 10.

The procedure starts with a calculation of the **overall mean of the average values:**

$$\bar{\bar{x}} = \frac{(\bar{\mathrm{X}}_{i,1} + \bar{\mathrm{X}}_{i,2} + \cdots + \bar{\mathrm{X}}_{i,m})}{\mathrm{m}}$$

Where:

$\bar{\bar{x}}$	=	the overall mean of average values
$\bar{\mathrm{X}}_{i,1} + \bar{\mathrm{X}}_{i,m}$	=	the overall value of the concentration of particles from individual samples
m	=	the number of locations for calculation of the average value

The equation 8.5 serves to determine the **standard deviation of the average values calculated**:

$$s = \sqrt{\frac{(\bar{\mathrm{X}}_{i,1} + \bar{\bar{x}})^2 + (\bar{\mathrm{X}}_{i,2} - \bar{\bar{x}})^2 + \ldots + (\bar{\mathrm{X}}_{i,m} - \bar{\bar{x}})^2}{(m-1)}}$$

Where:
s = standard deficiency of the average values calculated

The equation 8.6 serves to calculate the **upper confidence limit of 95%**

$$95\%UCL = \bar{\bar{x}} + t_{0.95}\left(\frac{s}{\sqrt{m}}\right)$$

Where:
$t_{0.95}$ = is a variable which depends on the number of sample locations

Specifically:

m	2	3	4	5	6	7-9
t	6.3	2.9	2.4	2.1	2.0	1.9

An example of a results reporting form for recording particle counts is shown in **Table 22**:

Table 22: Example of table for particle counting test

<table>
<tr><td>Room Number: XXXX</td><td colspan="2">Room Area: XXX (m^2)</td><td colspan="2">Zone Sampling Area</td><td>Class ISO 7</td></tr>
<tr><td colspan="2">Data Collection Point</td><td colspan="2">≥0.5µm</td><td colspan="2">≥5.0µm</td></tr>
<tr><td colspan="2">1</td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td colspan="2">2</td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td colspan="2">—</td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td colspan="2">9</td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td colspan="2">Mean of Averages</td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td colspan="2">Standard Deviation</td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td colspan="2">95% of UCL</td><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td colspan="4">Acceptance criteria achieved, write clearly YES or NO</td><td colspan="2"></td></tr>
<tr><td colspan="6">Comments:</td></tr>
<tr><td>Completed:</td><td colspan="2">Date:</td><td colspan="2">Checked:</td><td>Date:</td></tr>
</table>

Example:

- Room Class according ISO 14644-1: ISO Class 7 (see **Table 23**)
- Number of air changes: 251/h
- Room area: $61m^2$
- Considered particles 0.5µm and 5µm
- Note: one sample per location will be tested

First, the cleanroom must be classified using following equation:

<table>
<tr><td rowspan="5">$Cn = 10^N \text{ x } \left[\frac{0.1}{D}\right]^{2.08}$</td><td>• Cn is the maximum permitted concentration of airborne particles that are equal to, or larger than the considered particle size. Cn is rounded to the nearest whole number</td></tr>
<tr><td>• Cn is the maximum permitted concentration of airborne particles that are equal to, or larger than the considered particle size. Cn is rounded on the nearest whole number. N is the ISO classification number, which shall not exceed the value of 9. An intermediate ISO classification number may be specified, with 0.1 the smallest permitted increment of N D is teh considered particle size in µm 0.1 is a constant with a dimension of µm</td></tr>
<tr><td>• N is the ISO classification number, which shall not exceed the value of 9. An intermediate ISO classification number may be specified, with 0.1 the smallest permitted increment of N</td></tr>
<tr><td>• D is the considered particle size in µm</td></tr>
<tr><td>• 0.1 is a constant with a dimension of µm</td></tr>
</table>

0.5µm	$Cn = 10^7 \ x \left(\frac{0.1}{0.5}\right)^{2.08}$		
5µm	$Cn = 10^7 \ x \left(\frac{0.1}{5}\right)^{2.08}$	$Cn = 2925$	

Hence for particles:

>0.5µm for ISO Class 7 is rounded to 352 000 particles/m^3

>5µm for ISO Class 7 is 2930 particles/m^3

Table 23: Selected airborne particulate cleanliness classes for cleanrooms and clean zones

ISO Class Number (N)	Maximum concentration limits (particles/m^3 of air) for particles equal to and larger than the considered sizes shown below [concentration limits are calculated in accordance with Equation (1) in 3.2 of ISO 14644-1]					
	0.1µm	0.2µm	0.3µm	0.5µm	1.0µm	5.0µm
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1000	237	102	35	8	
ISO Class 4	10000	2370	1020	352	83	
ISO Class 5	100000	23700	10200	3520	832	29
ISO Class 6	1000000	237000	102000	35200	8320	293
ISO Class 7				352000	83200	2930
ISO Class 8				3520000	832000	29300
ISO Class 9				35200000	8320000	293000

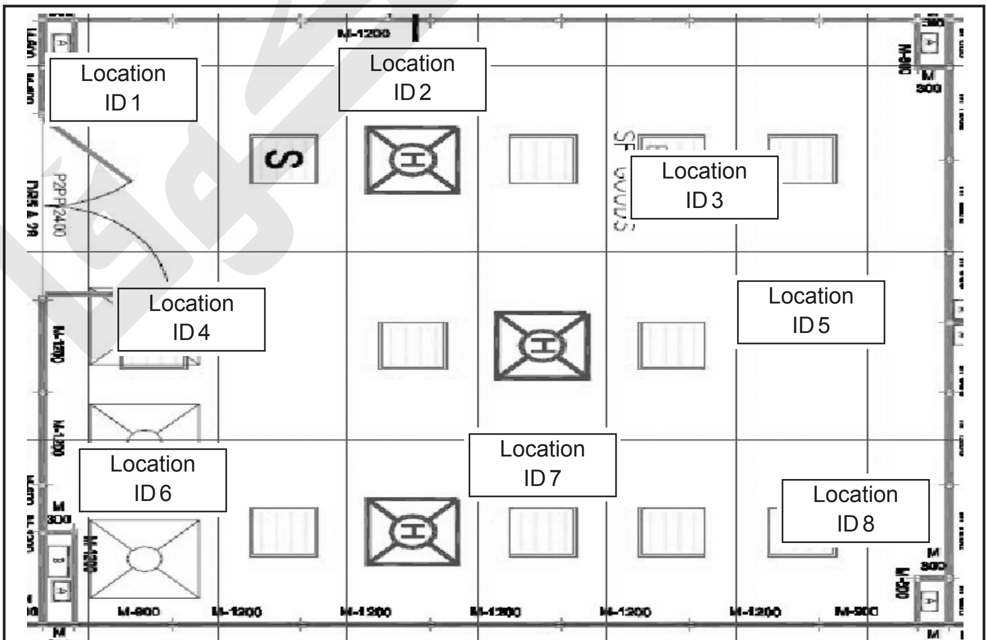


Figure19: *Locations for Particle counting*

$N_L = \sqrt{A}$	$N_L = \sqrt{61}$	$N_L = 7.8$

Minimum number of locations:

$V_s = \frac{20}{2930}\ x\ 1000$	$V_S = 6.82$	(Minimum 1 minute sample)

Is rounded on **8 locations** (see **Figure 19** and **Table 24**).

- Minimum volume to be taken as a sample:

Table 24: Taken measurements from different locations within the cleanroom

Room Nº xxxx	Particles/m^3	
	≥0.5µm	≥5µm
Location ID 1	10314	2816
Location ID 2	11760	2323
Location ID 3	11478	2816
Location ID 4	12643	2534
Location ID 5	9252	2710
Location ID 6	12572	2851
Location ID 7	10700	2886
Location ID 8	16632	2499

- Overall mean of averages

≥0.5µm	$\bar{\bar{x}} = \frac{(10314 + 11760 + 11478 + 12643 + 9252 + 12572 + 10700 + 16632)}{8}$

≥0.5µm	$\bar{\bar{x}} = 11919$

≥5µm	$\bar{\bar{x}} = \frac{(2816 + 2323 + 2816 + 2534 + 2710 + 2851 + 2886 + 2499)}{8}$

≥5µm	$\bar{\bar{x}} = 2679$

- Standard deviation of the average values

≥0.5µm	

S^2 =	$\frac{[(10314-11919)^2 + (11760-11919)^2 + (11478-11919)^2 + (12643-11919)^2 + (9252-11919)^2 +}{7}$

S^2 =	$\frac{+ (12572-11919)^2 + (10700-11919)^2 + (16632-11919)^2]}{7}$

S =	2222

≥5μm	

S^2 =	$\frac{[(2816-2679)^2 + (2323-2679)^2 + (2816-2679)^2 + (2534-2679)^2 + (2710-2679)^2 + (2851-2679)^2 +}{7}$

S^2 =	$\frac{+ (2886-2679)^2 + (2499-2679)^2]}{7}$

S =	204

Upper confidence limit of 95%

≥0.5μm	$95\%UCL = 111919 + 1.9 \left(\frac{2222}{\sqrt{8}}\right)$

≥0.5μm	13411 √	Obtained result confirms that number of particles are within the classification limits defined as described in the standard ISO 14644-1, and the classification table 8.23

≥5μm	$95\%UCL = 2679 + 1.9 \left(\frac{204}{\sqrt{8}}\right)$

≥5μm	2816 √	Obtained result confirms that number of particles are within the classification limits defined as described in the standard ISO 14644-1, and the classification table 8.23

More examples of different situations which occur during the particle testing are given in ISO 14644-1:1999. Tables of maximum permissible concentration of particles/m^3 for a specific class and according to ISO 14644-1[3] (Table 1) and EU GMP[2] (Table 2) is provided in Annex 1.

7.4.11 Room recovery test

This test should confirm that the ventilation system has the capacity to maintain the cleanliness level in the cleanroom in question. The objective is to calculate the time needed for a cleanroom to recover after a contamination event has occurred. That is, to determine if an elevated number of particles can be reduced to a level within the cleanroom class within a given period of time.

The room recovery test is conducted in at as built or at rest occupancy states. According to ISO 14644-3, 100:1 room recovery time for classes such as ISO classes 8 and 9 is not recommended. This is because generating a sufficiently high level of particles is very difficult. Furthermore, this test is beneficial only for non-unidirectional cleanrooms.

ISO 14644-3 defines the test with 100:1 recovery time or cleanliness recovery rate.

The 100:1 recovery time test starts by determining the concentration of particles at a level which is 100 times or more above the level as found in the cleanroom. The values for this test are noted in a chart (semi-log chart) where the ordinate in the log scale represents the concentration and the time when that concentration is measured is given at the abscissa in a linear scale.

The part B.12 of the ISO 14644-3 standard provides a complete definition of the method for obtaining 100:1 recovery time. A key issue before this test is started is to determine the concentration of particles which is 100 times or more above the initial one.

The particles in the room can be generated with the same particle generator which is used during the filter integrity test. In some facilities the area is contaminated by a simple switching off and on the AHU unit after a certain period of time. Several deficiencies can occur during the use of this method, for example:

- Once the unit is switched on again, the airflow volume will be achieved after a certain period of time. The recovery test can only be started once the required air quantity is reached; in other words this means that the concentration which was 100 times or more above the initial one has already changed in relation to the value when AHU was switched off.
- Parameters such as airflow rates and differential pressures must be confirmed at every system activation because very often divergences occur in the airflows.

The standard however, determines that the size of these particles should not be smaller than 1μm. The particle counter should be set to enable a measurement at one minute intervals and a time delay not greater than 10 seconds when taking each sample. It is noted when the concentration reaches the value of 100 x target concentration t_{100n} and when the value reaches the target cleanliness level t_n, the 100:1 recovery time is given as $t_{0.01} = (t_n - t_{100n})$.

EU GMP defines that after completion of an operation within the cleanroom, the number of particles determined for the class in question in at rest occupancy state must be achieved in a period of 15 to 20 minutes.

Example:
The number of particles measured in an area defined as ISO 7 Class ≥0.5μm is 10314 particles/m^3. Particles of ≥0.5μm are 1,250,326 particles/m^3, and are generated with an aerosol generator using dispersed oil (such as Shell EL Ondina Oil™). This will provide a concentration in conformity with the requested 100:1 recovery time, ie. the recovery time is to be 100 or more times above the initial levels. The decrease of the particles depending on the time is given in **Table 25** and a semi log chart (**Figure 20**) is given to show the slope value of the line.

An example data set is:

Table 25: Particle concentration during the time of recovery test

Concentration particles/m^3	Minutes (min)	Concentration particles/m^3	Minutes (min)
1250326	0	116031.1	7
1031819	1	76106.8	8
819666	2	61069	9
552197.9	3	35829.5	10
398431.1	4	30993.4	11
330125.6	5	18532.5	12
191502.5	6	16944	13
		11296	14

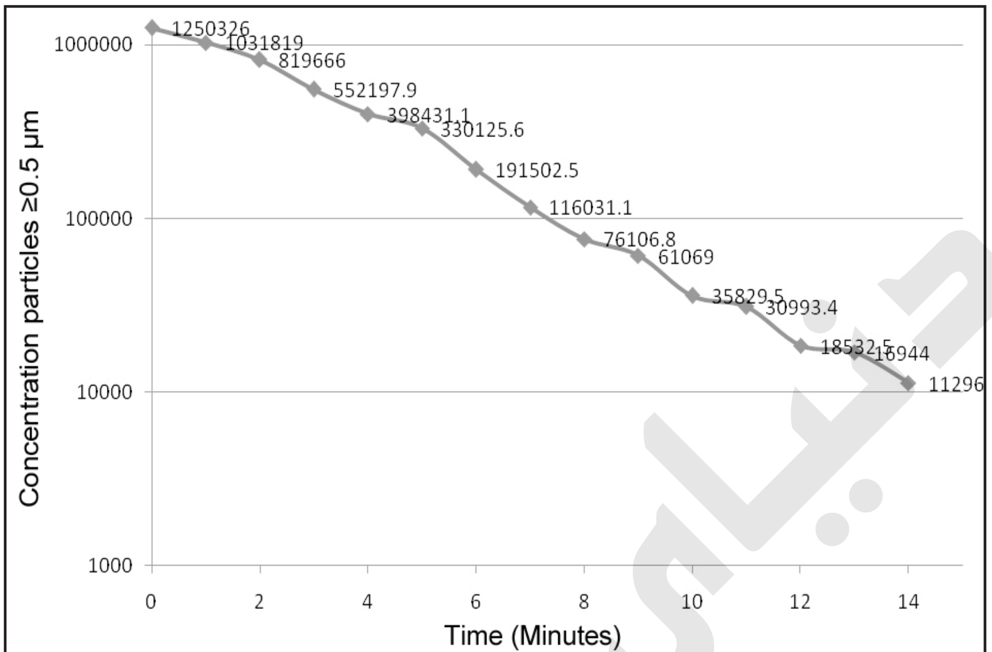


Figure 20: *Time/concentration curve for recovery testing.*

The illustration also provides an example of how a table, in which the results are inserted, should look like. It should contain the following:

- Definition of the area, the occupancy state and its class
- Method for the test, the instruments being used to conduct the test and their valid calibration certificates
- Number of locations

This is illustrated in **Table 26**:

Table 26: Recovery time/concentration table

Room Nº	Location Nº	Class ISO 14644-1	Start		End		Recovery Time **Tn-T100n** (min)
			Time **T100n** hh:mm:ss	Concentration Particles/m³	Time **Tn** hh:mm:ss	Concentration Particles/m³	
XX	*1*	*Class 7*	*10:40:00*	1250326	*10:54:00*	*11296*	*14*
XX	*2*	*Class 7*					
Acceptance criteria achieved (YES or NO)							
Comments:							
Completed by:		Date:		Accepted by:		Date:	

Room recovery rate is evaluated by using 100:1 recovery time:

$$n = -2.3 \times \frac{1}{t_1} \log_{10} \left[\frac{C_1}{C_0}\right]$$

- n – the cleanliness recovery rate
- t_1 – time between first and second measurement
- C_0 – initial concentration
- C_1 – concentration after time t_1

Equipment used for conducting the recovery test:

- Aerosol generator
- Diluter – if necessary
- Discrete Particle Counter

Because of the sensitivity of the particle counter where after a certain period of time and due to its exposure on big concentration of aerosol particles, the validation period of this instrument can quickly expire. That is why some cleanroom societies which actively participate in the development of the cleanroom test methods recommend conducting this test using a photometer.

7.5 Conclusion

Cleanrooms required a considerable amount of testing and qualification in order to ensure that they operate satisfactorily[6]. For this purpose, the chapter has detailed a practical approach for the commissioning and qualification of cleanrooms and clean air devices. Although the tests presented are described in the international cleanroom standards, the chapter has enhanced these by drawing upon the experience of practitioners involved with cleanroom testing and has illustrated the approach for testing cleanrooms with appropriate case studies.

The aim of the chapter is the practical one of providing the reader with a straightforward framework for cleanroom testing. Theoretical aspects of cleanroom operations are covered in several of the other chapters in this book. In relation to the tests outlined in this chapter, it is important that such tests are conducted by personnel who are highly familiar with all standard requirements. For this, many companies elect to use an independent service and validation provider who is not associated with any manufacturer or supplier, and to have the tests independently verified by the client. After completion of all the necessary testing, the most important aspect is the completion of documentation so that auditors and regulatory personnel can be satisfied that the requirements of Good Manufacturing Practice have been met.

7.6 References

1. EN 1822-1: High efficiency air filters (EPA, HEPA and ULPA) Part 1: Classification, performance testing, marking, European Committee for Standardisation (December 2009).
2. Eudralex. The Rules Governing Medicinal Products in the European Community, Annex 1, published by the European Commission, 2009.
3. ISO 14644-1: Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness. International Organisation for Standardisation ISO, Geneva, Switzerland (May 1999).
4. ISO 14644-2: Cleanrooms and associated controlled environments – Part 2: Specifications for testing and monitoring to prove continued compliance with ISO 14644-1. International Organisation for Standardisation ISO, Geneva, Switzerland (September, 2000).
5. ISO 14644-3: Cleanrooms and associated controlled environments – Part 3: Test methods. International Organisation for Standardisation ISO, Geneva, Switzerland (December, 2005).
6. Whyte W. Cleanroom Technology , Fundamentals of Design, Testing and Operation, London: Wiley-Blackwell, London, UK, 2001.

Cleanroom certification and ongoing compliance

Tim Sandle, Head of Microbiology, Bio Products Laboratory, UK
Madhu Raju Saghee, Assistant Manager – QA, Novartis India Ltd, Thane, India

8.1 Introduction

Cleanrooms and clean zones, as this book has emphasised across several chapters, form the structural basis for all types of pharmaceutical processing and for many other applications within healthcare (such as pharmacies and surgical units) and life sciences sector. Cleanrooms are designed to provide environments which control the number of particles, many of which will be microbial carrying particles, in the air and to reduce the possibility of microbial contamination[1]. If the operator of the cleanroom does not have an understanding, supporting data, or indeed confidence, that the cleanroom is operating satisfactorily then the risk of product or patient contamination will be greater. The term 'operating satisfactorily' can only be assessed through the use of a quality standard for cleanrooms. Described elsewhere within this book is the long and, at times, convoluted history of cleanroom standards. This chapter is, however, concerned with the current cleanroom standard and how this is applied for the purpose of cleanroom certification and for the re-assessment of established cleanrooms. This standard is ISO 14644.

Although there are additional regulations and guidances which must be understood and adopted in relation to cleanroom operations under the auspices of Good Manufacturing Practice (where EU GMP, WHO GMP and FDA aseptic filling guidance provide the dominant regulatory missives), this chapter does not focus on these aspects of operation (and importantly, classification *is not* the same as monitoring). Instead, the chapter is concerned with the requirements for assessing a newly built cleanroom prior to use and for an annual (sometimes, for ISO class 5 areas, six-monthly) re-assessment of cleanrooms through the use of the international standard. The chapter is not written from the perspective of 'how to classify a cleanroom'; the intention of the chapter is to outline the required tests and provide guidance for cleanroom managers and quality personnel.

The international standard for cleanrooms is ISO (International Organisation for Standardisation) 14644. This standard is sometimes 'adopted' by regional standards bodies so prefixes like 'EN' (for European Norm) or 'BS' (for British Standard) will sometimes be seen. These prefixes do not indicate any differences in content and ISO 14644 stands as an international standard to be used for all cleanrooms. The history and development of ISO 14644 has been described in this book (refer to Chapters 2 and 3). The standard became effective in 1999 (although it was not adopted by the USA. until 2001).

The ISO 14644 standard is applicable to all types of cleanrooms, including pharmaceuticals and microelectronics. Thus not all parts of the standard are relevant to all types of industry. As a related point, it is important to note that ISO 14644 in itself is not a regulatory document. Parts of it are, however, recommended by regulatory agencies to be used to design and test cleanrooms, and these cleanrooms will be subject to regulatory review.

Within ISO 14644 'clean', in relation to 'cleanroom', is defined by a maximum permitted concentration of airborne particles according to an assigned class. The standard states that a cleanroom is:

> *"A room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimise the introduction, generation and retention of particles inside*

the room and in which other relevant particles inside the room and in which other relevant parameters, e.g. temperature, humidity and pressure, are controlled as necessary."

In relation to class, cleanrooms are classified as 'ISO Class 1' through to 'ISO Class 9'. These classes are expandable which means that it is permissible to have subclasses. This allows, as different chapters of the book allude to, EU GMP Grade A to be expressed as equivalent to ISO class 4.8, for example. Whilst the US FDA accepts and uses these classes (2), for EU GMP[3] and the World Health Organisation (WHO)[4] the ISO 14644 classes must be related to a grade (which has an alphabetic notation, the same notation is used by both EU GMP and the WHO). Care must be taken when attempting to align different standards for the ISO classes relate to any operational state whereas EU and WHO GMP define different maximum permitted levels of particles for different operational states (for example, there is a different particle state for Grade B 'at rest' and Grade B 'in operation'). **Table 1** and **Figure 1**, below, outline how the EU GMP guidance and ISO 14644 standards relate:

Table 1: EU GMP grades compared with ISO 14644 classes for the 'at rest' and 'in operation' conditions.

	At Rest			
Particle size	**0.5µm**		**5.0µm**	
EU GMP Grade	Annex 1	ISO 14644-1 (Class) Count	Annex 1	ISO 14644-1 (Class) Count
A	3,520	(5) 3,520	20	(4.8) 20
B	3,520	(5) 3,520	29	(5) 29
C	352,000	(7) 352,000	2,900	(7) 2,930
D	3,520,00	(8) 3,520,000	29,000	(8) 29,300
	Operational			
Particle size	**0.5µm**		**5.0µm**	
EU GMP Grade	Annex 1	ISO 14644-1 (Class) Count	Annex 1	ISO 14644-1 (Class) Count
A	3,520	(5) 3,520	20	(4.8) 20
B	352,000	(7) 352,000	2,900	(7) 2,930
C	3,520,000	(8) 3,520,000	29,000	(8) 29,300
D	Not Defined		Not Defined	

With the definition of 'clean', the concentration of airborne particles is the critical parameter for cleanrooms, clean zones, and controlled areas. Particle concentration must be measured at representative locations for classification and monitored routinely or continuously at critical locations during operation. Many factors besides airborne particulate cleanliness must be considered in the design, specifications, operations and control of cleanrooms and other controlled environments. This chapter outlines these various requirements and concludes with a discussion about the industry concern with energy use

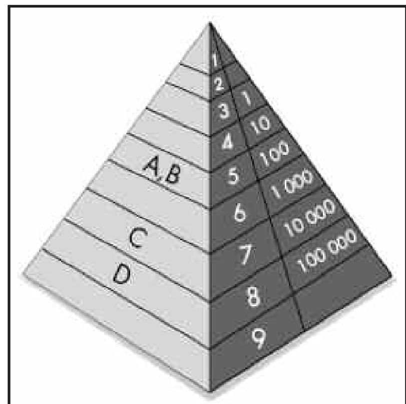


Figure 1: *Illustration of the interrelation of EU GMP grades and ISO 14644 classes*

and whether, through a risk management process, cleanroom operational parameters can be adjusted whilst, at the same time, continuing to conform with the standard.

8.2 Cleanrooms

Before examining the ISO 14644 test requirements it is useful to consider cleanrooms in general since understanding the design and function of cleanrooms helps with the understanding of the standard. A cleanroom is an environment that has a low level of environmental pollutants such as dust, airborne microbes, aerosol particles and chemical vapours. This level of contamination is specified by the number (or concentration) of particles per cubic meter at a specified particle size. The basic unit of measurement within a cleanroom is a micron (μm) which is one millionth of a metre. Within the life science sector the particle size against which concentrations are examined is 0.5μm. As a reference point, a human hair is around 100μm.

Different types of cleanrooms require different levels of control, based on the permitted number of a particular size of particle counts. The required standard of cleanliness of a room is dependent on the task performed in it and it follows that the more susceptible the product is to contamination, the better ('cleaner') the standard[5]. Thus for different activities cleanrooms are assigned classes or grades. For aseptic filling, for example, the class is ISO class 5 (which is approximately equal to EU GMP Grade A); whereas for final formulation of a sterile product, the cleanroom class is commonly class 8 (equivalent to EU GMP Grade C in operation).

To achieve the appropriate level of control, the air entering a cleanroom from outside is filtered to exclude dust, and the air inside is constantly recirculated through high-efficiency particulate air (HEPA) or ultra-low penetration air (ULPA) filters to remove internally generated contaminants. Air filters are commonly described and rated based upon their collection efficiency, pressure drop (or airflow resistance), and particulate-holding capacity. HEPA filters are normally 99.97% or more efficient in removing particles greater than 0.3μm from the room air supply[6]. Filtration of particles relies on four main principles: inertial impaction, interception, diffusion, and electrostatic attraction.

Air filters are constructed of filter media, sealants, a frame, and sometimes a faceguard and/or gasket. Common types of media include glass fibre, synthetic fibre, non-woven fibre, and PTFE (polytetrafluoroethylene). These various components of the filter indicate why a regular assessment of air filters for leaks is of importance (this is discussed below).

Other design aspects of cleanrooms reduce contamination risks[7]. Most cleanrooms used within the pharmaceutical sector are kept at a positive pressure so that if there are any leaks, air leaks out of the cleanroom instead of unfiltered air coming in. The air pattern within the cleanroom may be unidirectional or turbulent. With cleanrooms, as opposed to unidirectional devices, the air within the cleanroom is more often turbulent. Turbulent (air flow describes an air pattern where the air in a cleanroom is held in constant motion, although not all of the air is moving in the same direction. The turbulent air seeks to trap particles that may be in the air and drive them towards the floor, where they enter extract filters and leave the cleanroom environment.

A critical factor in cleanroom design is controlling the number of air changes per hour (this is also known as the air-change rate). This refers to the number of times each hour that filtered outside air replaces the existing volume in a cleanroom. In a normal home, an air-conditioner changes room air 0.5 to 2 times per hour. In a cleanroom, depending on classification and usage, air change occurs anywhere from 10 to more than 600 times an hour. Achieving the optimal air change rate requires good ceiling fan coverage. A further factor integral to maintaining cleanliness is fan-generated air speed. Again, higher airflow velocity results in a "cleaner" cleanroom. The term "ventilation efficiency" refers to the speed of filtered air passing through the cleanroom in addition to the number of air changes per hour.

To ensure cleanliness standards are maintained, other protective measures are in place. These measures include staff entering and leaving through airlocks (sometimes including an air shower stage. In the air shower, high velocity air blows off debris that could contaminate the cleanroom environment. Sometimes de-ionisers are used to remove static thereby increasing the effectiveness of removing hair and lint); and wearing protective clothing such as hoods, face-masks, gloves, boots and coveralls[8]. Furthermore, equipment inside the cleanroom should be designed to generate minimal air contamination and be easy to clean and disinfect[9]. To eliminate the majority of microbial contamination on surfaces, robust cleaning and disinfection regimes are in place[10].

For activities requiring the cleanest concentration of air, clean zones are used. These are unidirectional airflow (UDAF) cabinets. Unidirectional airflow systems direct filtered air downward or horizontally in a constant stream towards filters located on walls near the cleanroom floor or through raised perforated floor panels to be recirculated. Stainless steel or other non-shed materials are used to construct unidirectional airflow filters and hoods to prevent excess particles entering the air.

8.3 Cleanroom certification to ISO 14644

As indicated above, ISO 14644 is the cleanroom standard for the certification and re-assessment of cleanrooms. It is a nine-part standard (and soon to be a ten-part standard). This chapter focuses foremost on parts 1 and 2 as these are directly applicable to cleanroom certification. The reader should note that both these parts are, at the time of writing, subject to international review and may lead to a modification to the classification approach.

The tests listed in ISO 14644-1 are divided into mandatory and optional tests. To these describes tests other tests may be added where these are of relevance to the cleanroom (such as an assessment of temperature and humidity). The range of tests may also differ where the cleanroom is undergoing validation in contrast to commissioning. These phases can be distinguished thus:

- Commissioning: Testing, proving and documenting a facility system to demonstrate and satisfy the safe functionality in accordance with the design criteria.
- Validation: Qualification practices, fully documented, to provide the necessary assurance, through pre-defined test/s to prove that the critical systems operate in accordance with the facility design criteria.

Readers are advised to refer chapter 7 for detailed information regarding cleanroom commissioning and qualification.

8.3.1 ISO 14644-1 (Part 1: Classification of air cleanliness)

Part 1 of the ISO 14644 standard provides the airborne particle limits for different classifications of cleanrooms[11]. This standard also provides the methods that should be used to measure the airborne particles when testing a cleanroom to determine its class.

Cleanrooms are certified according to principles outlined in ISO 14644 Part 1 and according to the methods described in ISO 14644 Part 2. To classify pharmaceutical grade cleanrooms either:

- The number of particles equal to and greater than 0.5μm is measured in one cubic metre of air and this count is used to identify the cleanroom class.
- Or, the class is assigned, and this is either proven correct or not through an assessment of the number of particles equal to and greater than 0.5μm measured in one cubic metre of air.

(With 0.5μm some users elect to include 5.0μm size particles as well; this is discussed below).

Selecting the approach will be dependent upon a local policy. For aseptic filling, the clean zone

must be ISO class 5/EU GMP Grade A. Therefore, the area is designed to this specification and an attempt is made to see if the device can meet this classification.

The ISO 14644-1 standard specifies the decimal logarithm of the number of particles 0.1µm or larger permitted per cubic metre of air. So, for example, an ISO class 5 cleanroom has at most 10^5 = 100,000 particles per cubic metre. The permitted numbers of particles are shown in **Table 2** below.

Table 2: ISO 14644-1 cleanroom standards

Class	Maximum particles/m³					
	≥0.1µm	≥0.2µm	≥0.3µm	≥0.5µm	≥1µm	≥5µm
ISO 1	10	2.37	1.02	0.35	0.083	0.0029
ISO 2	100	23.7	10.2	3.5	0.83	0.029
ISO 3	1,000	237	102	35	8.3	0.29
ISO 4	10,000	2,370	1,020	352	83	2.9
ISO 5	100,000	23,700	10,200	3,520	832	29
ISO 6	1.0×10^6	237,000	102,000	35,200	8,320	293
ISO 7	1.0×10^7	2.37×10^6	1,020,000	352,000	83,200	2,930
ISO 8	1.0×10^8	2.37×10^7	1.02×10^7	3,520,000	832,000	29,300
ISO 9	1.0×10^9	2.37×10^8	1.02×10^8	35,200,000	8,320,000	293,000

Airborne particles are measured using a discrete particle counter[12]. This is a light-scattering instrument that is used to determine the concentration of airborne particles, equal to and larger than the specified sizes, at designated sampling locations. Although they come in a wide variety of sizes from heavy bench top units to hand-held counters, all counters have the same basic components of sensor, vacuum source (for sample flow) and control electronics. Counters used for classification are required to have been calibrated against the ISO 21501-4 standard[13]. This standard states:

> *"Monitoring particle contamination levels is required in various fields, e.g. in the electronic industry, in the pharmaceutical industry, in the manufacturing of precision machines and in medical operations. Particle counters are useful instruments for monitoring particle contamination in air. The purpose of this part of ISO 21501 is to provide a calibration procedure and verification method for particle counters, so as to minimise the inaccuracy in the measurement result by a counter, as well as the differences in the results measured by different instruments."*

Particle count assessments can be made in different operating states. In cleanrooms, particulate concentration changes over time: from the construction and installation of equipment to its operational status. The certification state of the cleanroom must be defined in advance of testing, by selecting one of three possible operating states:

As Built: a completed room with all services connected and functional, but without production equipment or personnel within the facility.
At Rest: a condition where all the services are connected, all the equipment is installed and operating to an agreed manner, but no personnel are present.
In Operation: all equipment is installed and is functioning to an agreed format, and a specified number of personnel are present working to an agreed procedure.

It is up to the user to define which operational state is appropriate at the time of classification. For a newly built cleanroom, the ‘as built’ assessment is often part of the operational qualification and is a means by which the cleanroom company hands over the cleanroom to the user. Of the other two states, for most areas, the operational state is preferred as this represents a greater challenge as the cleanroom has equipment running and people are present[14].

Cleanrooms are classified according to the number and size of particles permitted per volume of air. ISO 14644-1 gives a method to classify cleanrooms by the concentration of airborne particles, the classification being based on the following equation:

$$C_n = \frac{(0.1)^{2.08}}{D} \times 10^N$$

Cn = Max conc^n

D = particle size in µm

$10^5 = 10$^ classification number = 5

Where:

- Cn is the maximum permitted concentration (in particles/m^3 of air) of airborne particles that are equal to, or larger, than the considered particle size. Cn is rounded to the nearest whole number, using no more than three significant figures
- N is the ISO classification number, which shall not exceed the value of 9. Intermediate ISO classification numbers may be specified; with 0.1 the smallest permitted increment of N
- D is the considered particle size in µm
- 0.1 is a constant with a dimension of µm

The exponent 2.08, the correlation between particle concentration and particle diameter, was selected based on its previous use within the withdrawn FS 209 E standard, possibly to ensure continuity with the previous standard[15].

The designation for cleanroom or clean zone certification is should include the following elements:

- The room classification number expressed as “ISO Class N”
- The cleanroom occupancy state
- The required particle size (in relation to pharmaceutical processing, 0.5µm is the required standard. Some users, who fall under EU GMP or WHO GMP regions, elect to include the additional 5.0µm size)

An example designation is:

1. ISO class 4; operation state; considered sizes; 0.2µm (2,370 particles/m^3), 1µm (83 particles/m^3)
2. The considered particle size(s) for which the concentration (s) will be measured, should be agreed upon by the customer and the supplier.
3. If measurements are to be made at more than one considered particle size, each larger particle diameter (e.g., D_2) shall be at least 1.5 times the next smaller particle diameter (e.g., D_1) e.g.: $D_2 \geq 1.5$ D

The standard provides the method by which the performance of a cleanroom may be verified by sampling locations and by sample volume.

Sample locations are selected according to the following equation (Annex B of ISO 14644-1):

Number of locations (NL) = √ area of room (A)

Where, NL is the minimum number of sampling locations (rounded up to a whole number) and A is the area of the cleanroom or zone in metres.

For situations in which the number of sampling locations involved is at least two but not more than nine (as might apply to smaller cleanrooms), the calculation of 95% upper confidence limits is required.

With sampling volume, at each sampling location, sample a sufficient volume of air that a minimum of 20 particles would be detected if the particle concentration for the largest considered particle size were at the class limit for the designated ISO class. The single volume (Vs) per location is determined by using the equation:

$$V_s = \frac{20}{C_{n,m}} \times 1{,}000 \quad (B.2)$$

Where:

- Vs: is the minimum single sample volume per location, expressed in litres
- Cn,m: is the class limit (number of particles per cubic metre) for the largest considered particle size specified for the relevant class
- 20: is the defined number of samples that could be counted if the particle concentration were at the class limit

The standard requires that the volume sampled at each location shall be at least 2 litres, with the minimum sampling time at each location of 1 minute. When Vs is very large, the time required for sampling can be substantial. By using the sequential sampling procedure (see annex F of ISO 14644-1), both the require sample volume and time required to obtain samples may be reduced.

It should be noted that the Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (PIC/S), to which EU GMP inspectors and the FDA are members, states in guide PE 009 that for routine testing the total sample volume should not be less than 1m³ for Grade A and B areas and preferably also in grade C areas[16].

On completion of the particle count classification, the average (mean) level of particles is calculated for the cleanroom. The average particle concentration(s) must not exceed the concentration limit for the particular ISO class. Therefore, the cleanroom or clean zone is deemed to have met the specified air cleanliness classification if the averages of the particle concentrations measured at each of the locations and, when applicable, the 95% upper confidence limit calculated (in relation to room locations). If the results of testing fail to meet the specified air cleanliness classification, testing may be performed at additional, evenly distributed sampling locations, The results of recalculation, including data from the added locations, shall be definitive and corrective and preventative actions (CAPA) are required.

The standard describes situations where the 95% upper confidence interval is used and where results may not indicate compliance. Where this occurs, if the noncompliance is caused by a single, non-random outlier value resulting from an erroneous measurement (due to procedural error or equipment malfunction) or from an unusually low particle concentration (due to exceptionally clean air), the outlier may be excluded from the calculation, provided that:

- The calculation is repeated, including all remaining sampling locations
- At least three measurement values remain in the calculation

- No more than one measurement value is excluded from the calculation
- The suspected cause of the erroneous measurement or low particle concentration is documented and accepted by both the customer and supplier

It is important to note that, when assessing outliers, that widely divergent values for particle concentrations among the locations sampled may be reasonable and even intentional, depending on the nature of the application of the clean installation under test.

8.3.2 ISO 14644-2 (Part 2: Specifications for testing and monitoring to prove continued compliance with ISO 14644-1)

The second part of ISO 14644 specifies requirements for periodic testing of a cleanroom or clean zone to prove its continued compliance with ISO 14644-1 for the designated classification of airborne particulate cleanliness[17]. It also specifies other test requirements to demonstrate that the cleanroom is functioning according to the appropriate standard. The following tables indicate mandatory and optional tests.

Table 3: Required cleanroom certification tests as per ISO 14664-2

Required testing (ISO 14644-2)

Schedule of tests to demonstrate continuing compliance			
Test Parameter	Class	Maximum Time Interval	Test Procedure
Particle Count Test	<= ISO 5	6 Months (*)	ISO 14644-1 Annex A
	> ISO 5	12 Months	
Air Pressure Difference	All Classes	12 Months	ISO 14644-1 Annex B5
Airflow	All Classes	12 Months	ISO 14644-1 Annex B4

(*) The standard states that the interval between tests can be reduced if there is a continuing monitoring system in place (such as a Facility Monitoring System). A facility monitoring system is a means of collecting data and turning it into information automatically. Over time, this information builds up knowledge of the process being monitored18. Systems can measure temperature and humidity, as well as particle counts. With the advent of 'real time' particle counters capable, at least in theory, of differentiating between viable micro-organisms and non-viable particles, these devices could also be incorporate into a facility monitoring system.

Particle count cleanroom test

The particle count test has been outlined above. This must be reassessed according to the required frequencies or when a cleanroom has been redesigned or in the event of a significant change (such as a change to a HEPA filter). The test requires that a set number of locations within the room are used to assess the number of particles within the required volume of air. The average count obtained must meet the required class. The reader should note that many regulatory authorities expect the cleanroom user to include additional locations should any location within the room be regarded as critical or presenting a product risk.

Air pressure differentials

In relation to air pressure, air in cleanrooms should always flow from high pressure to low pressure areas. Normally the desired flow path should be from the area of cleanest, to less clean, to less-contaminated, and then to dirty areas. For this, rooms are required to be held at different pressures.

The static pressure between cleanrooms of different class, and cleanrooms and unclassified areas can be established and maintained using various airflow balancing techniques. These include both active/automated and passive/manual systems. Pressurisation is defined as a technique that air

pressure differences are created mechanically between rooms to introduce intentional air movement paths through room leakage openings. With this, the relative quantities of air are delivered and removed from each space by the ducted air system, air transfer system and losses. These openings could be either designated, such as doorways, or undesignated, such as air gaps around doorframes or other cracks.

ISO 14644 recommends that the pressure differential between adjacent cleanrooms or clean zones of different cleanliness level should lie typically in the range of 5Pa to 20Pa, to allow doors to be opened and to avoid unintended cross-flows due to turbulence. Pressure should be assessed through regular monitoring.

Airflow

Airflow is usually specified either as average air velocity within the room or as air changes per hour. Air volume is concerns the air supply. This is an important consideration given that the cleanliness of cleanrooms is directly dependent on the volume of clean air supply to the room. Three methods of measuring air supply volumes are used, these being the Pitot static, anemometer and hood method. The most common way of measuring the air supply to a cleanroom is by means of a measuring hood. With the hood, the measurement grid is at the exit of the hood and a hood has a set of holes facing the direction of airflow which measures the total pressure, and a set of holes facing away from the airflow that measures the static pressure. The difference between these two pressures, which is measured by a pressure micromanometer, is the velocity pressure, which can be easily converted to velocity. Understanding the surface area of the hood exit, the air volume being supplied can be calculated.

Air change rate is a measure of how quickly the air in an interior space is replaced by outside (or conditioned) air. For example, if the amount of air that enters and exits in one hour equals the total volume of the cleanroom, the space is said to undergo one air change per hour. Air flow rate is measured in appropriate units such as cubic feet per minute (CFM) and is given by:

Airflow rate = Air changes x Volume of space/ 60

For clean air devices air velocity requires a separate assessment. Air velocity is a determining factor in achieving airflow uniformity under unidirectional airflow devices. Air velocities in those cases may vary with the configuration of the equipment downstream of the air filters. For ISO class 5 and EU/WHO GMP Grade A devices, the airflow velocity must fall within the range 0.36 – 0.54 metres per second (sometimes written as 0.45m/s ±20%). The velocity is assessed using an anemometer, a device for measuring wind speed.

Table 4: Optional cleanroom certification tests as per ISO 14644-2

Schedule of additional optional tests			
Test Parameter	Class	Maximum Time Interval	Test Procedure
Installed Filter Leakage	All Classes	24 Months	ISO 14644-1 Annex B6
Containment Leakage	All Classes	24 Months	ISO 14644-1 Annex B4
Recovery	All Classes	24 Months	ISO 14644-1 Annex B13
Airflow Visualisation	All Classes	24 Months	ISO 14644-1 Annex B7

Installed filter leakage

The risk of filter leakage is of importance and must be measured in an appropriate way. Filter leakage refers to the filters that supply air directly into the cleanroom (normally HEPA filters within the pharmaceutical sector). This is an important assessment for, should a HEPA filter leak, then an excess level of particles could enter the room. The purpose of performing regular leak tests is to ensure the filter media, filter frame and filter seal are free from leaks.

Leakage is checked by challenging each filter with an aerosol of particles dispersed upstream of the filter and scanning over the downstream face to ensure that there are no leaks that exceed a specified level of penetration. The aerosol selected for HEPA leak testing should not support microbial growth and should be composed of a sufficient number or mass of particles. Guidance is provided in ISO 1822[19].

The challenge aerosol presented to the upstream side of the filter should be stable, homogeneous and have a concentration of between 20µg/l and 50µg/l in accordance with ISO 14644-3:2005. For a stable test the aerosol providing particles should have the following distributions:

- More than 20% by mass of particles less than 0.5µm
- More than 50% by mass of particles less than 0.7µm
- More than 75% by mass of particles less than 1.0µm

When performing this task, it is important to ensure that the aerosol supply is homogenous. To maintain homogeneity, the aerosol should be injected into an upstream duct at a distance that is at least 15 duct diameters from the upstream face of the filter.

Leaks are assessed though the use of scanning equipment, the most common of which is an aerosol photometer. The photometer is used to measure the upstream aerosol concentration as well as the downstream penetration of the HEPA filter by the aerosol. By the use of light scattering instrumentation, upstream and downstream particle concentrations can be measured. In essence, if 10,000 x 0.3 micron particles are blown into a HEPA air filter, only 3 particles are allowed to pass through. This would give the filter a 99.97% efficiency at 0.3 micron rating

Filters that can be face scanned are relatively straightforward to test. However, filters that are inaccessible or housed within complex installations, are difficult to measure and require specialist contractors.

Containment leakage
The containment leakage test is designed to show that air moves in the desired direction, from a clean to a less clean area.

Recovery
The recovery study is sometimes referred to as the clean-up time study. The test is designed to show that if the level of particles rises above the required class limit due to an event that the room can ‘recovery’ (that is the level of particles comes back down to a level below the class limit) within a reasonable time period. This time period is normally within 15-20 minutes. The test is undertaken by measuring the particle level over time.

Although this test is optional, it is a useful test to undertaken for aseptic filling areas. Understanding how the room recovers in the event of a rise in particles can help with risk assessments into HVAC (heating ventilation and air-conditioning) system failures.

Airflow visualisation
Airflow visualisation is sometimes referred to as an air-pattern or smoke study. The test involves blowing smoke around the cleanroom to ensure that the flow is satisfactory (with aseptic filling this is the air moving away from the critical zone). The air pattern, visualised with the smoke, is assessed for undesired movement such as counter drafts, stagnant areas, areas where air rises (especially after striking a surface), turbulent areas (within areas that are designed to have unidirectional airflow) and cross currents.

Airflow studies are covered in considerable detail in Chapter 16 of this book.

These mandatory and optional tests required for cleanroom certification can be summarised in the following diagram:

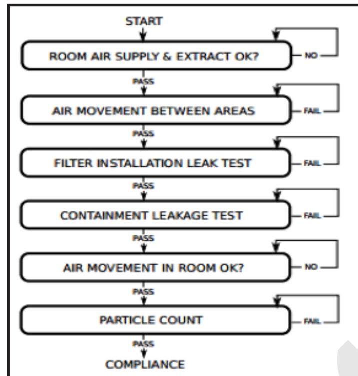


Figure 2: *ISO 14644-2 cleanroom certification tests*

8.3.3 Other parts of the ISO 14644 standard

As indicated earlier, the ISO 14644 cleanroom standard has several parts. The parts relevant to cleanrooms in the pharmaceutical sector (particularly parts 3, 4, 5, and 7) are discussed below.

- Part 3: Test methods.

  This gives a description of the test methods that should be used to test the cleanroom to show that it is working correctly. ISO 14644-3 specifies test methods for designated classification of airborne particulate cleanliness and for characterizing the performance of cleanrooms and clean zones. These test methods are specified in the document for two different types of cleanrooms and clean zones; unidirectional flow and non-unidirectional flow. The most important objectives of this document are to provide an internationally common basis of measurement and evaluation of cleanrooms and, at the same time, not to prevent the introduction of new technologies.

  ISO 14644-3 places emphasis on the 14 recommended tests used to characterise cleanrooms and clean zones. These tests are:

  1) Airborne particle count for classification
  2) Airborne particle count for ultrafine particles
  3) Airborne particle count for macroparticles
  4) Airflow
  5) Air pressure difference
  6) Installed filter system leakage
  7) Flow visualisation
  8) Airflow direction
  9) Temperature
  10) Humidity
  11) Electrostatic and ion generator
  12) Particle deposition
  13) Recovery
  14) Containment leak

  As identified in ISO 14644-1 and ISO 14644-2, some of these tests are mandatory, although most are voluntary. The key controlling factor is the quality level the cleanroom owner desires

and what measurements are necessary to help achieve that level. For a pharmaceutical cleanrooms, tests 1, 4, 5, 6, 7, 8, 9, 10 and 13 are of the most relevance. Some of the other tests described are applicable to the electronics sector.

- Part 4: Design, construction, and start-up.

  This gives general guidance as to how a cleanroom should be designed, constructed and made ready for handing over to the user. ISO 14644-4 specifies requirements for the design and construction of cleanroom and clean air devices, as well as requirements for start-up and qualification, but does not prescribe specific technological nor contractual means to meet the requirements. This document is intended for purchasers, suppliers, and designers of cleanroom installations.

- Part 5: Operations

  This gives general advice on how to run a cleanroom. ISO 14644-5 provides the basic requirements for operating and maintaining cleanrooms and associated controlled environments. This standard addresses requirements that are basic to the operation of all cleanrooms regardless of the application. Topics include the operational systems that must be in place; selection and use of appropriate cleanroom garments; training and monitoring of personnel and activities; installation and use of equipment; requirements for materials used in the cleanroom; Maintaining the cleanroom environment in a clean and usable condition conforming to design standards.

- Part 7: Separative enclosures (clean air hoods, gloveboxes, isolator, mini environments).

  This part of ISO 14644 specifies the minimum requirements for the design, construction, installation, testing and approval of separative devices in those respects where they differ from cleanrooms as described in ISO 14644-4 and 14644-5. Separative devices range from open to closed systems.

8.4 Reporting

The ISO 14644 standard provides a recommended best practice for the reporting of cleanroom certificate results. This takes the form of:

- The name and address of the testing organisation, and date on which the test was performed
- The number and year of publication of this part of ISO 14644. For example: ISO 14644 -1 : date of current issue
- A clear identification of physical location of cleanroom or clean zone tested (include reference to adjacent areas if necessary), and specific designations for coordinates of all sampling locations
- The specific designation criteria for the cleanroom or clean zone, include the ISO classification, the relevant occupancy state (s), and the considered particle size(s)
- Details of the test method used, with any special conditions relating to the test or departures from the test method, and identification of the test instrument and its current calibration certificate
- The test results, including particle concentration data for all sampling location coordinates

8.5 Risk-based approach in cleanroom commissioning and qualification

Cleanrooms are highly energy intensive to operate. Because the air volumes supplied to the cleanrooms are many times (10-100) greater than those supplied to conventionally ventilated rooms, the capital and operating costs for the construction of cleanrooms can be very high. It is possible to review the level of cleanroom certification undertaken and the way in which cleanrooms are designed and operated. This involves reaching a careful balance between saving energy and maintaining compliance. To achieve this, the guidance provided in ISO 14644-4 has helped to shape some best practice thinking on ‘green’ lifecycle sustainability cleanroom design and energy efficient approaches in GMP operating practice. From this, a risk-

based approach to cleanroom commissioning and qualification exercises can be constructed[20]. The standard states[21]:

> *"Consideration may be given to incorporating in the design energy conservation considerations, such as provisions to reduce or close down temperature and humidity control and to reduce airflow during periods in which there is no activity. The ability to recover operating conditions in a defined recovery period should be demonstrated."*

Higher air change rates equate to higher airflows and more energy use. In most cleanrooms, people are the primary source of contamination. Once a cleanroom is vacated, lower air changes per hour to maintain cleanliness are possible allowing for setback of the air-handling systems. Variable speed drives can be used on all recirculation air systems allowing for air flow adjustments to optimise airflow or account for filter loading. On this basis, for energy conservation reasons, airflow of the ventilation systems may be reduced to low levels during non-operating periods. Energy savings in cleanrooms can be realised by reducing air changes. To assess the risk, strict monitoring of air quality and cleanliness of cleanroom components is important to assure the environment is still suitable for the operations carried out. However, if systems are turned off, the potential for unacceptable room contamination to occur must be risk-assessed[22].

BS EN 16001 is a European energy standard which is sometimes used to benchmark cleanrooms (implemented via European Union directive: 206/32/EC on energy end-use efficiency and energy services (EEESD)). In addition, national and international standards are being developed to outline the energy requirements for cleanrooms, covering key performance indicators (such as CO_2 emissions) through to metrics to review where energy levels are reduced.

Savings to the operation and hence classification of cleanrooms can be made with the use of isolators. Such devices also confer advantages in terms of contamination control when undertaking critical activities like aseptic filling[23]. An isolator is a mini-environment that uses a physical barrier (usually a plastic film, plastic sheet or glass) to isolate the susceptible or critical part of the manufacturing process from the rest of the room. The critical manufacturing area is kept within the isolator and provided with large quantities of very clean air, the rest of the room being provided with lower quantities of air. Isolators are examined in Chapter 9.

From this starting point, there have been considerable advancements with cleanroom design. These are aimed at ensuring that the cleanroom is designed in a way which ensures that it meets the requirements of the user and is designed in the optimal way to ensure contamination control. Some of these risk-based initiatives are examined in Chapter 26. The implications for microbiologists and for contamination control is that the enthusiasm of altering the operation of HVAC (heating ventilation and air conditioning) parameters is that these may impact upon the level of non-viable particles and viable counts. It is therefore important that microbiologists are consulted in any decisions relating to cleanroom parameters.

8.6 Conclusion

This chapter has provided an overview of the tests required for the certification and classification of cleanrooms. The chapter has not been written so much as a practitioner's guide but in a way that the cleanroom manager and quality assurance personnel can understand what is required and what is the purpose behind the test. For this the chapter has set out to describe the purpose cleanrooms and their contamination control measures.

In describing the cleanroom tests, both mandatory and optional, most of the focus has been upon the international cleanroom standard ISO 14644. This standard has set out the requirements for cleanrooms, as a universally accepted guideline, since 2001. It has been noted that, at the time of this book's publication (2013), that some parts of cleanroom standard are subjected to revision.

The chapter has also outlined where variations to the ISO standards could be considered. This is with particular regard to the growing interest in energy saving. Whilst the chapter, and indeed the views of the editors, supports such initiatives, it is important to make any changes through formal change control and to consider any contamination risks. Cleanrooms in the healthcare and pharmaceutical sector are in place to protect the product and patient from microbial contamination.

8.7 References

[1] Sandle T, Saghee MR and Ramstrop M. Environmental Monitoring and Cleanrooms, IDMA-APA Guideline, Technical Monograph No.5*, Indian Drug Manufacturers Association*, Mumbai, India, 2010.

[2] FDA. Guidance for Industry. Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice. Rockville, MD, USA, Food and Drug Administration, 2004.

[3] Eudralex. The Rules Governing Medicinal Products in the European Community, Annex 1, Brussels: Belgium: European Commission, 2009.

[4] Good manufacturing practices for sterile pharmaceutical products. In: WHO Expert Committee on Specification for Pharmaceutical Preparations. Thirty-sixth report. Geneva, World Health Organisation, Annex 6 (WHO Technical Report Series, No. 902), 2002.

[5] Halls NA. *Achieving Sterility in Medical and Pharmaceutical Products*. New York: Marcel Dekker, 1994.

[6] Whyte W. *Cleanroom Technology: Fundamentals of Design, Testing and Operation*, Wiley, London, 2001.

[7] Ljungqvist B and Reinmüller B. *Clean room design – Minimizing Contamination Through Proper Design*. Interpharm Press, Buffalo Grove IL/USA, 1997.

[8] Ljungqvist B and Reinmüller B. Modern cleanroom clothing systems: people as a contamination source. *PDA Journal of Pharmaceutical Science and Technology* 2003;**57**(2):114-125.

[9] Sandle T. The use of polymeric flooring to reduce contamination in a cleanroom changing area. *European Journal of Parenteral and Pharmaceutical Sciences* 2006; **11**(3):75-80.

[10] Sandle T. *The CDC Handbook: A Guide to Cleaning and Disinfecting Cleanrooms*, Grosvenor House Publishing, Surrey, UK, 2012.

[11] International Organisation for Standardization, ISO 14644-1: Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness. Geneva, Switzerland, 1999.

[12] Sandle T. The assessment of air-cleanliness in cleanrooms and clean zones using optical particle counters. *The Journal, Institute of Science and Technology*, Winter 2009, pp4-7.

[13] International Organisation for Standardization. ISO 21501-4: Determination of particle size distribution – Single particle light interaction methods – Part 4: Light scattering airborne particle counter for clean spaces, Geneva, Switzerland, 2007.

[14] Reinmüller B. *People as a Contamination Source – Clothing Systems.* In: Dispersion and Risk Assessment of Airborne Contaminants in Pharmaceutical Cleanrooms, Royal Institute of Technology*, Building Services Engineering, Bulletin No. 56*, pp54-57, Stockholm, Sweden, 2001.

[15] Möller ÅL. International standards for the design of cleanrooms. In: Whyte W. (Ed.). *Cleanroom Design*, 2nd edition. Chichester, John Wiley & Sons; pp21-50, 1999.

[16] PIC/S Pe 009-9. Guide to Good Manufacturing Practice for Medicinal Products. Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme, Geneva, Switzerland, 2009.

[17] International Organisation for Standardization. ISO 14644-2, Cleanrooms and associated controlled environments – Part 2: specifications for testing and monitoring to prove continued compliance with ISO 14644-1, Geneva, Switzerland, 2000.

[18] Eaton T. Annex 1 of the EC Guide to Good Manufacturing Practice (EC GMP) and continuous particle monitoring – help or hindrance of cleanroom manufacturing? *European Journal of Parenteral and Pharmaceutical Sciences* 2007; **12** (2): 3-7.

[19] International Organisation for Standardization 2009. ISO 1822-4. High efficiency air filters (HEPA and ULPA). Determining leakage of filter elements (scan method), Geneva, Switzerland, 2009.

[20] Sandle T. and Saghee MR. Inside the Box. *Pharmaceutical Manufacturing and Packaging Sourcer*, Summer 2012, pp54-57, 2012.

[21] International Organisation for Standardization 2001. Cleanrooms and associated controlled environments – Part 4:Design, construction and start-up, Geneva, Switzerland, 2001.

[22] Whyte W and Eaton T. Microbiological contamination models for use in risk assessment during pharmaceutical production. *European Journal of Parenteral and Pharmaceutical Sciences* 2004;**9**(1):11-15.

[23] Agalloco J, Akers J, Madsen R. Choosing technologies for aseptic filling: Back to the future, forward to the past? *Pharmaceutical Engineering* 2007;**27**;1:8-16.

Fundamentals of pharmaceutical isolators

Brian Midcalf, Assistant PTQA Course Director (ret.), UK
John Neiger, Editor, Clean Air and Containment Review, UK
Tim Sandle, Head of Microbiology, BioProducts Laboratory, UK

ketabton.com

9.1 Introduction

The isolator concept had several different origins. These include the development of glove boxes for containment in the nuclear industry, the development of large isolators for housing germ-free mice or other small mammals in biomedical research, the development of hospital patient isolators for both immuno-compromised patients and patients with highly infectious diseases and the development of lead-lined containment isolators for radiopharmaceuticals. The developments of isolators continued with an ever wider range of applications for pharmaceutical manufacture and hospital aseptic production, processing and dispensing, followed by isolators for sterility testing.

This chapter starts with the very basic fundamentals of the isolator concept and then develops through why an isolator is required, isolator applications, isolator types and design, construction, ventilation aspects, access and transfer devices, controls and monitoring.

9.2 Isolator basic concepts

This basic explanation of an ‘isolator’ is aimed at those who have very little idea of what an isolator is. The explanation begins with a simple analogy and then immediately contradicts this to come in line with the current definition of an isolator. It then goes on to consider some of the essential components which are dealt with in more detail later in this chapter.

An ‘isolator’ might, at first sight, be thought to be a ‘sterile box’. This is however an inaccurate concept as an isolator should not be regarded as ‘sterile’ or a ‘box’. The space inside an isolator is defined and contained. Therefore because of numerous and varied configurations, the isolator is better described as a ‘separative device’.

The concept of sterility is more subtle and will be addressed later but suffice it to say that an isolator cannot be regarded as a pressure vessel that can be hermetically sealed. It therefore cannot be regarded as a sterile device or container. The special environment in an isolator is designed to be free from contamination. This means the ‘controlled work space’ is free of all particulates, including micro-organisms, down to specified levels. Aerodynamic or physical separation or a combination of these is used to achieve and then maintain this controlled environment. As may be realised, this concept is not so simple in practice and numerous things can go wrong which in turn can compromise the controlled workspace.

The current *definition* of an ‘isolator’ as given in *Pharmaceutical Isolators*[1] is: “A separative device as defined in ISO 14644-7:2004[2] and used for pharmaceutical and related applications. It utilises constructional and/or aerodynamic means to enclose a controlled workspace”. An important note is added in italics: *“An isolator is not a sterilising device”*.

Mention has been made of particle-free air contained within a device. The air is rendered free of particles by an air filter which is of the High Efficiency Particulate Air (HEPA) type. This filter, by definition, removes a very high percentage of particulates from the air passing through it. Filters come in various grades, classified according to the percentage penetration of the most penetrating

particle size (MPPS), which is typically between 0.1 – 0.3μm. More information on HEPA filters is provided in section 9.5.5. Clearly there now follows a need to supply this filtered air to the controlled work space. This is usually done by a centrifugal fan, forcing air through the HEPA filter at a specified flow rate to give an air change rate determined by the system's design. As we now have a flow direction through a HEPA filter, we also have a pressure differential relative to the background environment. This pressure differential may be positive or negative, depending, in simple terms, on whether the fan is pushing the air into the controlled workspace or drawing it out. Both the air flow rate and the differential pressure should be displayed on the isolator to confirm that the isolator is functioning correctly.

The types of isolator available are quite varied. In basic construction they may have either a flexible film barrier or a rigid physical barrier. The controlled workspace may be positive or negative pressure relative to the background environment and the airflow inside may be unidirectional (sometimes incorrectly known as laminar flow) as shown in Figure 1 or non-unidirectional (turbulent) as shown in Figure 2. Isolators require transfer devices to enable items to be passed into or out of the controlled workspace and access devices to permit manipulation of the items inside.

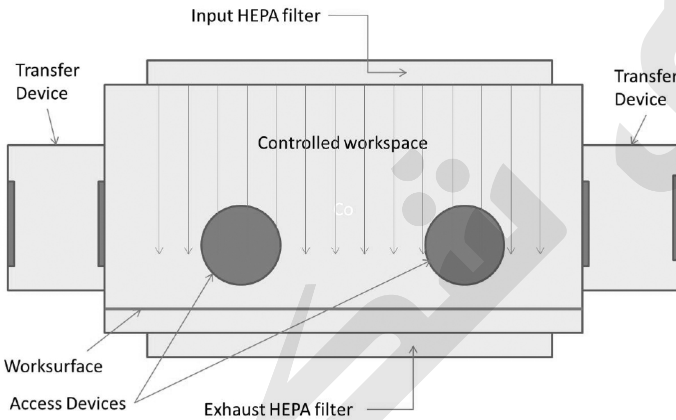


Figure 1: *Basic schematic layout of a unidirectional airflow isolator.*

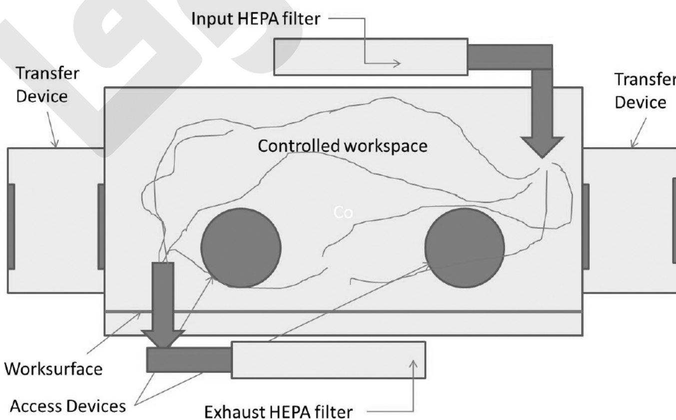


Figure 2: *Basic schematic layout of a non-unidirectional airflow isolator.*

Different types of transfer device, including simple doors, air-purged chambers and rapid transfer ports are defined and classified in ISO 14644-7 and described in section 9.5.2 of this chapter. Access devices, including gauntlets, glove/sleeve systems and remote controlled robotic devices, are also described in ISO 14644-7 and in section 9.5.3 of this chapter. It may be realised that the introduction of transfer devices and access devices adds to the risk of compromising the controlled work space due to poor design (e.g. selection of transfer device type) or poor operation or, more usually, through leaks. Therefore maximum acceptable levels for leakage rates must be set and maintained, as it is through uncontrolled leaks that the controlled workspace can become compromised and trouble can ensue. It should be noted that this is the main respect in which isolators differ from Restricted Access Barrier Systems (RABS). RABS are, as their acronym implies, barriers containing access devices such as gloves or gauntlets. No attempt is made to seal the controlled work space. The integrity of the controlled workspace relies on the HEPA filtered airflow alone and therefore separation is by aerodynamic means.

In addition to its physical and dynamic components, all pharmaceutical isolators require a means to achieve sanitisation of the controlled workspace and the items in it and entering it. Sanitisation is the process of reducing the number of micro-organisms in a space or in an inanimate matrix, by the action of an agent on their structure or metabolism, to a level judged to be appropriate. Sanitisation can be achieved by surface decontamination using alcoholic sprays and/or swabs or by dunking or by gaseous fumigation of controlled zones within an isolator system. It is normal to validate the process of sanitisation to determine its effectiveness. Cleaning and sanitisation are covered in sections 9.5.4, 9.11 and 9.12.

So now we have an introduction to what constitutes an isolator and its vital elements.

9.3 Why isolation technology is required

This section explains how isolators can be applied for product protection, containment, cross-contamination control and energy efficiency.

Having established that a pharmaceutical isolator is a separative device that separates a pharmaceutical process or activity from the operator and the surrounding background environment, we need to see why we may need one. There are indeed several reasons, and very often more than one of these may apply for a given application.

Product protection or contamination control

For applications that require the process to be in a classified clean or classified aseptic environment, protected from microbial and/or non-viable contamination arising from the operator and the surrounding environment, a suitably designed isolator has advantages over cleanrooms and open-fronted unidirectional airflow cabinets and enclosures. In particular, the isolator provides a physical barrier between the process and human beings who are the greatest source of potential contamination.

Containment or operator protection

For applications that involve toxic substances and substances of unknown toxicity, a suitably designed isolator, usually negative pressure in relation to the surrounding environment, will minimise the risk of exposure of operators and the surrounding environment to such substances. Again, the physical barrier provided by the isolator is more effective than the aerodynamic barriers provided by, for example, class II type microbiological safety cabinets and powder containment booths.

Cross-contamination control or control of process generated contamination

Cross-contamination is when a product or process becomes contaminated by another product or

process that is taking place nearby or that has taken place previously at the same location. The avoidance of cross-contamination is vital in the production of pharmaceuticals and biologicals as well as in other healthcare applications such as nuclear medicine (patient blood and elemental radionuclides). An isolator offers no additional advantages over other solutions for controlling cross-contamination, but the key is to design the interior of the isolator so it can be easily cleaned and decontaminated between process campaigns. A factor to note is that unidirectional airflow reduces the transfer of contamination across a working area in operation.

Energy efficiency

An isolator provides the opportunity to design a compact and more local controlled space around a particular process. This is inevitably more energy efficient and also supports the 'process core' concept set out in ISO 14544-4[3].

9.4 Key application of Isolators

This summary of isolator applications starts with two applications outside the pharmaceutical area. Descriptions of some of the most common industrial and hospital applications follow.

There is a very wide range of applications for isolators. In some of the applications, a high level of containment is required; in others, none. In some, the quality of the internal environment is the vital criterion. In others, it is of no importance. Often a combination is required. Each application therefore needs to be assessed to determine the level of containment and the quality of the internal environment that is required so that a solution can be engineered accordingly. Manufacturers of isolators should be able to justify their design in relation to the application.

9.4.1 Non-pharmaceutical applications

Biomedical research

Large isolators are used to house germ-free mice and other animals used in biomedical research. The essential requirement is for clean air so that the animals can be bred to be free from disease. A secondary requirement is for the safe control of waste products including airborne material.

Nuclear engineering

Gloveboxes, which are in effect isolators, are required to provide a very high level of containment. These normally have a significant amount of lead shielding against radiation as they are habitually used to process or handle radionuclides.

9.4.2 Pharmaceutical industry applications

Primary manufacture

Applications in primary manufacturing include: keg sampling, weighing and dispensing, mixing and blending, bulk charging and discharging of reactors, blenders, granulators and similar equipment, drying and filtering, milling and explosion-free handling.

Secondary manufacture

Applications in secondary manufacturing include crystallisation, micronising, tablet compression, tablet coating, sterile liquid operations, aseptic filling, filling and packaging machines, blow-fill-seal processing machines, aseptic access to depyrogenation ovens, autoclaves, freeze dryers (lyophilizers) and vacuum dryers and other aseptic handling of terminally sterilised components.

Sterility testing

For pharmaceutical manufacturers, sterility testing is perhaps the most exacting of all sterile applications as a false positive can result in the rejection of a very high value production batch (unless the sterility test can be invalidated through the failure investigation)[4]. The level of air cleanliness for sterility testing should be at least as good as that of the production process.

Therefore the use of an isolator, with a better assurance of a controlled contamination-free environment, helps guarantee a reduction of false positive results. There is an absolute requirement to transfer sterility test samples aseptically or without any risk of contamination of the product which, if it occurred, could give rise to an expensive false-positive result to the sterility test.

Table 1 shows the some typical industrial applications and the design aspects that might apply. GMP and hazard control should be in accordance with local regulatory requirements.

Table 1: Industrial isolator applications

APPLICATION	Positive pressure	Negative pressure	Flexible film	Rigid isolator	Unidirectional airflow	Turbulent flow	Gas sanitised
Powder weighing		✓	✓	✓	✓	✓	
Micronising		✓	✓	✓	✓	✓	
Keg-sampling		✓	✓	✓		✓	
Reactor loading		✓		✓		✓	
Reactor unloading		✓		✓		✓	
Filling lines (3)							
Pharmaceutical	✓	✓	✓	✓	✓	✓	✓
Medical products	✓	✓	✓	✓	✓	✓	✓
Sterility Testing	✓		✓	✓	✓	✓	✓

Note:

1. Some turbulent flow applications may require an inert atmosphere.
2. Unidirectional airflow can be total or local.
3. Filling line isolators are usually bespoke and therefore the subject of a design specification.
4. All isolators should avoid stagnant air pockets.
5. Rigid isolators can be painted mild steel, stainless steel or plastic depending on application.

9.4.3 Hospital pharmacy applications

In hospitals, isolators are used for:

- Central Intravenous Additive Service (CIVAS) with and without antibiotics
- Preparation and dispensing of cytotoxic drugs
- Compounding of Parenteral Nutrition (PN), Total Parenteral Nutrition (TPN) or Enteral Nutrition solutions
- Sterility testing of medicines
- Manipulation of monoclonal antibodies and gene therapy products
- Any other aseptic drug reconstitution and syringe, vial, bag or device filling
- Specialist radiopharmaceutical applications requiring protection of the radionuclide source. This also includes preparation of radionuclide labelled diagnostic agents and of patient's blood labelled with radiopharmaceuticals

With hospital isolators generally, the batch sizes handled are relatively small compared to those used in industry. Response times for individual patient prescriptions are more critical and shelf lives can be anything from 'for immediate use' to longer-term commercial batch storage. Hospital applications tend to be served by a variety of standard isolator designs compared to isolators for industry which are likely to be customised for specialised applications.

Table 2 shows the different key features of isolators that can be used for some typical hospital applications. GMP and hazard control should be in accordance with local regulatory requirements.

Table 2: Hospital isolator applications

APPLICATION	Positive pressure	Negative pressure	Flexible film	Rigid isolator	Unidirectional airflow	Turbulent flow	Gas sanitised
Aseptic preparation							
Parenteral nutrition	✓		✓	✓	✓	✓	
CIVAS	✓		✓	✓	✓	✓	
Cytotoxic	✓	✓	✓	✓	✓	✓	
Radiopharmaceutical		✓		✓		✓	
Aseptic batch production							
Parenteral nutrition	✓		✓	✓	✓	✓	✓
CIVAS	✓		✓	✓	✓	✓	✓
Cytotoxic	✓	✓	✓	✓	✓	✓	✓
Gene therapy	✓	✓	✓	✓	✓	✓	✓
Live virus		✓	✓	✓	✓	✓	✓
Blood products		✓	✓	✓	✓	✓	✓

Note:

1. Unidirectional airflow can be total or local.
2. All isolators should avoid stagnant air pockets.
3. Rigid isolators can be painted mild steel, stainless steel or plastic depending on application.

Summary

Traditionally, many of the above applications have been carried out using cleanroom technology. The use of isolators has enabled the operator, the largest source of contamination, to be separated from the process. Sporicidal gas generators have enabled high levels of bioburden reduction to be achieved and validated. In certain designs, the full cycle for gaseous sanitisation, including aeration, can be lengthy but the development of rapid gassing systems has made possible total cycle times as low as 20 minutes. Liquid sanitisation (spraying and wiping) still remains a viable option in hospital applications where a quick response is important, and the capital cost is also much lower. The decision whether to use a rapid gassing system or a spray-wipe system should be based on risk assessment.

Hazardous chemicals and microbial cultures are becoming more common and operators need assurance of their personal safety. Isolators can be designed to provide high containment as well as high microbial decontamination. Isolators can therefore help to overcome many current risk factors.

9.5 Isolator design

This section describes the design options available for the main components of an isolator system.

The recommended procedure for designing an isolator is for the user to draw up a User Requirement Specification (URS), distribute it to a shortlist of potential vendors and then develop it in detail with the selected vendor.

9.5.1 Construction of main enclosure and transfer devices

General

Isolators should be constructed from materials and components which are durable, capable of

maintaining a good air-tight seal, non-corroding, and resistant to agents selected for decontamination. All internal surfaces should be accessible to the operator for decontamination purposes, preferably without needing to open the isolator, i.e. accessible through access devices. Supporting structures and all external surfaces should also be easy to clean and corrosion-resistant. Construction materials can include flexible film, stainless steel, coated steel, glass and a wide range of plastics.

Flexible film

High quality 0.5mm transparent PVC may be used as a relatively inexpensive material for forming the envelope of an isolator designed for applications including aseptic dispensing, sterility testing, research and pilot-scale production. Optical quality PVC is normally specified for the areas that require clear visibility for the operator. A rigid external framework will normally support the flexible film envelope. The best currently available material for the framework is tubular stainless steel for which proprietary joining pieces are available. The envelope is fastened to the frame using welded flaps and either Velcro or press-stud type fastenings. Floors or work surfaces made of flexible film are not recommended unless the level of usage is to be extremely light. A major advantage of isolators constructed of flexible film is operator comfort.

Stainless steel

This may be used to form the enclosure of isolators for applications where there is a high rate of wear and tear. It may also be used for transfer chambers and similar parts. All parts need to be constructed so that cleaning can be carried out thoroughly and easily. This can be assisted by incorporating radiused (rounded) corners and smooth edges. Welded joints should be free from crevices and non-porous. The standard of internal finish should be specified. According to *Pharmaceutical Isolators*[1], most isolators have a finish in the region of 0.8 Ra (roughness average) which is equivalent to a grit size of 120. Smaller components, such as work trays can be electropolished but this is more difficult for larger items such as, for example, the main chamber of the isolator.

- 304 grade stainless steel is generally specified only for non-product-contact areas such as the enclosure itself but 316L grade stainless steel is increasingly being specified for these areas.
- 316L grade stainless steel is generally required by the pharmaceutical regulatory authorities, such as the MHRA and the US FDA for product contact areas such as containers, vessels and pipework.

It is strongly recommended that a certificate of conformity of the grade of stainless steel should be obtained.

Coated mild steel

Mild steel with a suitable polymer coating may be used in relatively light applications where non-corrosive and non-abrasive materials are to be handled. The coating process involves the electrostatic application of polymer powder followed by oven baking to cure the coating.

Rigid plastics

These may be used in relatively light applications. Users should be aware of possible hazing, crazing or cracking when plastics are exposed to some chemical solvents. It should also be noted that the use of plastics could give rise to a release of toxic fumes in the event of a fire.

Windows in rigid isolators

Windows may be made from safety plastics or laminated safety glass. Opening windows may be fitted for access to equipment. They should form an airtight seal when closed. They must, for safety, be interlocked to any moving machinery inside the isolator. Top-hinged windows should be supported safely when open.

Gaskets and seals, including pneumatic seals

Because isolators must be leak-free to a specified level, the correct selection of seals and gaskets is of great importance. Gaskets are used as an interface between the main fixed components of an isolator, such as the transfer chamber with the main chamber, and seals are used for components that open, such as doors and visors, and removable components, such as access panels and HEPA filters.

All seals and gasket should be made of a high quality material that is resistant to the cleaning and disinfecting agents to which they might be exposed. They should also be made of a material that has a 'memory', i.e. returns to its uncompressed state when components are taken apart or opened.

Pneumatic door seals which might leak or discharge into the internal controlled work space of the isolator should have suitable in-line filtration for the air supplies. Care should be taken as this filtration will not remove contamination introduced during assembly and re-assembly of the seal. Gas supply services to the isolator for other purposes should be similarly filtered.

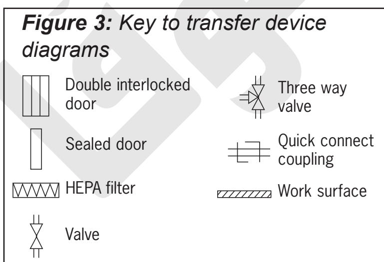
If an isolator is designed to be sealed, for example as part of a sporicidal gassing cycle, the manufacturer should be consulted to ensure that the isolator is fitted with all the necessary safety devices.

9.5.2 Transfer devices

Transfer devices are specifically designed to allow materials to be transferred into or out of the controlled workspace of an isolator whilst maintaining the separation of the controlled workspace from the background environment. Types of transfer device are classified from A to F for descriptive purposes in ISO 14644-7 and in *Pharmaceutical Isolators*. The classification does not imply any kind of ranking. For example a Type A transfer device is not inferior to a Type F transfer device but it is very different and has an entirely different use. Another important point is that some Types of transfer device are specifically designed for the transfer of materials between the controlled workspace of the isolator and the background environment and other Types of transfer device are specifically designed for the transfer of materials between 'sterile' areas.

For the purposes of this book, the classification used in *Pharmaceutical Isolators* will be used, but the differences with ISO 14644-7 will be noted.

Figure 3: *Key to transfer device diagrams*

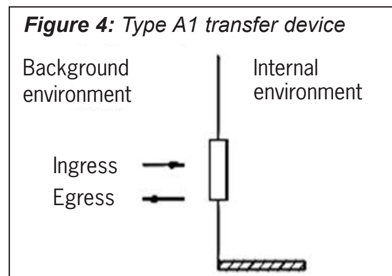


Type A1 transfer device

This is a simple door shown diagrammatically in **Figure 4**. There are two uses for a simple door:

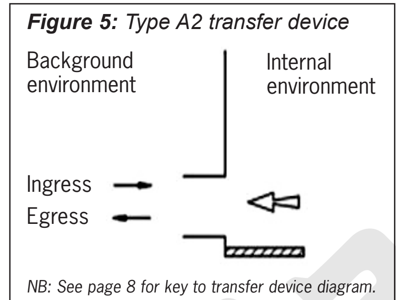
1. Loading an isolator with product prior to a sporicidal gassing process,
2. Occasional access for installing, maintaining or removing large equipment.

Figure 4: *Type A1 transfer device*



Type A2 transfer device.
This is a carefully engineered opening that relies on an outward flow of air to prevent the transfer of airborne contamination from the background environment during the material transfers. In the case of filling line isolators, the Type A2 transfer device is sometimes known as a mouse hole or dynamic hole. It is shown diagrammatically in **Figure 5**.

***Figure 5:** Type A2 transfer device*

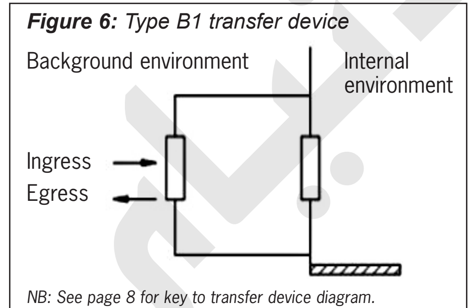


NB: See page 8 for key to transfer device diagram.

Type B1 transfer device
This is a simple chamber with two doors and nothing more. The chamber is attached to the isolator so that one door opens into the controlled workspace of the isolator and the other door opens onto the background environment.

It is important to note that where a transfer device has two doors, both doors should not be open at the same time. The opening of doors of such transfer devices often controlled by timed interlocks to prevent simultaneous opening. This applies to Type B2, C1, C2, D and E transfer devices.

***Figure 6:** Type B1 transfer device*

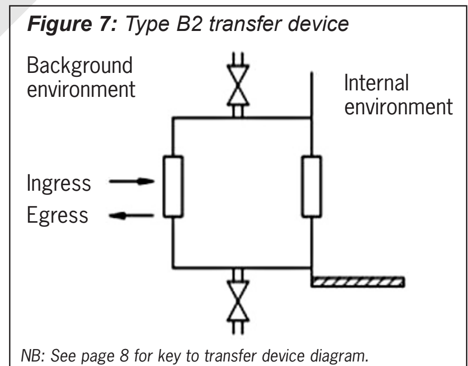


NB: See page 8 for key to transfer device diagram.

The type B1 is very rarely used as there is no means of preventing the transfer of potentially contaminated air into or out of the controlled workspace during material transfers. It is shown diagrammatically in **Figure 6**.

Type B2 transfer device
This is very similar to the Type B1 transfer device, but has gas connections that allow the space to be purged with inert gas or sporicidal gas, depending on the application, when both doors are closed. It is shown diagrammatically in **Figure 7**.

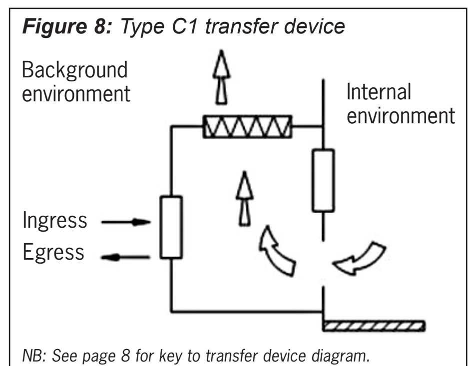
***Figure 7:** Type B2 transfer device*



NB: See page 8 for key to transfer device diagram.

Type C1 transfer device
This is a ventilated transfer chamber with an outer door, a HEPA filter in the side or top of the transfer chamber and an engineered opening alongside the inner door which leads to the controlled work space of the isolator. The opening allows unfiltered air from the controlled workspace to pass out into the transfer chamber and the HEPA filter allows the air to continue into the background environment when the outer door is closed. The Type C1 transfer device, widely used in the early days of hospital pharmacy isolators, can only be used with positive pressure isolators. Because of a theoretical concern that, with the outer door open, there is a path whereby airborne contamination from the background environment can reach the controlled workspace (against the direction of airflow), Type C1 devices

***Figure 8:** Type C1 transfer device*



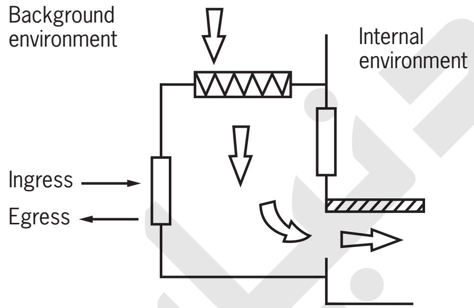
NB: See page 8 for key to transfer device diagram.

were eventually superseded by Type D transfer devices. As a point of interest, the equivalent concern does not exist with Type A2 mouse hole devices provided they are validated. The Type C1 device is shown diagrammatically in **Figure 8**.

Type C2 transfer device

This is also a ventilated transfer chamber with two doors and an engineered opening, in this case designed for a particular kind of unidirectional airflow negative pressure isolator that has a removable work tray with air slots to allow the air through from the controlled workspace to a plenum under the work tray before continuing into the isolator's return air and filtration system. The opening from the transfer chamber is into the plenum beneath the work tray. When the outer door of the transfer chamber is open, background air is drawn into the transfer chamber and then through the opening into the plenum under the work tray, where it joins the air from the controlled workspace to be filtered and either exhausted or recirculated. When both doors are closed, any contaminated air is rapidly purged from the chamber. As with the C1 device, because of the theoretical path (through the slots in the work tray against the airflow), Type C2 devices have been replaced by Type D transfer devices. The Type C2 device is shown diagrammatically in **Figure 9**.

Figure 9: *Type C2 transfer device*

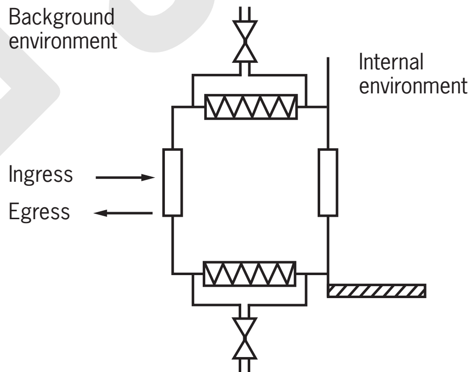


NB: See page 8 for key to transfer device diagram.

Type D transfer device

This is a ventilated transfer chamber with two doors and two HEPA filters. When either door is opened and then closed, contaminated air is rapidly purged from the chamber. The diagram shows that the Type D device has its own separate air supply and return, but *Pharmaceutical Isolators* recognises that in some isolator designs, Type D transfer devices may form part of the filtered airflow system of the isolator. Interlocking of the doors is important and ISO 14644-7 defines Type D1 and Type D2 devices, both with interlocks, the only difference between them being that the interlocks in the Type D2 devices are timed, which is good practice anyway. The Type D transfer device is shown diagrammatically in **Figure 10**.

Figure 10: *Type D transfer device*

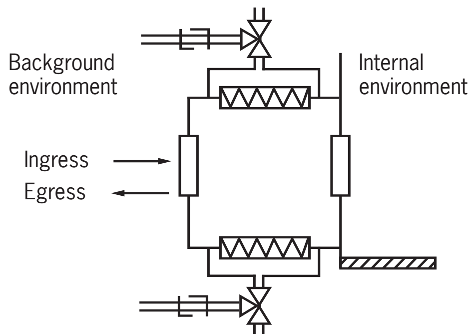


NB: See page 8 for key to transfer device diagram.

Type E transfer device

This is a Type D transfer device fitted with additional inlet and outlet gas connections for sporicidal gassing. Such devices, shown

Figure 11: *Type E transfer device*



NB: See page 8 for key to transfer device diagram.

diagrammatically in **Figure 11**, may be used for rapid sporicidal gassing or for sporicidal gassing where it is carried out in the whole isolator.

Type F transfer device

A Type F transfer device is a chamber or container that can be 'docked' onto an isolator in such a way that it can be opened into the isolator without allowing air from the background environment either into the isolator or into the transfer container. They have various names including docking port, rapid transfer port, DPTE (*double-portede transfer étanche*) and high containment transfer port. The operation of a Type F transfer is best described by reference to **Figure 12**. In the first sketch, the sealed container is being brought towards the closed docking port on the isolator. In the second sketch it has 'docked on' and in the third sketch the door of the docking port has been locked onto the lid of the container and both have been opened into the isolator. There is a 'ring of risk' where a small area of the annular sealing arrangement might be exposed to the both the background environment and the internal environment of the isolator.

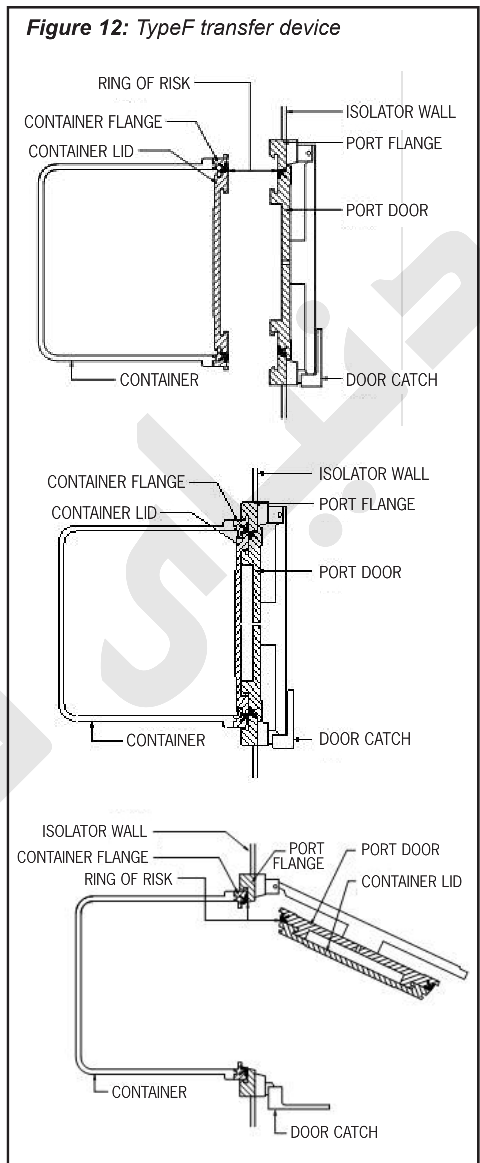
The application for a Type F transfer device is where materials have to be transferred between two 'sterile' areas without becoming contaminated.

Leak testing and material flow

It is considered practice to have a transfer device which can be leak tested both individually and as part of the whole isolator system.

Having a transfer device at each end of an isolator can permit a logical flow pattern of raw materials and components in at one end and finished products out at the other end thus avoiding cross-over.

Figure 12: *TypeF transfer device*



9.5.3 Access devices

Access devices are specifically designed to allow manipulations inside the controlled workspace of an isolator whilst maintaining the separation of controlled workspace from the background environment. They can range from glove-sleeve systems to robots. By contrast, a device which allows the transfer of materials between the inside and the outside of the isolator is classed as a transfer device.

Access devices should be compatible with the work to be carried out. Ergonomics and material compatibility are the principal factors in selecting the appropriate device. The position and mounting of glove sleeve systems or gauntlets is largely determined by ergonomics.

Gloves

The simplest and most common access devices are gloves, which are fitted to glove/sleeve systems. Gloves are the most critical component of an isolator because:

- They contain the operators' hands which are potentially contaminated (even after hand sanitisation) and may be sensitive to toxic materials.
- They are likely to be in contact with process items and with surfaces in the isolator.
- Leaks in gloves may be difficult to detect.

Gloves are the most vulnerable component of the isolator envelope or barrier because they are:

- Made of thin materials
- Prone to wear and tear
- Prone to physical damage
- Prone to chemical damage
- Prone to damage as they are changed, which may be at frequent intervals

Gloves should be of an appropriate design for operators to carry out the required manipulations. Most glove manufacturers will be able to advise on this. Important considerations are:

- They should be made of thin materials to provide good operator touch sensitivity.
- They should be made of a material that is sufficiently flexible for operator dexterity.
- They should be made of a material that is sufficiently elastic for the glove change system.
- They should be free of leaks.
- They should not be capable of contaminating the product in aseptic applications.
- They should be powder-free and sterile.
- They should provide the required level of operator protection. For toxic substances, they should have sufficient impermeability. For radiopharmaceuticals they should have a sufficient degree of radiation protection.
- They should be resistant to any chemicals or cleaning agents likely to be used, including, where applicable, gaseous sanitising agents. They should be capable of being cleaned.
- They should be made of a material which has sufficient strength and durability.
- They should be made of a material that is non-allergenic.
- They should be made of a material that has suitable electrostatic properties if required.
- Where double gloving is used, i.e. where the operator is already wearing a glove before entering the isolator glove, the materials of the respective gloves should not bind or adhere to each other.
- They should be the correct size for the operator and a range of sizes should be accommodated by the glove change system.
- They should be suitable for the glove change system. Usually this means that they should be beaded and should have a sufficient wrist length. (Care is needed as this is a variable).
- They should be as comfortable to wear as possible.
- They should be cost effective. Gloves that are changed frequently should cost considerably less than gloves that are sufficiently durable for long-term use.
- They should carry a CE Mark and, if particular requirements are specified, suitable certification should be available.

Compromises will need to be made, for example operator dexterity should be balanced against glove strength and thickness. Ambidextrous gloves may be cheaper than handed gloves. It should

be noted that cleanroom or isolator glove properties are not necessarily the same as those for gloves used directly in medical or surgical procedures.

Glove material

Gloves are available in a wide variety of materials and the **Table 3** provides information on the most commonly used materials.

Information about some of these and other materials is given in ISO 14644 Part 7.

Manufacturers should be expected to supply test data on their gloves but additional testing or evaluation may be performed by the user to determine the most suitable material for a particular process application.

Table 3: Glove materials

Material	Advantages	Disadvantages
NATURAL RUBBER LATEX	Exhibits softness and feel not yet achieved with other synthetic polymers. Resists most moderate chemicals, organic acids, alcohols, ketones and aldehydes. Is relatively inexpensive.	Attacked by ozone, strong acids, fats, most hydrocarbons and degrades with sterilising processes. In particular is fairly susceptible to hydrogen peroxide vapour. Can provoke an allergic reaction.
NEOPRENE	A general-purpose elastomer, resists moderate chemicals, acids, ozone, oils and solvents. Has a high tensile strength and flexibility and is good for sensitivity and dexterity.	Attacked by strong oxidizing acids, esters, chlorinated aromatics and nitro-hydrocarbons.
NITRILE	Has good solvent resistance, is very durable and has good puncture resistance.	Resists hydrocarbons and many chemicals, is attacked by ozone, ketones, esters, chlorinated and nitro hydrocarbons. Can be less elastic.
'HYPALON'	Has good resistance to oxidising chemicals, ozone, many acids, HPV (Hydrogen Peroxide Vapour) sterilisation, heat and abrasion. Very durable, has a high tensile strength can be produced in varying thicknesses down to 0.008" (0.02mm).	Is attacked by concentrated acids, esters, chlorinated and nitro-hydrocarbons. Relatively expensive.
BUTYL	Has a very low permeation of water vapours, gases and toxic chemicals, is resistant to oxygenating solvents and most oxidising chemicals and is flexible at low temperatures.	Is attacked by petroleum solvents, coal tar solvents and aromatic hydrocarbons.
'VITON'	Has excellent resistance to permeation from many chlorinated, aliphatic and aromatic hydrocarbons, most oils and acids. Is good for dexterity.	Is attacked by ketones, low molecular weight esters and nitro containing compounds.
EPDM (Ethylene Propylene)	Is soft and has ozone and vegetable oil resistance.	Is attacked by mineral oils and solvents.

Glove sizes

Gloves are still manufactured in accordance with National Codes and Standards. These may vary from country to country in respect of thickness, length, diameters and other features such as finger dimensions. International Standards are now being established and manufacturers are moving towards common dimensions and features.

In Europe, gloves generally comply with BS EN 420[5] and are produced either as anatomical, i.e. different left and right hand, or as ambidextrous, i.e. suitable for either hand.

It is better to have the fingers of the operator reaching the end of the glove without any "slack". Factors that govern glove size selection are:

- Physical characteristics of the operators
- Male or female operators
- Loose fit or form fitting
- Use of glove liners
- Use of double gloving
- Need for ventilation
- Operator comfort

Sleeves

General properties

Gloves are usually used with sleeves which are sealed to shoulder rings set in a window or visor, or, in the case of flexible film isolators, the envelope. Occasionally the sleeves are welded to the envelope so they form part of it. The sleeves are fitted with cuff rings to carry the gloves, which may be changed by various means including proprietary devices.

The logic here is that the gloves are the most vulnerable part of the isolator system and should be easily changeable, preferably without breach of containment. The next most vulnerable parts are the sleeves, and these too should be easily changeable, though not necessarily without breach of containment.

The material of sleeves should be tough enough to withstand continual folding and stretching, but flexible and supple, so that they do not restrict the user. The surface exposed to the isolator should be smooth and easily cleaned, whilst the operator side should be pleasant to the touch and neither sticky nor abrasive.

Some types of sleeve, made up from two separate un-bonded layers of material, are designed so that if either layer becomes punctured, then the two layers separate visibly, making the sleeves self-indicating of leaks.

Accordion sleeves

Sleeves can be constructed in an accordion or corrugated format to enhance movement and extension. This concept may be considered for some pharmaceutical isolators. Its main features are:

- Prevention of reversing outwards in positive pressure environments.
- Enhancement of operator comfort by holding the sleeve off the operator's arm and permitting airflow.

However, an accordion sleeve is not easy to clean and thus may not be suitable for aseptic applications.

Cuff rings and glove changes

Cuff rings are normally of moulded or machined plastic, with grooves to retain the beaded gloves

and sleeves and any security o-rings. They should be as light and unobtrusive as possible, but strong enough to withstand the glove change process.

Changing gloves

The simplest cuff rings only retain the sleeve and glove but do not permit gloves to be changed without breaking the barrier. It is sometimes necessary to change a glove whilst processing materials in an isolator. This is not possible whilst using the simple glove system and a more advanced glove change design should be used, details of which are given below. In changing a glove, it is very important to maintain the integrity of the isolator controlled workspace and not to introduce a breach of the isolator barrier.

Slightly more complex rings allow aseptic gloves to be changed by the glove-over-glove method to maintain the barrier. In this case, the bead of glove to be changed is first moved to the forward groove. Then a new glove is placed entirely over the old one, with its bead in the rearward groove. With the new glove in place the bead of the old glove is eased out of its groove and into the sleeve for removal. The isolator manufacturer may provide special sleeves, with widened wrists to allow for the change process.

Where hazardous substances such as cytotoxics are present, a safer variation of the aseptic glove change may be used. Here the operator should be wearing a protective glove before starting the glove change. The glove to be changed is inverted into the sleeve and held before removal. An impregnated wipe is used to clean the contaminated surface of the old glove around the cuff area and is then inserted into the inverted old glove for disposal. The new glove is then placed over the old glove and secured and the old glove removed. Finally the operator removes his own protective glove, turning it inside out to enclose the old contaminated glove and impregnated wipe for safe disposal.

Another type of cuff ring has a telescopic action, with the sleeve retained on an outer ring and the glove retained on an inner ring. Glove changes can be made by displacing the old inner ring and glove and pushing a new inner ring and glove into place. This system maintains the barrier and allows glove changes in either aseptic or containment isolator mode depending on the direction from which the new inner ring with glove is introduced.

Leak testing

Since the gauntlet or glove/sleeve assembly is a critical part of the system, isolator manufacturers often supply a device for leak testing the assembly independently from the body of the isolator. Such a device usually consists of a disc, which seals onto the shoulder ring, and a micromanometer to monitor pressure decay. The initial pressure differential may be the existing pressure differential between the inside and the outside of the isolator, or may be increased by inflating the sleeve. The value of this simple test should not be underestimated. The integrity of the isolator is partially dependent on the effectiveness of a gauntlet or glove/sleeve system in maintaining a physical barrier with the background environment so the initial evaluation of integrity of the access device and its continuation in the event of a glove change is important. The leak test should be effective, easy to perform and the staff able to demonstrate their ability to perform the test whenever required.

Shoulder rings

As with cuff rings, these are normally of moulded or machined plastic. In rigid isolators, they are usually sealed into the isolator window, although occasionally into the isolator wall. When used with flexible film isolators that do not have built in sleeves, they are fixed and sealed onto the envelope.

For negative isolators, the shoulder ring is mounted facing out of the isolator and the sleeve or gauntlet is changed from outside the isolator.

For positive pressure isolators, the shoulder ring is mounted facing into the isolator and the sleeve or gauntlet is changed from within the isolator. Because it is difficult to change sleeves in this configuration, they may be mounted outside as for negative pressure isolators, even for aseptic operations. This may inhibit penetration of sanitising agents and especially sanitising gas, to the area of the sleeve close to the shoulder ring.

Where shoulder rings are fitted to form access ports for infrequent use, beaded port seals or 'blanking caps' are available which avoid the need to leave glove-sleeve assemblies in the potentially degrading environment of the isolator.

Gauntlets

A gauntlet is effectively a one-piece sleeve and glove combined. It may be full or mid-arm length and the length is defined accordingly.

Gauntlets are particularly suitable for hazardous containment situations and can be produced in many materials including laminated materials and radiation protecting materials.

Gauntlets are produced in various sizes and configurations to the same European standards as gloves. As with gloves, they may be shaped to be anatomical or ambidextrous.

Gauntlets are not as sensitive in use as gloves and are generally used for more arduous or intensive operations where frequent changes are not appropriate. They are often used by more than one operator. In such situations, for hygiene, it is recommended that operators don suitable 'liner' gloves before entering the gauntlets.

The frequency of changing for gauntlets is normally extended to the maximum practical period. This is due to their high cost and the difficulty of carrying out a change without breaking the barrier. Gauntlets for use with isolators are thicker and more durable than gloves as they are intended to be used for longer periods. A gauntlet has a defined operational life and should be changed well before this is reached.

Manual gauntlet changes are possible without breaking containment. There are also several proprietary mechanical designs that facilitate changes in toxic applications, but these are all essentially heavy nuclear systems that have not found use in pharmaceutical applications.

Half-suits

Large isolators, both rigid and flexible film, may be designed to incorporate a half-suit. This enables an operator to be inside the isolator controlled work space, protected by a flexible film top half of a suit accessible (for entry) from outside the isolator, usually from under the work-surface. Half-suits are often welcomed by operators as they have greater flexibility and greater reach whilst working inside the isolator as well as a separate breathing air supply. Standard gauntlets and glove/sleeve assemblies give a working radius of about 500mm from the isolator window and a normal lifting capacity of up to 5kg. By comparison, use of a half-suit inside the isolator extends the reach to around 1,200mm with a lifting capacity of perhaps 15kg. Operators usually work in a standing position, but a tall chair or stool can be used to allow a comfortable seated operation.

The half-suit is normally mounted on the isolator base-tray or floor and may be on a raised or angled plinth to make entry and exit easier. The half-suit is fitted with a clear vision helmet which may be a separate, changeable item. It is also fitted with cuff rings on its sleeves to carry gloves. Half-suits need to be light, yet tough and flexible. A platform may be provided underneath the isolator so that operators of shorter stature can work comfortably. This can take the form of a fold-down platform or an adjustable powered platform.

All half-suits have a supply of breathing air which is fed to the neck region and also the wrist region. Some suits have a double-layer structure in which the inter-space forms the air duct. Others have air ducts formed in the flexible film over the surface of the suit.

Breathing air is most often supplied by a centrifugal fan. Air from the background environment may pass through a HEPA filter for added safety and comfort of the operator.

Operators should never enter a half-suit when the air supply is switched off. Should the supply fail at any time, the operator should leave the suit immediately. All operators should be trained in this procedure.

Operators are advised not to enter the suit during gas sanitisation of the isolator. There is a remote risk that sanitising gasses may enter the half-suit by permeation or leakage through the flexible film, so it is folly not to take this precaution. After gassing of the isolator is complete, the air supply to the suit should be run for an approved minimum time, probably at least 30 minutes, before the operator enters. This is because the sanitising gas can reach relatively high concentrations in a half-suit by diffusion depending on the gassing time.

Half-suits should be supplied with means of support when unoccupied to prevent collapse and to allow for free circulation during gas sanitisation. Such devices should be simple to operate and should not encumber the operator. Entering a half-suit whilst the isolator is under positive pressure is not entirely easy and consideration should be given to suits with means to aid entry, such as inflatable support rings to hold the half-suit off the operator.

Communication from half-suits is not easy and consideration should be given to the provision of intercom systems. Half-suits should be introduced to operators carefully to avoid adverse reaction such as claustrophobia. Compared with open-bench operation, half-suits are encumbering devices but some operators prefer them to glove/sleeve systems. They can be seen as advantageous as operators have their own air supply, good communication devices and a better ergonomic environment as they can reach more items without potential back or arm strain.

Provision for gassing

In applications where gas sanitisation is used, there should be provision for glove/sleeve assemblies, gauntlets or half-suits to be supported in an extended position during sanitisation. Suitable devices include inflatable inserts and floor-mounted supports.

9.5.4 Sanitisation systems

The principal methods of sanitisation and decontamination are:

- Surface decontamination by liquid cleaning, sanitisation or disinfection by spraying, swabbing or dunking. The currently recommended process is to spray, wipe and probably spray again. This is a relatively quick process that takes 1 to 10 minutes depending upon the number of units to be processed.
- Sporicidal gassing with bactericidal, fungicidal and sporicidal agents. The currently favoured gassing medium is hydrogen peroxide vapour. This gives a greater level of sterility assurance than liquid cleaning. cGMP expectations for pharmaceutical applications would be for a sporicidal gassing system as such systems can be validated more easily and can be shown to be reproducible. Rapid cycling systems are now available that have reduced cycle times down to 20 minutes or less.
- Clean in Place (CIP) by mechanical or automated cleaning systems.
- Sterilise in Place (SIP) by heat or other sterilising methods – this would normally only apply to interfaces with the isolator or to process equipment inside it.

9.5.5 Filtration and airflow

Filters

All isolators have HEPA (High Efficiency Particulate Air) filters. These remove viable and non-viable particles and aerosols from air passing through them. For air supplied to isolators, they are used to provide the required level of air cleanliness, typically EU GMP Grade A, in the controlled work space and also in certain types of transfer device. For exhaust air leaving isolators, they are used to protect personnel and the external environment from undesirable contamination that has arisen inside.

A HEPA filter is made of a sheet of highly specialised glass paper folded back and fore over spacing devices, as shown in **Figure 13**, and then sealed in to a frame, which is usually extruded aluminium. This construction allows a very large filter area to be contained within a relatively small frame. Several types of HEPA filter are available. Mini-pleat filters are typically between 24mm and 75mm deep and are therefore very compact. Deep pleat filters are usually between 150mm and 300mm deep and can therefore contain much more filter paper than a mini-pleat with the same face area. This means that for the same face area and volume flow rate the pressure drop is much lower. Cartridge HEPA filters, which are cylindrical, are suitable for some applications.

The performance of a HEPA filter is defined by its percentage efficiency or percentage penetration at the most penetrating particle size (MPPS) for that filter. The MPPS is a most useful characteristic of a HEPA filter and is due to the different mechanisms of filtration that take place, including straining, impaction, interception and diffusion, each of which is effective over a different range of particle sizes. HEPA filters therefore have a greater efficiency or lower penetration for particles that are smaller as well as larger than the MPPS.

In Europe, the standard that governs HEPA filters is EN 1822:2009 – High efficiency air filters (EPA, HEPA and ULPA)[6]. This is in five parts and it is Part 1 that contains the classification table

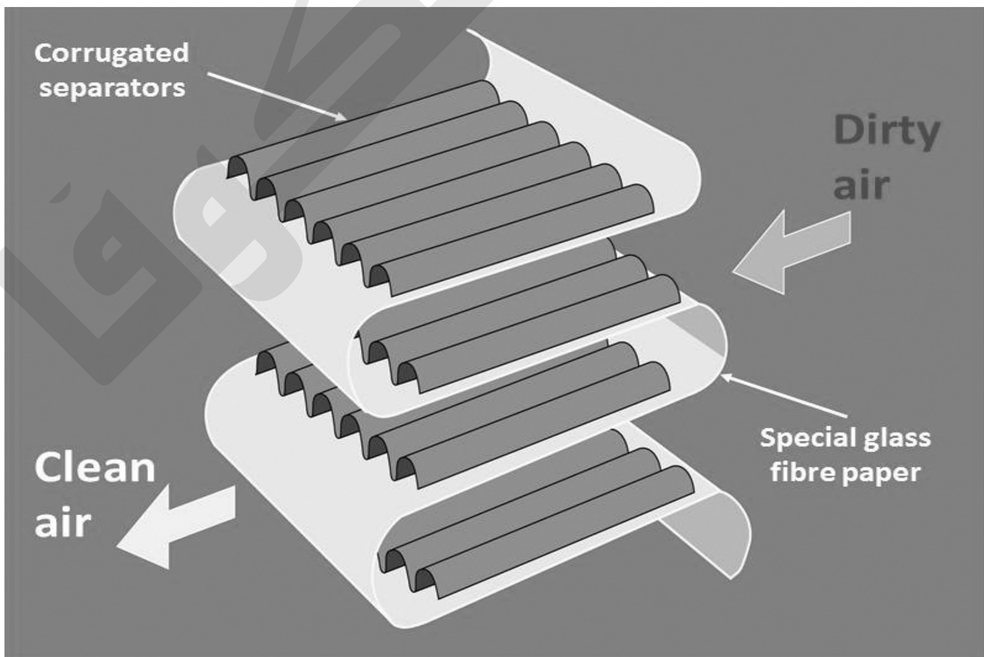


Figure 13: *How a HEPA filter works.*

Table 4: Classification table from Part 1 of EN 1822:2009

Filter Group Filter class	Integral value		Local value[ab]	
	Efficiency (%)	Penetration (%)	Efficiency (%)	Penetration (%)
E 10	≥85	≤15	---c	---c
E 11	≥95	≤5	---c	---c
E 12	≥99,5	≤0,5	---c	---c
H 13	≥99,95	≤0,05	≥99,75	≤0,25
H 14	≥99,995	≤0,005	≥99,975	≤0,025
U 15	≥99,999 5	≤0,000 5	≥99,997 5	≤0,002 5
U 16	≥99,999 95	≤0,000 05	≥99,999 75	≤0,000 25
U 17	≥99,999 995	≤0,000 005	≥99,999 9	≤0,000 1

[a] See EN1822-4
[b] Local penetration values lower than those given in the table may be agreed between supplier and purchaser
[c] Group E filters (Classes E 10, E 11 and E 12) cannot and shall not be leak tested for classification purposes

shown as **Table 4**. The lowest grades of filter called EPA (Efficient Particulate Air Filters) and prefixed ‘E’ cannot be tested for local leaks. The next grades of filter are called HEPA filters and are prefixed ‘H’. The highest grades of filter are called ULPA (Ultra Low Penetration Air) filters and are prefixed ‘U’.

Most isolators use H14 HEPA filters. At their rated volume, these have a maximum overall penetration of 0.005% at MPPS and a maximum local penetration (i.e. for a single leak) of 0.025%. The MPPS is usually between 0.1μm and 0.2μm. It is important to note that, if the volume flow rate through the filter is greater than the rated volume, then the performance of the filter deteriorates rapidly.

It is good practice to protect any HEPA filter that is likely to be subjected to a high level of airborne contamination with a coarse grade inexpensive pre-filter placed upstream of the HEPA filter.

As a high proportion of the energy consumed by an isolator is that applied to pushing or drawing air through the HEPA filters, thus should be a major consideration in the selection of filters.

Airflow systems

It is the airflow system of an isolator that determines whether it is positive pressure or negative pressure in relation to the surrounding environment and how effectively process generated contamination is removed. The airflow system also affects the internal air quality achieved and whether there are any eddies or dead zones where airborne contamination can linger. Positive pressure isolators are recommended where the predominant requirement is for product protection (protection of the product from airborne contamination). In the simplest isolators, the positive pressure is achieved by blowing filtered air through the isolator. Negative isolators are recommended where the predominant requirement is for containment or operator protection (protection of the operator from airborne contamination). In the simplest isolators negative pressure is achieved by drawing the air through the isolator.

An important option is the choice of unidirectional airflow or non-unidirectional airflow. Unidirectional airflow delivers air straight from the HEPA filter to the critical process area and also removes any process generated contamination from it. The definition for unidirectional airflow is:

> *‘Controlled airflow through the entire cross-section of a clean zone with a steady velocity and approximately parallel airstreams’*

A further note adds:

‘This type of airflow results in a directed transport of particles from the clean zone.’

A typical negative pressure unidirectional airflow isolator is show in **Figure 14**. It can be seen that the air enters the work chamber through a large HEPA filter in the roof of the chamber and leaves via the gap around the work tray. The transfer chambers are also purged with HEPA filtered air and the two airstreams meet under the work tray. All the air returns up the airway behind the work chamber. Part is then recirculated through the downflow filter and part exhausted.

Non-unidirectional flow is where the air supply entering the clean zone mixes with the internal air. Process generated contamination is also mixed with the internal air where it circulates until it is removed in the isolator exhaust.

A typical non-unidirectional airflow isolator is shown in **Figure 15**. HEPA filtered clean air is drawn through the transfer chambers and the working zone of the isolator by means of a remote fan. This is a much simpler arrangement than the unidirectional airflow design.

The main advantage of this type of isolator compared with a unidirectional airflow isolator is that it is simpler and more compact. It is also more easily adapted to different sizes and to incorporate built in equipment such as centrifuges and ionisation chambers. Disadvantages are that there is nowhere where clean air straight from the filter can be guaranteed and purge times in both the working zone and the transfer chambers are significantly longer.

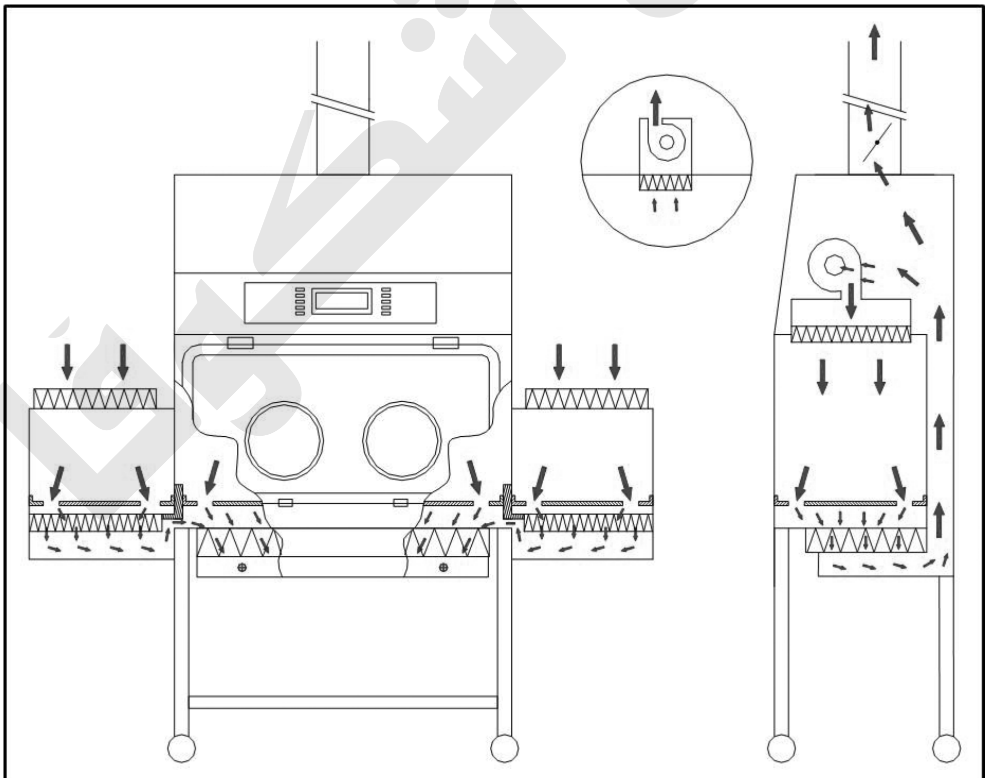


Figure 14: *Airflow diagram for a negative pressure isolator with unidirectional airflow. Diagram courtesy of Envair Limited.*

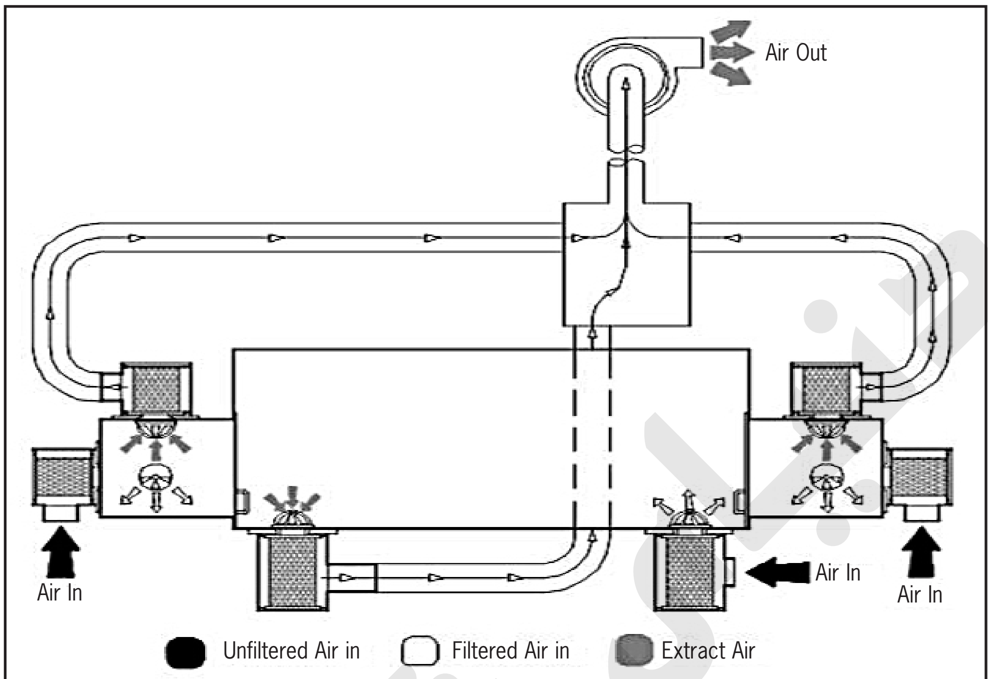


Figure 15: *Airflow diagram for a negative pressure isolator with non-unidirectional airflow. Diagram courtesy of Amercare Limited.*

9.5.6 Leakage and leaktightness

All isolators leak to some extent. It is therefore important to understand the possible effects of leaks in all types of isolator in order to quantify and rationalise a leakage rate that is acceptable in each case. If the leakage rate is set at too relaxed a level, then excessive contamination might pass through the leak to the detriment of the performance of the isolator. Conversely, if it is set at too tight a level, the cost of the isolator and its ongoing monitoring might be unnecessarily high. The maximum acceptable leakage rate should be determined for every design of isolator in relation to its intended application. Guidance on this is given in *Pharmaceutical Isolators* and the relevant page numbers are given in the text below.

The glove/sleeve and/or half-suit systems are the most likely components of an isolator to suffer wear and tear and thus develop leaks. As they are they are likely to be in the critical zone, they warrant special attention at the design stage and when monitoring protocols are developed.

Leakage in positive pressure isolators

In positive pressure isolators, leaktightness is primarily important where sporicidal gassing is used or where hazardous materials are handled. Any leakage from the isolator may be hazardous to the operator and the occupational exposure limit (OEL) for the material must not be exceeded. Sporicidal gassing systems may include provision for isolator leak testing and the maximum acceptable leak rate may be determined during development of the gassing cycle, when the air change rate in the room should be taken into account.

Where there is no gassing, it is possible for positive pressure isolators to go negative momentarily during the removal of hands from gloves. Therefore, a value for leakage of 1% volume per hour

(equivalent to a pressure decay of 25Pa from 250Pa in $1^1/_2$ minutes) is widely used. ['*Pharmaceutical Isolators* p115]

Leakage in negative pressure isolators

In negative pressure isolators, leaktightness is primarily important because of contaminated air from the background environment may enter the controlled workspace and compromise the aseptic environment. For turbulent flow isolators with a low air change rate of the order of 180 air changes per hour, an arbitrary value for leakage of 0.25% per hour may be used. ['*Pharmaceutical Isolators*' p118-119]. For unidirectional airflow isolators where the air change rate is much greater at around 1800 air changes an hour, a more relaxed value for leakage of 1.0% volume per hour is widely used. [*Pharmaceutical Isolators* p115]. The rationale for this is based on the greater dilution and the more rapid removal of any airborne contamination that might enter the controlled workspace of the isolator. This dilution is not maintained if the airflow stops as in an electrical supply failure.

It should be noted that even at moderate operating pressures (depressions), a leak in the isolator envelope could result in a gradually dispersing jet of air moving at several metres per second. This jet will only break up fully when it strikes something solid.

Isolator manufacturers should estimate the rate of entry of contamination at a given leak rate and its rate of removal in the isolator airflow.

High pressure integrity/low hourly leak rate isolators

A further leakage class of 0.05% volume per hour is specified for high containment enclosures such as Class III microbiological safety cabinets and nuclear gloveboxes ['*Pharmaceutical Isolators*' p 116]. This leakage class is not normally specified for pharmaceutical isolators.

Gloves, sleeves and half-suits

Leaks in gauntlets, gloves, sleeves and half-suits are likely to present a greater risk than those in the main body of an isolator. This is because these components are:

- the least robust components of the isolator
- the point of closest contact between the process and the operator
- even when not in use, possibly close to critical parts of the process

The donning of gloves and gauntlets may produce a pumping action that can raise the pressure inside them and force trapped air into the isolator.

For these reasons, it is important to:

- specify gauntlets, gloves, sleeves and half-suits that are of good quality and have an appropriate resistance to wear and tear
- specify suitable test protocols for frequent monitoring for leaks

Internal leakage

Internal leakage can occur between different sections of an isolator, for example across internal doors. Designers should consider how such leakage should be tested at validation and re-validation and make suitable provision.

HEPA filter seals may leak. It is possible to mount them in a fail-safe manner as described in BS EN 12469[7], (Performance criteria for microbiological safety cabinets) which states that 'HEPA filters shall be mounted in such a way that no air can bypass the filter medium'. This would ensure that any leakage across a seal does not release background air into positive pressure isolators or release potentially contaminated air from negative pressure isolators.

9.5.7 Isolator controls

Having described the main elements of an isolator, it is appropriate to describe the controls that are required.

After the on-off switch, it is clear that the most important controls are the fan speed controllers as they govern both the air change rate and the isolator pressure. Other controls may include transfer chamber door opening and door interlocks and semi-automatic or automatic pressure decay tests. ‘Hidden’ controls are often used to control the fan start-up sequence, to avoid overloading the power supply on start-up, and safety interlocks.

Isolator controls are usually integrated with displays of the key parameters, visual or audible alarms that activate when any of the key parameters go out of specification and basic data recording.

The key parameters that should be displayed are:

- Isolator pressure relative to the background environment
- Pressure differential across the main HEPA filters, (i.e. those most likely to become blocked)
- Isolator downflow velocity (unidirectional airflow isolators)
- Isolator volume flow rate (non-unidirectional airflow isolators)
- Particle concentration (where monitoring system fitted)
- Pressure decay leak testing sequence

Note: Both downflow velocity and volume flow rate can be converted to air change rate which some users may prefer.

The isolator and its control system must be provided with all the connections and access points necessary for all the tests and calibrations that need to be carried out from time to time, including connection points for micromanometers and access for measuring the upstream challenge and scanning the clean downstream side of every filter for installed HEPA filter leak tests.

Apart from the simplest isolators, which have the most basic of controls and indicators such as on-off switches for the fans and dial type diaphragm differential pressure gauges for internal pressures, most pharmaceutical isolators now utilise PLCs (programmable logic controllers) in conjunction with pressure and air velocity sensors. PLCs are highly versatile and can be programmed to control, indicate and alarm the parameters as required for any particular isolator design, whether a widely used standard design or a unique special design.

A PLC has other important features as well. Firstly it can be programmed to have various levels of access which may be password protected. For example, operator access might be limited to start-up and transfer chamber door activation; management access might be limited to altering the transfer chamber interlock timing delay, whilst engineer access would allow access to everything.

The second important feature is that a PLC can be programmed to activate controls of other equipment that is separate from the isolator, or even link with the PLCs of such equipment. A good example is where isolators are sporicidally gassed. Here it is possible not just to open and close the gassing valves, but also to adjust the mode of the isolator during the gassing cycle so that it is appropriate for each individual part of the cycle.

The regulator authorities now have a great focus on automated controls and computerised systems. PLCs come within the scope of the regulations that apply, namely GAMP®[8], 21 CFR Part

11[9] and EU GMP Annex 11[10]. PLCs can be standard, configurable (or customisable) or custom (bespoke). Obviously these require different levels of validation, with the standard PLC requiring validation to the point where it is established that every standard unit will perform in the same way, and every custom PLC requiring individual validation.

9.5.8 Waste removal

Waste is generated from the processes involved in the use of an isolator. For pharmaceutical manipulations it can consist of packaging material from gloves, syringes, needles, ampoules, vials, administration devices, tubing and any test equipment that may be used at the time. All these items have packaging to protect them from contamination and therefore have to be unpacked either inside the isolator controlled work space or in the transfer device. At this stage the packaging becomes waste material.

The removal of waste material can become a problem unless it is planned and managed. It needs to be placed in a receptacle or container which can then be removed from the isolator controlled work space without compromising this space. Removal can be through the same transfer device as is used for product removal or a special dedicated transfer device, may be preferred.

One such special transfer device is a ‘bagging out port’ shown in **Figure 16**. This consists of a rigid tube protruding from the isolator and opening into it, over which is fed a long length of polyethylene ‘lay-flat’ tubing. This tubing, which will have been sterilised (probably by gamma irradiation), is taped to the rigid tube at the end nearest the isolator and sealed at its outer end to form a dedicated waste receptacle that can be drawn off as required. A radio frequency heat sealing device is provided to double seal the outer end of the polyethylene tubing to create sealed bags of waste which can be removed (by cutting between the seals) leaving a new clean receptacle for the next batch of waste. This arrangement would be classified as a type A1 transfer device and allows waste to be removed without at any time compromising the controlled work space of the isolator.

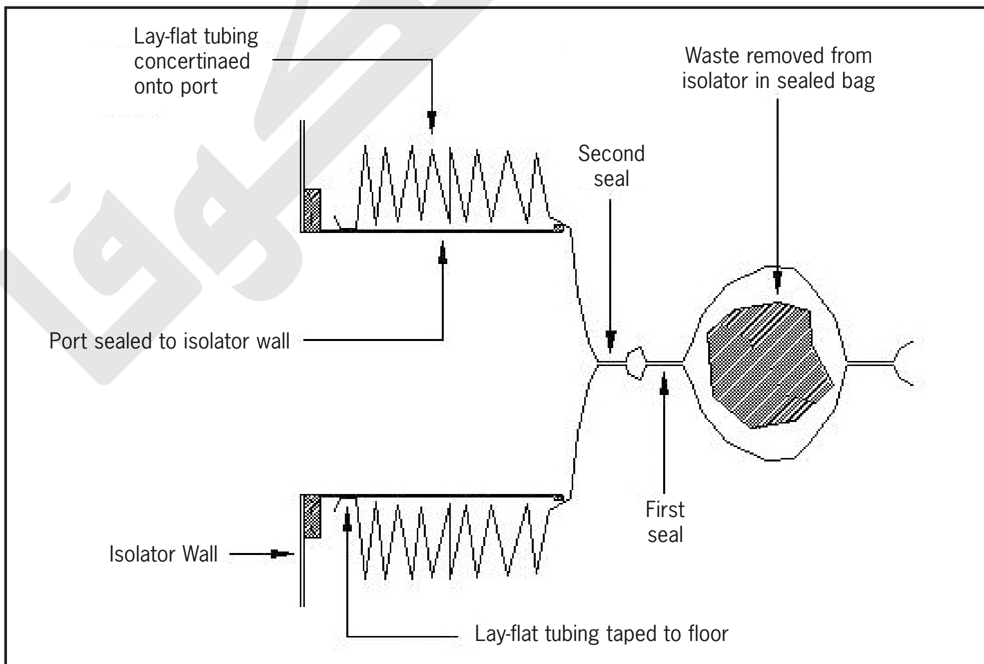


***Figure 16:** Bagging out port.*

Sharps need to be managed separately in order prevent operators from contaminating themselves with needle stick injuries. A standard sharps container should be positioned in the isolator in a safe and accessible place where it is convenient for dropping in the needles or other connecting devices. The full sharps container should be removed through whatever transfer device is provided, so as not to compromise the isolator controlled work space.

All waste should be disposed of carefully and in accordance with local waste disposal regulations. There may be specific regulations for sharps.

Waste management in an isolator, needs to be carefully planned as part of the material flow. Without planning, the controlled work space of the isolator would get seriously congested, usually with waste or used packaging material, making normal operation difficult or impossible. The amount of waste material generated may be more than initially anticipated and therefore its handling needs to be planned. This is a good opportunity to talk rubbish during a new isolator planning session!

9.6 Isolator siting

This section covers the background environment required for a cleanroom and the reasons for choosing that background environment. It also describes the protective clothing required in the two classes of cleanroom considered. Finally there are brief sections on cleanroom recovery testing and isolator exhausts.

9.6.1 Siting

It is important to note that an isolator is not a substitute for a cleanroom but can be sited in a lower grade cleanroom compared with for example, an open-fronted unidirectional airflow device. A well-known medicines inspector said in 1995 “An isolator that is not in a cleanroom is operating on the edge of failure.” Subsequently this became a requirement of the various issues of EU GMP Annex 1, which currently states that for an isolator used for aseptic processing, the air classification for the background environment should be at least grade D. It should be noted that GMP isolators are sited in cleanrooms, not just for regulatory reasons, but also because it makes sense to provide a clean background environment for handling materials into and out of the isolator. When isolators are used for sterility testing there is no formal requirement for them to be placed in a Grade D environment, although it is advisable to do so in order to add a further level of protection against the risk of contamination and to reduce false positives, hopefully to zero. It is indeed logical that the environment for sterility testing should be better than the environment in which the product to be tested has been manufactured.

Pharmaceutical Isolators gives general guidance for the siting of isolators. Some of the key recommendations include:-

- Isolators should be sited in a dedicated cleanroom, used only for the isolator(s), ancillary equipment and related activities.
- Segregation can be important and isolators used for incompatible activities, where cross-contamination between products must be avoided, should have separate dedicated rooms.
- Isolator cleanrooms should not be susceptible to contamination from services or equipment located close by, especially drains.
- Internal surfaces, including walls, floors, ceilings and work surfaces should be smooth, impervious and free from cracks and open joints. The materials used should be non-shedding, sealed and resistant to repeated treatment with cleaning agents and disinfectants.
- There should be a minimum of ledges. Shelves, cupboards and equipment in the room should be easily cleanable and free from dirt traps. There should be no uncleanable areas such as crevices or inaccessible recesses.

- False ceilings with lay-in tiles are not recommended, but if they have to be used, they must be of a non-shedding material and the tiles sealed in.
- Sinks or hand washing machines should not be sited in isolator cleanrooms.
- Access should be through a change room with interlocked doors.
- For isolators used for aseptic applications, the isolator room should be at least EU GMP Grade D.

EU GMP Grade D is much less stringent than Grade B in which open-fronted unidirectional airflow devices, such as unidirectional airflow cabinets and Class 2 microbiological safety cabinets, have to be sited and this is where the financial case can be made. In the cleanroom itself, although the fabric and construction are not much different, the air change rate can be lower down to a minimum of 20 per hour. In addition a Grade D cleanroom only requires a single stage change area whereas a Grade B cleanroom normally requires two stages.

9.6.2 Clothing regimes

The biggest saving of all is in the clothing regime. EU GMP Annex 1 states:

Grade D: Hair and, where relevant, beard should be covered. A general protective suit and appropriate shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination coming from outside the clean area.

Grade A/B: Headgear should totally enclose hair and, where relevant, beard and moustache; it should be tucked into the neck of the suit; a face mask should be worn to prevent the shedding of droplets. Appropriate sterilised, non-powdered rubber or plastic gloves and sterilised or disinfected footwear should be worn. Trouser-legs should be tucked inside the footwear and garment sleeves into the gloves. The protective clothing should shed virtually no fibres or particulate matter and retain particles shed by the body.

9.6.3 Cleanroom testing

This is covered extensively in chapters 8 and 10, but there is one point that confuses many people, which is why it is necessary to carry out a particle count in a room in the 'at rest' state when the critical state is the 'operational state'. The reason is very simple. If carried out in accordance with EU GMP Annex 1, the 'at rest' condition should be attained within 15 – 20 minutes of operations ceasing. In other words the test is to determine how effectively the room air cleans up. This is known as the recovery test in ISO 14644-3[11]. It has been known for cleanroom users to ask for the 'at rest' state to be carried out first thing in the morning when the cleanroom has had a chance to settle down overnight. Such a test is meaningless as it gives no indication of the clean-up effectiveness of the cleanroom airflow system.

9.6.4 Isolator exhausts

A factor that needs to be considered alongside siting is how to deal with isolator exhausts.

Gassed isolators and isolators used for handling hazardous materials and materials of unknown toxicity should ideally be ducted to atmosphere and each isolator should have its own dedicated exhaust. Exhaust ducts should be under negative pressure, which is achieved by placing the exhaust fan near the end of the duct, usually on the roof. *Pharmaceutical Isolators* suggests the discharge level of the exhaust stack is 2m above the height of the nearest building and the UK NERC (National Environmental Research Council) specifies (for fume cupboards, which are analogous) a high velocity discharge with a minimum discharge velocity of 10m/s[12].

If ducting is not possible then double exhaust HEPA filtration should be used. This should deal with

aerosols and particulates. However HEPA filters do not stop vapours, which might be toxic, and therefore carbon filters are sometimes specified. It should be noted that according to some unreported work at the University of Bath, UK, carbon filters will only remove some, but not necessarily all, toxic vapours from the airstream. For cytotoxic isolators at least, everything points to the use of ducting to atmosphere.

9.7 Performance, monitoring and testing

The assurance that an isolator is working in accordance with its design specification is provided by testing after installation and thereafter by regular monitoring and periodic testing.

Before an isolator can be tested at all, there are three essential steps that need to be taken at the design stage. These are:

1. Establish the values for the key performance parameters. These will be the values against which all testing and monitoring will be carried out.
2. Ensure that the isolator control system design includes all the necessary measuring sensors and read-outs.
3. Ensure that the isolator design includes all necessary access points and connections for external instrumentation.

Testing after installation is often known as Operational Qualification (OQ). Periodic testing usually follows a similar pattern and both are carried out by engineers, who might be independent, or from the manufacturer, or, in the case of larger companies, from the user. Regular monitoring is arranged by the user's organisation and is carried out by the operator, by supervision, by the Quality Assurance (QA) department or by a combination of these.

Regular monitoring will indicate trends and provide forewarning of items that might need attention. Periodic testing will provide verification of the monitoring results and will be an opportunity to calibrate the on-board sensors and instruments. Some tests, such as leak tests of installed HEPA filters, can only be carried out at periodic testing.

A typical schedule for testing and monitoring is shown in **Table 5** overleaf.

Many of the tests shown in **Table 5** are essentially similar to the tests carried out on a cleanroom, which are described in Chapters 6, 7 and 8. However the one test that is unique to isolators is the pressure decay test which is carried out to demonstrate that the leakage of air out of a positive pressure isolator or into a negative pressure isolator is within specified limits.

Leakage is often expressed as the percentage of isolator volume lost per hour, although the ratio of volume lost to isolator volume, expressed as h^{-1}, is easier to work with mathematically and is used in standards. ISO 14644-7[2] designates four levels as shown in **Table 6** overleaf.

Most isolator manufacturers specify their own maximum permissible leak rates which should be accompanied by a rationale. Mostly the leak rates are class 2 or class 3 as class 1 is considered too stringent and too difficult to measure and class 4 is considered inadequate.

There are several ways of locating leaks including the soap bubble test, helium testing and DOP testing. These tests do not quantify the leak rate and are generally used to locate leaks when the specified leak rate is exceeded. The most common method for measuring the leak rate is the pressure decay test. In this test the isolator has its supply air and exhaust air sealed off at a given pressure differential, which can be 250 Pa during operational use or 1,000 Pa for acceptance

Table 5: Typical schedule for testing and monitoring

Items to be tested/monitored	Factory Test	OQ Engineer	Periodic Engineer[1]	User Monitoring
Isolator pressure	Yes	Yes	6 months	Continuously[2]
Transfer chamber pressure (where applicable)	Yes	Yes	6 months	Continuously[2]
Pressure differential across the main HEPA filters, (i.e. those most likely to become blocked)	Yes	Yes	6 months	Continuously[2]
Installed HEPA filter leak test	Yes	Yes	6 months	–
Isolator downflow velocity (unidirectional airflow isolators)	Yes	Yes	6 months	Continuously[2]
Isolator volume flow rate(non-unidirectional airflow isolators)	Yes	Yes	6 months	Continuously[2]
Isolator exhaust volume flow rate	Yes	Yes	6 months	–
Uniformity of airflow (unidirectional airflow isolators)	Yes	Yes	6 months	–
Uniformity of mixing (non-unidirectional airflow isolators)	Yes	Yes	6 months	–
Recovery, or clean-up time	Yes	Yes	6 months	–
Particle concentration (where no monitoring system fitted)	Yes	Yes	6 months	–
Particle concentration (where monitoring system fitted)	Yes	Yes	6 months	Continuously[2]
Breach testing	Yes	Yes	6 months	–
Pressure decay leak testing	Yes	Yes	6 months	Weekly
Glove testing				Sessional
Functional testing including alarm settings	Yes	Yes	6 months	Weekly

1. Engineers will use their own calibrated instruments for their tests and use these to calibrate the instruments on the isolator where necessary.
2. Most of these readings will be displayed continuously. They should be recorded daily.

Table 6: Classification of isolator leak rates from ISO 14644-7:2004

Class	% leak rate per hour	Hourly leak rate h^{-1}
1	≤0.05	$\leq 5 \times 10^{-4}$
2	<0.25	$<2.5 \times 10^{-3}$
3	<1	$<1 \times 10^{-2}$
4	<10	$<1 \times 10^{-1}$

Table 7: Pressure decay times

Class	Hourly leak rate h^{-1}	Minimum time for pressure to decay 25 Pascals
1	$\leq 5 \times 10^{-4}$	30 minutes
2	$<2.5 \times 10^{-3}$	6 minutes
3	$<1 \times 10^{-2}$	1.5 minutes
4	$<1 \times 10^{-1}$	n/a

testing if ISO 14644-7 is being followed. The pressure is obviously negative in the case of negative pressure isolators. The time taken for the pressure differential to decay by 25 Pa is then noted. Values are shown in **Table 7**.

It should be noted that the decay time can be grossly distorted if during the test changes occur to either the internal temperature of the isolator or the external barometric pressure. If the barometric pressure changes by 0.1mbar during the course of a test, this will affect the isolator pressure by 10 Pa. Such a change can be due to a passing weather system but, more likely, due to nearby doors opening and closing. If the internal isolator temperature changes by 0.1°C, then the effect on internal pressure is 35 Pa. Such a temperature change can come from heat generated by internal lights or fan motors when the airflow has stopped. It has been reported by one practitioner that simply shining a floodlight into an isolator caused a big jump in pressure! It is therefore clear that, for the longer test of 30 minutes, precautions have to be taken either to ensure that isolator is in a stable state during a pressure decay test or to compensate for changes in internal temperature and barometric pressure by measuring these at the start and finish of the test and calculating the compensation. As the test can be affected by isolator volume in the same way, it is recommended to cap off glove openings before starting the test.

ISO 14644-7 states that the hourly leak rate of the transfer device shall not be greater than the hourly leak rate of the separative device. It is therefore important to be able to carry out appropriate leak tests on transfer devices.

Isolators are also subject to microbiological monitoring in the same way as cleanrooms.

9.8 Isolators used in pharmaceutical manufacturing

One application of isolators is for use in pharmaceutical manufacturing. This application has increased from the early part of the 21st century. There are particular advantages for using isolators for the filling of sterile injectable products due to the risk of contamination from the environment and from personnel.

The barrier isolator provides an advantage because:

a) The entire inside of the isolator can be decontaminated through a proven cycle using a surface decontaminating agent (such as hydrogen peroxide), The effectiveness of the decontamination cycle can be quantitatively assessed (through the use of biological indicators) and reproduced, in a way not possible within a cleanroom. The manual cleaning and disinfection of a cleanroom cannot be as effectively assessed and is time consuming.
b) The operators are separate from the product. Only the drug and material for manufacturing come in direct contact with the processing system.
c) Cross contamination is avoided by assembling the equipment prior to running a decontamination cycle.
d) The product is afforded greater protection through the containment and through unidirectional airflow.
e) The use of barrier isolators for sterile drug filling is favoured by regulators. In the 2004 aseptic filling guidance, the FDA states that:

   "A well-designed positive pressure isolator, supported by adequate procedures for its maintenance, monitoring, and controls, offers tangible advantages over traditional aseptic processing, including fewer opportunities for microbial contamination during processing…a process conducted in an isolator ... can have a low risk of contamination because of the lack of direct human intervention and can be simulated with a lower number of units as a proportion of the overall operation."

In developing isolators for manufacturing, care should be taken with ergonomic design, particularly ensuring that all critical aspects of the filling line can be accessed through glove ports.

The capital costs for isolators used for manufacturing are often substantial, given the size of the isolators required. Such costs can be recovered through the reduction in contamination events, the reduced time required in recovering from a power loss compared with cleaning or fumigating a

conventional cleanroom and the less rigorous cleanroom clothing regime that is required for working with an isolator – see 9.6.2.

9.9 Regulatory aspects on isolators – EU GMP, US FDA, PIC/S, Pharmacopoeia

This review presents an overview of isolators as referenced in GMP (Good Manufacturing Practice) documentation and in the pharmacopoeia. The review captures the key points raised in each of the guidance documents and highlights some of the differences between them. The review examines the European Union, World Health Organisation and the US Federal Drug Administration guidance documents.

9.9.1 EU GMP

Isolators are referenced in Annex 1 of the EU GMP Guide[13]. The guide indicates that the use of isolators provides a significant advancement in terms of contamination control measures:

“The utilisation of isolator technology to minimise human interventions in processing areas may result in a significant decrease in the risk of microbiological contamination of aseptically manufactured products from the environment”

Little mention is made regarding the operational requirement of isolators but it is noted that laminar flow [sic] may not exist in the working zone of all such devices. Furthermore, it is not explained how Grade A air is maintained without unidirectional airflow. However, reference is made to the need to validate isolators prior to use. The validation requirements are listed as: air quality, sanitisation process, material transfer and integrity testing.

In terms of the background environment, reference is made to the background environment needing to be appropriate (a minimum of EU GMP Grade D) so that the environment within the isolator is maintained at the required classification (namely EU GMP Grade A).

The guide focuses on the weaker aspects of isolator operation. The guide notes that isolators are prone to leaks (especially in relation to gloves and sleeves) and highlights that an area of concern is the transfer of material into and out of the isolator. Due to these weaknesses the guide states that routine monitoring, based on leak testing, should be undertaken.

9.9.2 FDA Aseptic Filling

The FDA aseptic filling guidance[14] includes a section on isolators. Like the EU GMP guide, the FDA guidance infers that the isolators provide additional assurances above those provided by a traditional cleanroom: “use of an isolator system further enhances product protection” which is based on the “lack of direct human intervention”. Unlike EU GMP reference is also made to the application of isolators for sterility testing, where the FDA guidance notes that such applications lower the possibility of false positive results occurring.

Significant information relating to isolators is found in Appendix I of the guide. Here there is a section on maintenance of isolators, which focuses on the risks posed from leaks to integrity of gloves, half-suits, and seams, and there is a section dealing specifically with glove integrity.

The second part of the appendix examines isolator design. In this part the following aspects are considered: airflow, materials of construction, pressure differentials.

With airflow, the guide notes that unidirectional airflow is a requirement for isolators used for aseptic processing whereas turbulent flow can be used for other applications (such as sterility testing)! In relation to pressure differential, the guide notes that the typical differential between the inside and outside of the isolator is 17.5 to 50 Pascals. The guide discusses air classification.

Unlike EU GMP, it specifically states that the internal environment must be ISO class 5 and that the surrounding room, for aseptic filling, must be classified at ISO class 8. No reference is made to the surrounding environment for isolators used for sterility testing.

The transfer of materials into and out of the isolator also has a section in the guide. The guide notes that some methods of decontamination in ports, such as ultraviolet light, are poor and should not be relied upon as effective decontamination processes. Instead the guide recommends localised HEPA (high efficiency particulate air) protection.

There is a section in the guide on decontamination. A note is made about the need for the decontaminating agent to reach all of the surfaces within the isolator (which requires attention to the layout within the isolator and the need to extend gloves fully). With the development of the decontamination cycle, importance is placed on validation for the cycle development. This is to be undertaken using biological indicators, placed in appropriate locations, with an acceptance criterion of four-to-six logs. The finalised cycle should have an 'over-kill' built in (that is the decontamination cycle should continue to some point in time after it has been demonstrated that the appropriate destruction of the microbial spores has been achieved). No reference is made to the most suitable types of biological indicator.

Further in relation to decontamination, the guide notes that decontamination should be undertaken regularly within the isolator and the frequency should be justified and documented. The verification of the isolator environment, especially when in use, must be examined through viable and particulate environmental monitoring.

The final part of the guide deals with training and the importance of aseptic technique for operators who use isolators.

Within the Code of Federal Regulations (CFR), occasional references are made to isolators. The most recent reference is with CFR 610.12[15], which covers the sterility test. A reference is made to isolators in relation to the FDA permitting no more than one repeat sterility test to be performed due to the isolator environment providing a higher level of assurance to protect the product under test from adventitious contamination.

9.9.3 PIC/S

The Pharmaceutical Inspection and Co-operation Scheme (PIC/S) has several references to isolators in its set of inspectorate guides. The most applicable, however, is 'Isolators Used for Aseptic Processing and Sterility Testing'[16]. Like the previous documents, the PIC/S document extolls the advantages afforded by isolators: "a more encompassing development of the barriers used in conventional clean rooms".

The PIC/S guide is far more detailed than either the EU GMP or FDA guidance (it is the only one of the three documents, for example, which draws a distinction between positive and negative pressure isolators). Isolators are defined as:

"An isolator is an arrangement of physical barriers that are integrated to the extent that isolators can be sealed in order to carry out a routine leak test based on pressure to meet specified limits. Internally it provides a workspace, which is separated from the surrounding environment. Manipulations can be carried out within the space from the outside without compromising its integrity."

The guide begins with a discussion about the surrounding environment for an isolator and draws a distinction between isolators used for aseptic processing (which are required to be housed within a classified environment) and those used for sterility testing (which do not require such an

environment). An importance is placed on airflow as the primary means for maintaining contamination control.

The guide proceeds to discuss the design considerations of isolators. These are divided into ergonomic requirements and having a design which can be appropriately decontaminated by means of a sporicidal process (using an agent which can kill bacterial endospores, fungal spores and vegetative micro-organisms). With the decontamination cycle this involves ensuring that all surfaces are reached by the gassing agent and that the material is compatible with the agent used. A fair amount of detail is provided in relation to decontamination cycle development, particularly in relation to gas distribution.

As with the FDA guide, importance is placed upon the development and qualification of the decontamination cycle and the requirement to verify this through the use of biological indicators. Whereas the FDA guide uses a four-to-six log range, the PIC/S guidance is for a minimum of six-log kill. The PIC/S document also states that the microorganism used as the biological indicator must have proven resistance to the sporicidal agent selected. The use of chemical indicators is optional. In relation to requalification, the guide recommends that biological indicator kill is assessed annually using the established cycle parameters.

Following the section on the decontamination cycle, the guide discusses the importance of preventing recontamination of the isolator environment. At this stage the guide emphasises the importance of air filtration and the maintenance of a positive pressure differential (a 10 Pa positive differential air pressure is required as a minimum, which is notably lower than that within the FDA guide). As with the FDA guide the areas of concern with respect to leaks are the integrity of gloves, sleeves and suits and, as with the other guides, the “transfer of material out of the isolator” which “should not compromise the critical zone.”

The guide also considers the importance of physical testing (where it is recommended that a risk assessment tool like Failure Modes and Effects Analysis is used) and for ‘in-use’ environmental monitoring (with an emphasis placed on particle monitoring). In terms of on-going monitoring, in relation to physical operation, the guide indicates that the following parameters should be assessed:

- Gas detection in the isolator/exhaust
- Gas concentration in the isolator/exhaust
- Flow rate in exhaust
- Gas inlet temperature
- Isolator pressure
- Pressure drop across filters
- Condensation detection
- Temperature of the external surface of the isolator
- Temperature of internal points in the isolator
- Absence of alarm conditions
- Correct operation and position of gas drivers such as fans, pumps and evacuation
- Displacement to expose occluded surfaces
- Gas concentration during ventilation
- Process step times

Whereas the FDA guide discusses aseptic technique, the PIC/S guide places more emphasis upon training in the use of the isolator and notes the inspectors will require staff to have an understanding of the operation of the isolator and particularly the gassing cycle.

9.9.4 World Health Organization

The World Health Organization (WHO) does not have a GMP document specifically for the use of isolators. However, reference is made to the use of isolators for sterility testing in the WHO guide for pharmaceutical microbiology laboratories[17]. The document makes reference to the need for environmental monitoring during sterility testing and the importance of six-monthly certification of key physical parameters, such as HEPA filter performance (leaks). The WHO guidance is not as detailed as that offered by the USP in relation to sterility testing (see below).

The WHO also makes reference to barrier technology in its quality assurance manual[18]. This is more in relation to the handling of cytotoxic products.

9.9.5 Pharmacopoeia

With the pharmacopoeias, the European Pharmacopoeia does not have a specific chapter on isolators. The primary reference made is in relation to biological indicator selection in Chapter 5.

With the United States Pharmacopeia reference is made to isolators in Chapter <797> 'Pharmaceutical Compounding—Sterile Preparations'[19], which deals with hospital pharmacies. This is in relation to selecting the appropriate environment for pharmaceutical preparations and no detailed guidance is given. A reference is also contained within the 'environmental monitoring' Chapter <1116> 'Microbiological Evaluation of Clean Rooms and other Controlled Environments'[20] in relation to the lower incidence of contamination expected from an isolator compared with a conventional cleanroom.

Far more detailed information is provided in the USP in relation to isolators used for the sterility test for which there is a specific chapter, Chapter <1208> 'Sterility Testing – Validation of Isolator Systems'[21]. In this chapter basic specifications are provided for air handling systems and for design issues, including location and transfer ports. Here some useful advice is provided in relation to installation qualification and performance qualification in a way which meets the current validation lifecycle approach.

The USP Chapter also provides reference to the validation of isolators through decontamination cycle development. There are aspects not covered in the GMP guides, such as the importance of ensuring that the sanitisation gas does not penetrate the packaging around consumables used within the isolator (this includes the requirement to perform growth promotion testing on the culture media used for the sterility test to verify that the sanitisation agent has not penetrated the medium). The chapter also makes reference to the requirement for environmental monitoring when the isolator is in use.

Summary

Considerable reference is made to pharmaceutical isolators in the EU and FDA GMPs, with supporting data provided from the PIC/S scheme where both the EMA and FDA are members. There is some additional information in the pharmacopoeias. Whilst the totality of the information is sometimes conflicting (notably in relation to the classification of the external environment and with regard to pressure differentials), and the detail is often lacking, the documents do provide an indication of the expectations of the regulatory authorities for design, physical test parameters and the validation of gassing cycles.

9.10 Overview on the ISO Standard for isolators

The principal standard that applies to isolators is ISO 14644-7:2004. This is one of the series of ISO 14644 standards described in Chapter 3. Because it is a generic standard and not industry specific, it utilises the general term 'separative device' to describe the devices covered in the standard which include 'clean air hoods, gloveboxes, isolators and mini-environments.

'Separative device' is defined in the normative section that covers terms and definitions, but a more helpful description is in Annex A. This reads:–

> *'A separative device utilises physical means, aerodynamic means or both to create improved levels of separation between the inside and outside of a defined volume'.*

The normative sections of the standard cover requirements to be agreed between customer and supplier; aspects of design and construction for consideration; access devices, which include gloves and gauntlets, remote manipulation and robotic handling; transfer devices; siting and installing and testing and approval.

Annex A describes the 'Separation continuum concept' which is a neat graphic explanation of how the 'assurance of maintaining separation' increases as the 'separation means' tightens from aerodynamic 'unrestricted air overspill' devices through to purely physical 'high pressure integrity/low hourly leak rate enclosures'.

Annex B gives guidance on 'Air-handling systems and gas systems' and Annex C gives guidance on 'Access devices'.

Annex D gives 'Examples of transfer devices'. These examples derive from 'Isolators for pharmaceutical applications'[22], which was written by the UK Pharmaceutical Isolator Working Party. The same working party went on to produce 'Pharmaceutical Isolators' in 2004, a comprehensive reference book, fully harmonised with ISO 14644-7 and the EU GMP.

Annex E covers 'Leak testing' by reference to ISO 10648-2:1994[23]. Pressure integrity is defined in terms of hourly leak rate but takes no note of isolator size! A method for estimating an acceptable hourly leak rate recognises the dilution effect of airflow in any space that would be contaminated by a leak.

9.11 Challenges with isolators

Brief discussion about current challenges with isolation technology.

Current challenges using isolators in the hospital environment relate to a number of factors including the site chosen for their use. The fundamental design of the isolator will relate to how current it is and when it was built. Many older designs have now been superseded with new concepts and this makes the isolator easier to use, easier to maintain and easier to keep clean and sanitised. So one of the current challenges may relate to managing an old design and making the best use of older technology. More recent designs of isolator and transfer devices will have fewer dirt traps or difficult to clean areas, interlocking devices which make it more fool proof to use, more effective hinges, closure devices and seals, and monitoring to indicate when the isolator is failing. Continuous monitoring systems will feedback to the operator or manager, the current status of the isolator including its differential pressure, air change rate, seal integrity and leak rate.

Another challenge relating to operating an isolator relates to the method of sanitisation. With the development of 'rapid gassing' techniques, gassing is becoming the preferred method of regulators as it gives a higher level of assurance of surface decontamination than can be obtained by spraying and swabbing. Gas sanitisation is approached in Chapters 11 and 13 and is worthy of serious consideration for anyone designing a new facility. If gas sanitisation is to be adopted, a new set of challenges emerges. These relate to the organisation of the work flow for the isolator. This requires very careful planning so that items for the next batch to be processed are gassed and ready for manipulation at the time specified and all items required are available when needed.

Isolator rooms are generally designed for a specific level of activity, therefore new challenges arise

when this level is exceeded. These challenges can be met by building extra facilities, extending the working hours or simply making better use of staff time in running the unit. Staff training may be another challenge and time and resources for comprehensive training will need to be allocated for aseptic services and isolator use. (See section 9.13 Training). The final challenge for management, having invested in this specialised training, is to keep trained staff available rather than have them seconded to other duties or responsibilities.

9.12 Cleaning and sanitisation

Pharmaceutical isolators require regular decontamination to facilitate product and operator protection. Decontamination normally comprises cleaning and sanitisation. These are different processes but may be combined.

This section includes some of the methods and practices available which are those currently in widespread use, as well as those of limited use or which are under development. To assist selection an overview is given on general methods, together with more detail on methods of choice, although this is not intended to be restrictive.

9.12.1 General considerations

The aim of cleaning is to reduce the contamination level of a surface. It should render it free from dust, organic or inorganic matter providing a visibly clean state. Cleaning will ensure that the bioburden on surfaces is as low as possible and give a greater assurance that any subsequent disinfection or gassing process will be effective. Please remember that an absolutely clean surface, one that doesn't have any contamination at all, must by definition be sterile. This ideal state is generally unobtainable practically.

If performed incorrectly, cleaning activities can actually increase the levels of contamination. Where possible, cleaning should be carried out by the operators who use the isolator as they will understand the importance of cleaning, what needs cleaning, and the most appropriate order of cleaning. Alternatively, cleaning may be carried out by specifically trained staff. Cleaning processes should follow validated SOPs (standard operating procedures). The effectiveness of cleaning processes should be validated, documented and regularly monitored.

The cleaning process applies to the controlled workspace of the isolator, the transfer devices associated with the isolator and the background environment, including the external surfaces of the isolator. Cleaning also applies to materials entering the isolator.

Isolators are used for a large range of manufacturing, preparation and dispensing processes. For aseptic work, cleaning is always either combined with or followed by disinfection (liquid sanitisation) or followed by gassing (gaseous sanitisation).

Opening the isolator for cleaning, by opening a front window for example, may give easier access for a thorough clean. This must then be followed by disinfection or gassing back in its closed state. Isolators used for hazardous materials such as cytotoxic drug dispensing or radiopharmaceuticals, may present a risk to operators if opened without taking suitable precautions.

Considerations when designing a cleaning process relate to the level and nature of contamination of materials as they enter the isolator and that of contamination generated by unpacking and processing. They also relate to the level of cleaning to be achieved and maintained and whether sanitisation is to be included as part of the cleaning process or carried out subsequently.

Identification of all surfaces which require cleaning is required, together with the surfaces that may be exposed during operations which will also include seal and gasket surfaces. The level of

acceptable residues needs to be determined as does the effect of cleaning on subsequent work to be carried out in the isolator.

The selection of cleaning agent to be used and the method and frequency of cleaning together with safety considerations for the use of such agents also needs consideration.

The development of SOPs and their validation and monitoring for cleaning is a natural development of this work. The effectiveness of the cleaning and any disinfection processes used should be regularly validated, documented and monitored. Cleaning will only be effective if it is carried out by trained staff, especially those who are involved in the aseptic processes, so relevant staff training becomes part of a cleaning regime.

9.12.2 Cleaning schedule or timing

The isolator controlled workspace and the transfer devices, should be cleaned before and after each manufacturing, aseptic preparation or dispensing session and between activities that may result in cross-contamination.

Cleaning of general areas, such as the background environment, should be carried out at defined intervals. This may be best done during natural breaks, such as meal times, or at the end of the working day.

Every time materials are introduced into an isolator from the background environment for aseptic processing, they should undergo a surface cleaning and disinfection process such as spraying and wiping with a sterile alcohol agent or other suitable, validated agents.

9.12.3 Cleaning methods

Isolators should be designed to facilitate easy cleaning and decontamination. Physical wiping is needed to assist in the removal of surface contamination. A progressing, overlapping, parallel wiping action should be used and circular movements avoided. Cleaning should progress from back to front, and from top to bottom. Non-shedding, low linting cloths should be used with an appropriate cleaning agent.

Special cleaning tools assist the cleaning of ‘out of reach’ areas when the isolator is closed. Such cleaning tools themselves must be capable of being decontaminated and any associated swabs or pads should be disposable. Procedures should be developed to prevent the risk of a tool introducing contamination or cross contamination due to poor technique. Cleaning materials such as wipes should be considered to be contaminated waste, which may present a hazard outside the isolator. Proper disposal procedures should be applied. Cleaning agents can present risks to the health of operators by inhalation or contact. Procedures should be developed to minimise these risks.

Where liquid agents are used, it is important that wetting is completed on all accessible surfaces. If sprays are used, these should be adjusted so that the aerosol droplets coalesce on the surface to give an even coverage. Over-wetting should also be avoided and use of a wipe after wetting can spread any contamination if there is too much liquid present. There are advantages in using a dry wipe after spraying. This will absorb any excess liquid, prevent spreading contamination and remove such contamination. The best method for cleaning needs to be validated for the type of process envisaged.

Surfaces should be dried after cleaning and before any subsequent sanitisation process. Drying the surfaces after cleaning may also be important to prevent corrosion.

Cleaning can be done manually or by an automated process – Clean in Place (CIP). With a manual method, it is difficult to achieve repeatability and assurance of full surface coverage, especially with

complex surfaces. This provides a challenge to validation. CIP processes as sometimes used in industrial isolators are more easily validated.

Operators play a key role in the success of all manual cleaning methods. SOPs are important training and control documents. Training of operators is a requirement of Good Manufacturing Practice.

9.12.4 Cleaning agents

Selection of cleaning agents is important to obtain an effective agent. The agent must also be compatible with all process and process equipment. In all cases the requirement is to remove a surface film of contamination. Whatever cleaning agent is used, it is essential that it is used at the manufacturers recommended dilution and according to the manufacturers recommended procedures. Failure to follow these guidelines may render the agent ineffective.

When preparing dilutions, the quality of the water used is important. Where cleaning agents are for use inside an isolator, water for injection (WFI) or similar purified water is recommended as the diluent.

Gross soil should be removed with the aid of a detergent. The types of soils that may be present should determine the choice of the detergent formulation. Detergent cleaners are usually classified into enzymatic and non-enzymatic, with the latter usually recommended for isolators. Non-enzymatic cleaners are further classified into alkaline, neutral and acid types. Alkaline cleaners are often a good choice for protein removal from a surface. The choice of a detergent will also depend on the compatibility with the isolator surfaces

If detergents are used to remove surface films, it is important to have a final rinse stage to ensure detergent residues are removed. Rinsing can be with purified water, water for injection (WFI), or sterile alcohol.

Sterile alcohol is frequently used as a combined cleaning and disinfecting agent and is recommended for cleaning non-gassed isolators. It should be noted that alcohol is not sporicidal. Alcohol based cleaning agents have been known to cause isolator enclosure materials to become ‘crazed’ or embrittled with time. This effect is reduced or eliminated if the surfaces are dried after treatment. Alcohol has the advantage that it removes surface films containing surfactants and some other contaminants. Alcohol may be rotated with aqueous detergents. For example a daily or pre-process ‘spray and wipe’ clean with 70% alcohol may be scheduled in combination with a monthly detergent clean. A detergent clean should always be followed by a purified water rinse to remove the detergent residues.

Surface films can harbour contamination and interfere with disinfection and gassing efficiency. Alcohols and aldehydes can fix organic material to surfaces. Therefore any spillages containing organic material, particularly proteins, should be cleaned up with a suitable detergent before disinfection or gassing. This is an example where alcohol should not be used as an initial cleaning agent.

Use of sterile impregnated wipes instead of sprays can reduce occupational exposure and wetting of surfaces. Spray alcohols represent a theoretical fire and explosion hazard if there is inadequate ventilation.

The EU GMP requires that disinfectants and detergents are monitored for microbial contamination. Diluted agents should be stored in previously cleaned containers for defined periods unless they are sterilised. Agents used to clean and disinfect should be sterile.

9.12.5 Cleaning validation

Cleaning validation for isolators is best limited to an assessment of surface contamination. This

can be either visual or with a 'white cloth' test. The white cloth should show no visible recovery when wiped over a surface following a cleaning routine. Quantitative methods with tracer chemicals, such as paracetamol or riboflavin, may also be used. Acceptance criteria for cleaning performance should be set on a case by case basis as different cleaning agents have different modes of action.

Cleaning agents should have technical data to support a suitable validation which should include a product specification and details of safety, compatibility and shelf life. It may be also necessary to validate the removal of chemical residues, including product and cleaning agent, which may have an impact on processed product quality or provide a source for cross contamination.

SOPs are required to set out the validated method of cleaning including agents recommended. SOPs should form the basis for operator training. They should contain instructions that are clear, practical and safe to put into effect.

9.13 Staff training

Operational guidance for staff is summarised with some pointers for development.

Structured training programmes should be provided for all staff working with isolators. Training should be appropriate to grades of staff, and their responsibilities and duties.

Although training is given under general headings, it should be based on the SOPs (standard operating procedures) that individuals are required to follow.
Training should meet the following performance objectives:

Principles of basic theory, design, and siting of isolators

Operators should have an understanding of:

1. Principles of isolator design
2. Different types of isolators
3. Different types of transfer devices
4. Airflow patterns inside isolators
5. Background environments for isolators
6. Entering, working in, and controlling the background environment

Procedures for the operation of isolators

Operators should understand and be competent in following SOPs for:

1. Pre-use checks
2. Entry, use and exit of access devices including glove/sleeves, gauntlets or half-suit
3. General operation and use of equipment inside isolators.

They should also be aware of maintenance and servicing, failure and troubleshooting protocols.

Procedures for materials transfer – non-gassed isolators

Operators should understand and be competent in following SOPs for:

1. Surface sanitisation processes
2. Transfer of materials into controlled and critical areas
3. Transfer of materials out of controlled and critical areas
4. Removal of waste including hazardous waste

Procedures for loading and sanitisation of gassed isolators
Operators should understand the principles of gaseous sanitisation as well as the choice of agents used and should understand and be competent in following SOPs for:

1. Loading patterns
2. Displacement (the extent to which the isolator volume is reduced by the materials and components being processed)
3. Appropriate temperature/humidity monitoring

Procedures for aseptic processing
Operators should understand and be competent in following SOPs for:

1. Standard aseptic processing techniques
2. Segregation and flow of products

Procedures for integrity testing
Operators should understand and be competent in following SOPs for:

1. Inspection of gloves, sleeves, gauntlets and half-suits
2. Leak testing of gloves, sleeves, gauntlets and half-suits
3. Repairs of sleeves and half-suits
4. Pressure decay tests
5. Detection of leaks in all other parts of the system

Procedures for routine glove changing
Operators should understand the risks presented to the integrity of the system when carrying out these procedures and be competent in following SOPs for:

1. Changing gloves
2. Changing gauntlets
3. Changing sleeves
4. Changing half-suits

Procedures for cleaning and sanitisation
Operators should understand the need to validate cleaning and sanitisation procedures with appropriate agents, and the potential need to vary the agents used. They should understand and be competent in following SOPs for:

1. Cleaning and sanitisation of background environments
2. Cleaning and sanitisation of isolators
3. Decontamination of isolators after use or spillage of hazardous materials

Environmental monitoring
Operators should understand potential sources of contamination and should understand and be competent in following SOPs for:

1. Particulate monitoring
2. Active microbiological monitoring
3. Passive microbiological monitoring
4. Chemical contamination
5. Radiation monitoring
6. Safety
7. Actions in the event of emergency e.g. loss of a complete glove

Assessment
The effectiveness of training should be assessed using any or all of the following methods:

1. Natural observation
2. Observation of outcomes
3. Process simulation
4. Oral questioning
5. Written questioning

On-going training and assessment
This should be undertaken after initial training, and at regular intervals.
After appropriate training and validation, individuals may be assessed as:

1. Operator – able to work to an SOP
2. Trainer – able to train to an SOP
3. Assessor – able to assess a trainee working to an SOP

Records
Training and assessment should be documented as they are carried out.
Individual records should be maintained for each member of staff. These record should include:

1. Performance objectives
2. SOPs noting that re-training is required if SOPs are modified
3. Documentation of assessment and further training
4. Date
5. Name of trainer
6. Trainee confirmation that training has been received

Other training
Staff may also require training in:

1. Health & Safety
2. COSHH
3. Handling of radio-active materials*[24]

* The preparation of radiopharmaceuticals requires that operators must have received 'adequate' training under The Ionising (Medical Exposure) Regulations[25].

Special consideration
It has been noted recently that some process technicians or operatives are being asked to note on batch records certain data concerning parameters that might affect the product. Such items include: Airflow readings, Pressure gauge readings, Production line speeds, Temperature and humidity readings. Where there is no recording device fitted readings may need to be taken. In such circumstances, there is clearly a need for training in taking the readings properly, including the correct use of any measuring instruments.

There is also a need for training in the correct action to be taken for out-of-specification results. There may be instances where an out-of-spec result is merely written down. There is no repeat reading, and no explanation as to why the out-of-specification reading may have occurred. As no special attention is drawn to it, the records are often signed off without further comment. It is important that managers are aware of this potential 'hole' in training. It is important that actions to be taken following abnormal readings are adequately covered in training regimes. It is naturally difficult to try to remember, perhaps weeks later, why something or some event occurred.

9.14 Glossary

Where possible, the definitions and terminology used in this chapter are the same as those used in 'ISO 14644.7 – Part 7: Separative devices (clean air hoods, glove boxes, isolators and mini-environments). However ISO 14644.7 is not application-specific. Therefore additional application-specific definitions and terminology are taken from other standards and guidelines such as the EU GMP[15].

9.15 References

1. Pharmaceutical Isolators, A guide to their application, design and control, Midcalf B, Phillips WM, Neiger JS, Coles TJ, ISBN 0 85369 573 3, Pharmaceutical Press, London, 2004.
2. ISO 14644-7:2004 – Cleanrooms and associated controlled environments – Part 7: Separative devices (clean air hoods, gloveboxes, isolators and mini-environments).
3. ISO 14644-4:2001 – Cleanrooms and associated controlled environments – Part 4: Design, construction and start-up.
4. US Pharmacopeia (USP) USP30 NF25 <1116> Sterility Testing – Validation of Isolator Systems, 2007.
5. BS EN 420:2003+A1:2009 – Protective gloves. General requirements and test methods.
6. EN 1822:2009 – High efficiency air filters (EPA, HEPA and ULPA).
7. EN 12469: 2000, Biotechnology – Performance criteria for microbiological safety cabinets.
8. ISPE GAMP® (Good Automated Manufacturing Practice) 5: A Risk-Based Approach to Compliant GxP Computerised Systems.
9. FDA CFR (Code of Federal Regulations) 21, Part 11: Electronic records, Electronic signatures.
10. EudraLex, The Rules Governing Medicinal Products in the European Union, Volume 4 Good Manufacturing Practice, Medicinal Products for Human and Veterinary Use, Annex 11: Computerised Systems.
11. ISO 14644-3:2005 – Cleanrooms and associated controlled environments – Part 3: Test methods.
12. NERC (National Environmental Research Council) Guidance Note: The safe use, maintenance testing of laboratory fume cupboards, December 2007.
13. EudraLex, The Rules Governing Medicinal Products in the European Union, Volume 16.4 Good Manufacturing Practice, Medicinal Products for Human and Veterinary Use, Annex 1: Manufacture of Sterile Medicinal Products.
14. US Department of Health and Human Services, Food and Drug Administration, Guidance for industry: Sterile drug products produced by aseptic processing – current Good Manufacturing Practice, 2004.
15. US Department of Health and Human Services, Food and Drug Administration, Federal Register/Vol. 76, No. 119/Tuesday, June 21, 2011/Proposed Rules, §610.12: CFR Part 610: Amendments to Sterility Test Requirements for Biological Products.
16. Pharmaceutical Inspection Convention/Pharmaceutical Inspection Co-operation Scheme, 2007, PIC/S, Recommendation: Isolators used for Aseptic processing and Sterility Testing: PI 014-3.
17. WHO Technical Report Series, No. 961, 2011: WHO good practices for pharmaceutical microbiology laboratories, World Health Organization, Geneva, Switzerland.
18. WHO Quality Assurance of Pharmaceuticals: a compendium of guidelines and related materials, Volume 2, 2nd updated edition: Good manufacturing practices and inspection, *World Health Organization*, Geneva, Switzerland, 2007.
19. US Pharmacopeia (USP) Revised General Chapter <797> Guidebook to Pharmaceutical Compounding—Sterile Preparations, 2008.
20. US Pharmacopeia (USP) USP30 NF25 <1116> Sterility Testing – Microbiological Evaluation of Clean Rooms and other Controlled Environments, 2007.
21. US Pharmacopeia (USP) USP30 NF25 <1208> Validation of Isolator Systems, 2007.
22. Isolators for pharmaceutical applications, ISBN 0 11 701829 5, HMSO, 1994, London UK.
23. ISO 10648-2: 1994, Containment enclosures – Part 2: Classification according to leak tightness and associated checking methods.

[28] UK HSE (Health and Safety Executive) Control of radioactive substances, HSE Information sheet: Ionising Radiation Protections Series No 8.

[25] UK DoH (Department of Health), The Ionising Radiation (Medical Exposure) Regulations 2000 (together with notes on good practice) The Ionising Radiation (Medical Exposure) Regulations 2000, (IR(ME)R 2000).

The choice for isolators: A risk-based decision

Didier Meyer, Marketing Manager for Isolation Technology, Getinge Life Sciences, France

10.1 Introduction

The term isolator as used today appeared in the late 1940s to raise germ-free rodents for research purposes[1]. From its beginnings, as an original stainless steel construction adjacent to an autoclave, isolator construction developed to the use of transparent flexible plastic materials in the late 1950s[2].

In addition to bio-medical applications with animal studies, another routine use for isolators was in hospitals for the caring of immune deficient patients, mainly babies and children[3]. The first use of isolators in the pharmaceutical industry, in the beginning of the 1980s, was for the compendial sterility testing of parenteral and ophthalmic products. Today isolators are considered as the primary separative enclosure device to be used when there is either a contamination risk to the product or a risk to personnel or both. Isolators provide a controlled environment in relation to a background room classification and in management of associated risks.

There is an increase in aseptic processing and more and more toxic or biologically active products that require some form of barrier technology to manage risks, of which isolators are a primary consideration.

The applicability of isolators for either protecting product or people (**Figure 1**) is outlined in PDA Technical Report 34[4].

Considering bio-contamination control, the controlled environment choices are between:

- Classified "cleanroom" – not a barrier Isolation technology (although may employ simple machine guards around process equipment).
- Restricted Access Barrier Systems (RABS)
- Barrier Isolation technology – Isolators

Of these cleanrooms are well defined in the ISO 14644 series, RABS have been characterised by ISPE[5] with further technical monograph development by the PHSS[6] and isolators are described in ISO 14644-7[7] and PDA Technical Report 34[4].

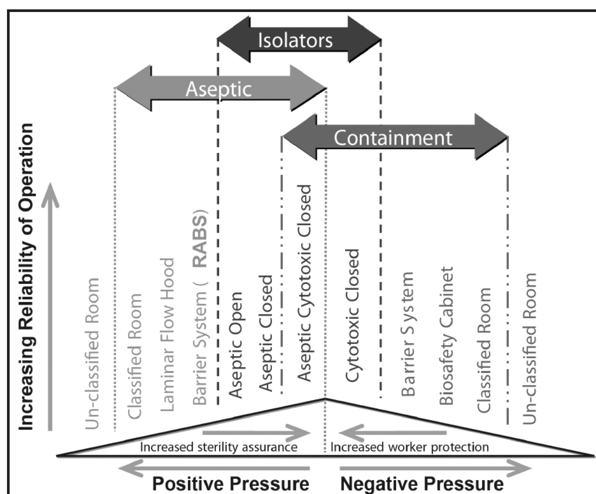


Figure 1: The isolation continuum (courtesy of Getinge).

The choice between other separative enclosures and isolators has to be made according to a risk based process. For this there must be a wide consideration of assessment of "risk", embracing

technical, economical and environmental considerations. This chapter addresses the risk based approach required to undertake such selections.

Definition of an isolator

The official definition of an isolator is provided in the international standard ISO 14644-7[7] as a type of "Separative Enclosure". Here the isolator is described as one of several types of separative devices. Another definition is given by James Akers[8]:

> *"Isolators are devices that provide for total separation between one environment and another. An isolator does not directly exchange air with the surrounding environment and all air must enter through a HEPA or ULPA filtration system. All transfer of material into the isolator must be accomplished while maintaining complete environmental separation. The interior of the isolator, and all equipment contained therein, must be sterilisable in a highly reproducible manner".*

The key phrases in this definition are: "total separation", "sterilisable in a highly reproducible manner". Separation is in the context of process to operator separation, via physical barriers, where process manipulation is maintained through means of manipulative devices such as barrier gloves, half or even full-suits.

To achieve "total separation between one environment and another" the enclosure which constitutes the body or shell of the isolator must be "leak tight". Total leak integrity or total separation is not possible but controlled levels of integrity can provide the minimum passage between the internal and external environments so that risks can be managed.

To be "sterilisable in a highly reproducible manner" is a term that sets an intent but in practice Isolators cannot be sterilised when considering the international acceptance of sterilisation, referenced in Pharmacopieas, as a penetrative process, typically moist heat, dry heat, ethylene oxide or gamma irradiation.

Isolators are possible to biologically decontaminate and validate with a reproducible sporicidal process that can achieve a high level of surface decontamination e.g. 6-log sporicidal reduction. Such biological reduction, with cycle overkill, can be considered surface sterilisation. For decontamination process to Isolators, when applying a gaseous, typically vapour phase, consideration has to be given to decontaminating gas agent containment hence leak integrity. Depending on the diffusion characteristics and operator exposure levels different leak integrities for decontaminating gas containment may be specified to that used as a controlled environment for a given process.

It is often the case when the application of the isolator is for an aseptic or product protection application the isolator is controlled at a positive pressure relative to the surrounding environment.

There are different levels of product handling risk depending on whether the product is in a liquid (stable), powder or volatile form and this will have significant impact on isolator pressure differential operation or specification.

It is not usual in aseptic processing of toxic products, at the filling stage, where stable solution products are handled that Isolators are run at a low positive pressure with other features specified in the isolator to manage risks of any toxic aerosol generation and capture with additional decontamination steps via spray wands or spray balls.

Isolators can also be used for containment purposes to protect the operators and the environment from highly toxic or biologically potent products[9]. Typically Isolators that have a primary

containment function are run at a negative pressure relative to the supporting environment. Often used for handling Active Pharmaceutical Ingredients (APIs) containment Isolators interface with process equipment or are used at powder dispensing or charging (reactors) steps.

Modern Isolators embrace automation to control and eliminate operator interventions or errors together with consideration to control and monitoring technologies as part of the current thinking relating to Process Analytical Technology (PAT). PAT is a system for designing, analysing and controlling manufacturing through timely measurements of critical quality and performance attributes of raw materials, in-process materials, manufacturing processes and the desired final product.

10.2 Selecting isolators

In order to put together a risk-based case for an isolator a number of steps should be followed, including:

i) *Developing a URS (User Requirements Specifications).*

ii) *Design considerations.*

The design of the isolator generally begins with a stepwise description of the process (the intended use of the isolator) and the critical points of use. The use of the isolator(s) should ideally be integrated into a Process Flow Diagram (PFD).

iii) *Selection of the type of isolator required.*

Relative to control of the isolator environment there are two key choices of isolator type: The pressure control operational requirement and whether the isolator has turbulent or uni-directional airflow.

An isolator can be used either to protect the product (operating in a positive pressure) or to protect the operator (working in a negative pressure). Such pressure differentials can be relative to the surroundings together with relation to different isolator modules that are interfaced. Pressure differential cascades can be set up for combined modules, in different combinations, to prioritise highest level of product protection (most positive) or highest level of containment (most negative). The intrinsic leak rate of the isolator and any potential accidental leakage provide the rationale for this technological choice. In theory, were a perfectly leak-tight isolator to exist, the choice would not be of any consequence.

Due to real (however slight) and potential leakage, there is a priority given either to protect the product (positive pressure for aseptic processes) or to the environment (negative pressure for toxic processes). The choice of turbulent flow or uni-directional is primarily set by standards and regulatory expectations e.g. EU Grade A for a critical process such as Pharmaceutical or Biopharmaceutical Filling would be uni-directional airflow with down flow velocities typically at a mean of 0.45 metres per second (other velocities can be validated).

A containment application used for upstream processing of an API as a powder, operated at negative pressure, would more than likely be turbulent airflow with less impact on powder dispersion and using filters that can be changed safety when contaminated. The choice is more difficult in the case of an aseptic processing of a toxic product. The two possibilities in this scenario are: a positive pressure isolator in a EU GMP Grade D/ISO Class 8 (minimum) surrounding environment or a negative pressure isolator in an EU GMP Grade B/ISO Class 7 (in operation) surrounding environment.

Both of these alternative approaches have to be successfully validated[10]; a rational way to choose between them would be to use a Hazard Analysis of Control of Critical Points (HACCP) approach.

This method allows various factors to be accounted for, such as:

- Material toxicity
- Quantity of material
- Open product exposure in the enclosure
- Filling speed (for aseptic filling)
- Number of operators
- Set up and process intervention procedures
- Design of the isolator considering turbulent or uni-directional airflow with associated air clean up and exchange rates
- Pressure control regime
- Leak integrity classification and monitoring regime
- Material used to construct the isolator – flexible or rigid wall
- Waste handling and containment of toxic hazards or bio-contamination
- Type and frequency of transfers
- Cleanliness class of the surrounding cleanroom
- Interventions in environmental monitoring

Ranking of the hazards and development of a decision tree can help with the selection and can also be used to explain the criteria for selection to both an internal project and operational teams (operators, safety engineers, quality assurance personnel) and external assessors or regulatory authorities (auditors, insurance consultants, inspectors).

iv) The functions of the isolator should be considered.

The routine usage of isolators in the pharmaceutical industry follows the usual steps undertaken to produce medications (refer to **Figure 2**):

- Fine chemical production
- Weighing, mixing and compounding
- Aseptic filling
- Sterility or final testing

v) Ergonomics

As a physical barrier provides the necessary separation of the operative to the process then access through this barrier via gloves, half suits, etc is inevitabily restrictive and places high importance on ergonomic study during the design qualification stage of the project. Firstly, all critical access points needs defining then associated barrier access devices (gloves etc) positioned. To validate selected positions during the design phase, it is typical to complete an erogonomic study with a mock-up of the isolator that can be used to complete an ergonomic process simulation.

The ergonomics in relation to operator comfort are important when reviewing manipulations for any given process. Over reach and strain, whether long periods of operation require a seated operating position and if the operator has to work against pressure differential are all considerations in ergonomic studies.

The operator has to be able to reach all the critical parts of the isolator without being obliged to open the isolator to the outside environment. The designer should ensure that the atmosphere inside is not mixed with the air outside of the isolator in order to provide the proper protection to the product and/or the operator.

The manipulation means that sleeves, half-suit or full-suit with their gloves, are the most important and the most fragile parts of the isolator. It is essential that the gloves are properly sized for the

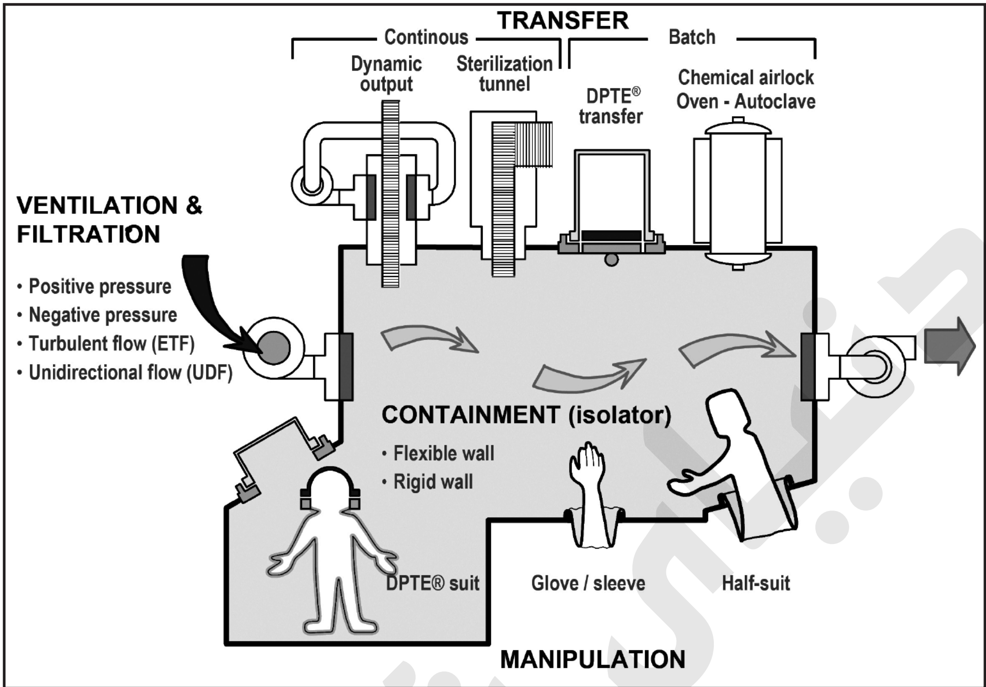


Figure 2: *Primary features that may be combined in a process isolator (courtesy of Getinge).*

hands of the operator, strong enough to be resistant to leaks and resistant to the decontaminating agent. At the same time they must also be light enough to allow full sensation for the tasks to be performed.

vi) Gloves and sleeves

The isolator gloves should be exchangeable without breaking the integrity of the isolator to accommodate various sizes of hands as well as for any replacement in the event of a tear or a leak to the glove. The half-suit and the full-suit must be provided with a sufficent level of filtered air to allow the operator to work comfortably for extended periods without interruption[11]. Additional gowning of the operators may include under-gloves and face-masks for hygienic purposes.

vii) Construction materials

A choice must often be made according to the requirements of the isolator application. The choice between flexible or rigid wall is often considered merely a competition of the two modes of construction notwithstanding that the manipulation devices (glove, sleeve, half-suit, and full-suit) are always flexible. However the choice of uni-directional or turbulent airflow is critical for any process and typically flexible wall Isolators are turbulent flow and have lower airchange rates that could impact particle clearance (important if continuous particle monitoring is used). Rigid wall Isolators are easier and more resilient to cleaning procedures and can cover the complete range of isolator applications.

The choice of barrier wall materials in an isolator (flexible or rigid) is based upon:

- The use of the isolator
- The pressure mode
- The ventilation mode
- The decontamination agent

Non toxic product sterility testing isolators are quite often constructed with flexible walls, operated at positive pressure with turbulent airflow to provide the operator with better comfort and a broader use of the natural light of the room. In contrast, automatic filling isolators are predominantly manufactured with stainless steel walls with uni-directional airflow allowing for continuity from the walls to the filling machine itself. There are, of course, exceptions to both of these applications.

Negative pressure isolators are usually made of rigid materials, avoiding the need for reinforcement of a flexible wall design. Where unidirectional flow is required with rather complex recirculation systems it is preferable to use rigid walls. Recirculation airflow can be routed using either airflow ducts located on outside of the enclosure or between double walls of the isolator. Typically in uni-directional airflow isolators the larger portion of the downflow air is continuously circulated together with the small portion exchanged with the outside environment, via air handling units.

The air exchange units can be used to control the isolator differential pressure (positive or negative) together with providing cooling effects to remove heat gain from the isolator air-handling system. If the amount of heat gain (from process equipment) is greater than the air exchange rate, using the surrounding environment air as the intake, then additional cooling systems may be required.

Turbulent flow air with between 5 to 40 air changes per hour can be readily installed for both flexible and rigid walls isolators. Such relatively low airchange rates do not provide rapid clean up of generated particles and may be considered too low if gaseous decontamination cycles e.g. bench mark process; hydrogen peroxide vapour, use the isolator ventilation to support gas residual removal (aeration) and fast cycle times are required.

Some plastic materials have a tendency to adsorb and then desorb the sterilant, especially hydrogen peroxide (H_2O_2). One of the considerations as to whether or not plastic walls and windows should be used relates to the risk of permanent chemical residues that can cross-contaminate the product[12].

viii) Leak tightness

No isolator is 'leak free' as they are based on a physical and practical construction (unlike a high vacuum vessel that uses robust engineering) so leak tightness has to be defined, managed and monitored. Depending on the level of process risks, different leak integrity levels can be applied. Typically levels of leak integrity are arbitrarily set values (possibly from an ISO standard), that the isolator is validated to comply with, as one of the control point measures around which a process is validated. A scientific basis for leak integrity is not generally practical.

ISO 10648 defines three classes of leak integrity for Isolators but as barrier technology develops wider considerations are required. The more simple the isolator the easier and practical it is to meet tighter levels of leak integrity. Conversely the more complex the isolator or interconnected modules that are interfaced with process equipment it is more likely that leakage is highly diffused around the system and meeting tight leak integrity levels is impractical.

A key aspect of isolators that is by no means absolute and raises questions of importance, level, location and measurement, is the "leak rate". Agalloco mentions, when comparing barrier systems and isolators[13], that the isolator – even the "open" ones – needs to be essentially leak-tight during their decontamination cycles. The magnitude of the leak rate has to be realistic relative to the materials of construction (the sleeves to which the gloves are attached are a part of the isolator wall) and the leak test method utilised. The risk of a leak has to be evaluated relative to its location and materials, including gaskets.

It is recommended that when considering leak integrity the following should be assessed, defined and used in routine monitoring:

- Leak integrity levels should be defined as a classification test to qualify the isolator integrity control state for a defined process, based on risk.
- Leak integrity levels should be set for containment of decontaminating gasses which may vary from the classification level. It should be noted that containment of hydrogen peroxide vapour, having poor diffusion characteristics, may require less integrity than that defined for a process operation or classification level.
- Leak test methods for classification and routine monitoring must be defined.
- Leak finding, diagnostic methods need defining to support a process in operation.

Classification: leak integrity
Considering the three classes of ISO 10648:

Class 1 (0.25% volume per hour) is a high integrity class and more appropriate to nuclear glove-boxes than pharmaceutical isolators. Such integrity is tested at relatively high pressures over extensive periods with compensation factors used to adjust for temperature and barometric pressures changes over the long test period.

Class 2 (0.5% volume per hour) is still a relatively high level that suits simple isolators, like a flexible film version, or is specified because leak integrity needs to be high. In pharmaceutical applications, high integrity may be specified because of a containment application or where the isolator is operated at negative pressure but the process is aseptic and product protection (from biocontamination) is required.

Class 3 (1% volume per hour) is a practical limit for stand alone isolators, including stainless steel construction and uni-directional flow versions. Such a level of integrity is practical to test as a pressure decay test (a simple test method that can be automated) and typically tested over short test durations to avoid influences of temperature and barometric pressure changes.

By specifying leak integrity by percentage volume per hour leak rates this provides a degree of independence of the starting test pressure. In classification integrity testing, higher starting (challenge) pressures may be used, with slightly longer test durations, but in monitoring it is recommended to use test pressures that are within the range 1.5 to 2 times the typical operating pressure.

Considerations in addition to ISO 10468, for more complex isolator systems.
When leakage is diffused over a complex isolator system the ISO 10468 Class 3 integrity level may be impractical to achieve. Two other leak integrity levels for isolators have been specified and are in routine use.

A leak integrity level of 2% volume per hour may be appropriate for some isolator applications, where process risks are not higher than the decontaminating agent gaseous vapour containment risk. Hydrogen peroxide vapour can be contained at such an integrity level if the decontamination cycle pressure is low, typically 30-40 pascals positive pressure and leakage is confirmed as diffused by measurement (scanning with a H_2O_2 sensor of suitable accuracy) that operators are not exposed to the operator exposure level (OEL) of one part per million (time-weighted average of 8 hours).

For complex isolator systems, such as filling lines, a leak integrity level of 5% volume per hour has been specified based on proof that leakage is diffused (for H_2O_2 vapour containment) and there is

non-compromise to the EU Grade A environmental control conditions. For completion of leak tests typically for testing at leak rates 0.5% volume per hour to 2% volume per hour, a pressure decay test is used. A pressure decay test is monitoring, from a set test pressure, the pressure drop (for positive) and rise (for negative) over a set time period.

The leak rate is dependent on acceptable time to drop a defined pressure and/or adjustment of time for the pressure decay. This test can be automated and in all cases needs a period of pressure stabilisation to allow for initial temperature changes as a result of pressurisation energy or movement (glove , etc).

For leak rates up to 5% volume per hour, a pressure hold test is more appropriate (but more difficult to automate). For a pressure hold test a small flow rate is applied to the isolator until a test pressure is reached, then the flow rate adjusted so the pressure holds at that test pressure. The flow rate to hold pressure is converted into a leak rate. Leak rates can be expressed as single hole equivalents (by calculation) and although this may provide some idea of leak hole possible scale it is of little practical use in complex isolators as leakage is typically highly diffused across the system. Typical single-hole equivalents are far greater in size than micro-organisms so it is difficult to correlate risk.

Where single-hole equivalents do become useful is in glove-sleeve testing. Being simple devices, a diffused leak is not expected so actual single hole leaks may be real. Test methods to measure single hole equivalence down to 80 microns have been developed and this is the limit of detection with pressure decay or hold methods in gloves. Relationship of leak rate expessed as % volume per hour and pressure drop over time plus volumetric flow rate (comparison with pressure hold). Typical pressure decay test pressures are two times the isolator operating pressure for monitoring tests but may be higher for classification tests. Single hole equivalent sizes are just for information, so no test is required.

Table 1: Three classes of isolators as per ISO 10648 based on leak integrity

Class of isolator (To 1SO 10468)	**3**	**2**	**1**
Hourly leak rate	$\leq 1X\ 10^{-2}\ h^{-1}$	$<2.5X10^{-3}\ h^{-1}$	$<5X10^{-4}\ h^{-1}$
Percentage volume change per hour	$\leq 1.0\%\ h^{-1}$	$<0.25\%\ h^{-1}$	$<0.05\%\ h^{-1}$
Standard decay time for 25 Pa drop	>1.5 mins	>6 mins	>30 mins
Volumetric leak rate (change per second)	$2.8\ X\ 10^{-6}\ m^3\ s^{-1}$	$0.70\ X\ 10^{-6}\ m^3\ s^{-1}$	$0.14\ X\ 10^{-6}\ m^3\ s^{-1}$
Single hole equivalent	464 micron	232 microns	103 microns

Monitoring leak integrity and frequency

Monitoring of leak integrity needs to follow and environmental monitoring program as leak integrity has an impact on bio-contamination control as well as safety in containment of decontaminating gaseous vapour.

The most used test method for monitoring leak integrity is pressure decay which is completed over a short test period to negate the impact of temperature changes.

The following should be considered when completing monitoring leak tests:

- Physics tells us that a 1ºC temperature rise results in a 350Pa change (stp).
- So a 0.07ºC temperature change results in a 25Pa change, so you need to measure to 0.01ºC. This degree of resolution is beyond the range of typically used temperature

measurement instruments that could monitor temperature change during leak tests.
- It is complicated to add compensation factors for temperature variation (as done in nuclear glove-box tests) so better to assume no temperature change.
- To limit impact of change, routine leak tests are carried out over a short test period (1.5 to 6 minutes) and no temperature measurements are taken and the tests are repeated if it fails in case the result was an impact of temperature change.
- Note that temperature changes may pass a leak test (increase pressure compensating leak) or provide a false fail result.
- The leak rate trend should be analysed. A very fast initial pressure drop that slows and levels may be the result of reducing temperature or structure movement (changing volume), hence the stabilisation period may not be long enough.

Leak test types and leak point finding methods

Several means of measuring the leaks are available for either making a quantitative or qualitative measurement. When considering the user's typical requirements and available equipment, the principal methods are:

- Pressure decay for the positive pressure isolator or glove-sleeve combinations – classification or monitoring test
- Pressure increase for the negative pressure isolator or RTP container – classification or monitoring test
- Pressure hold test for high leak rate isolators
- Oxygen concentration for the glove (hand piece only) on positive pressure isolator
- Detection of an indicator gas (helium, ammonia, Freon™ [dichlorodifluoromethane]) outside the isolator. A leak point finding test (used when leak tests fail)
- Ultrasonic detection of leak locations. A test of limited use

	Test pressure (Pa)	Specifications	Equivalent leak rate (Std cm^3/sec)	Flow rate at working pressure of 50 Pa (cm^3/sec)
Isol.	150	0,5% vol/h	1,38	0,8
	100	0,1% vol/h	$2,7\ 10^{-1}$	0,2
	100	0,1% vol/h	$2,7\ 10^{-1}$	0,09
glove	-3000	500 PPM	$5,8\ 10^{-2}$	$7,75\ 10^{-3}$
DPTE®	-4000	10^{-4} to $5\ 10^{-4}$ Pa.m^3/sec	$4,9\ 10^{-3}$ to $9,8\ 10^{-4}$	$1,58\ 10^{-3}$ to $3,2\ 10^{-3}$

Leak rate given for a 1 m^3 volume isolator (dark grey)

Leak rate given for the DPTE® transfer system 105 & 350 (light grey)

1 cm^3/sec = 2,12 10^{-3} Ft^3/min

1 Pa.m^3/sec = 9,87 Std cm^3/sec

***Figure 3:** Comparison between high integrity isolators and leakage of Rapid Transfer Port (RTP) containers and glove-hand pieces (courtesy of Getinge). For isolators, other leak rates apply as detailed in this chapter.*

Frequency of leak testing
The isolator work zones in the configuration used for processing should be leak tested ahead of process operations and in some cases (depending on risk) directly after to confirm steady state of integrity.

In some applications, isolators are held for an aseptic hold period and in this case before and after hold tests are completed together with in-process glove leak testing.

Leak testing should be undertaken prior to each decontamination cycle and this is typically an automated leak integrity test that acts as a safety interlock pre-gassing, hence the cycle will not advance to the gassing phase if a leak test fails.

Glove leak integrity testing
There are many examples of glove test devices. Some devices even include wireless Lan control, 21 CFR 11 compliant test data capture and ability to simultaneous test of a number of gloves so a single test duration of 15 minutes has limited impact on production downtime.

When considering devices with pressure decay test principles it is recommended the test pressure used is above 500pa (needed to detect pin holes) and visual inspection is also completed. The availability of a commercially available device for leak testing makes leak control easier[14].

In addition to the main isolator, the isolator gloves that are widely recognised as the weakest part of the isolator can be leak checked with the isolator before the decontamination process begins and if necessary after processing to confirm glove integrity has been maintained.

The most important risks associated with leaks occur during the production process carried out inside the isolator. The in-process (if required) glove leak check should be performed without compromising the inside quality of the enclosure, as a part of the test. In the instance of an isolator intended for aseptic operations, this requirement eliminates any leak test method that might introduce non-sterile air from the outside of the sleeve or glove. The oxygen leak test method reverses the glove within a tight chamber filled with nitrogen and allows a precise measurement to be made without the risk of contamination occuring.

A further area where leaks can occur is with the connection of an isolator to another isolator or to a transfer port. Ports normally involve the connection of one part to another (male-to-female or alpha-to-beta). The shape, construction materials of the gasket and the volume itself depend upon the use of the port connection. The leak tightness of the port assembly depends upon the volume and the port head connection. The leak tightness of the volume can be measured by one of the described methods. The port head, which includes a flange, a lid and a gasket, can be measured as a whole with a pressure increase. Comparative values of the leak tightnesses of various parts of an isolator shows that ports are usually more leak tight than the constitutive shell of the isolator.

10.3 Isolator sterilisation and decontamination
Isolators in the pharmaceutical industry were originally considered as “mini–cleanrooms” and were only wiped with disinfectant solutions prior to use in aseptic processing or sterility testing. Beginning in the early 1980s one of the goals of isolator users was the attainment of a more reproducible biologically lethal treatment for the internal environment. To demonstrate the effectiveness of these treatments, isolators are challenged with resistant spores prior to a liquid spray or evaporation of a chemical sporicidal agent[15]. At first the only available sterilant was diluted peracetic acid. Since the beginning of the 1990s, evaporation of aqueous solution of hydrogen peroxide (H_2O_2) has become the method of choice. This is partly because the by-products of H_2O_2 degradation are relatively safe: water and oxygen. There are some alternative decontamination

methods. A recently introduced treatment uses gaseous chlorine dioxide (ClO_2[16]). Ozone, increasingly used as a sterilant in water systems, also has potential for use with isolators. All of these sterilants are accepted by the various pharmacopoeias (such as in USP <1208>[17]).

The spores used for validation of the microbial treatment depend upon the mode of sterilisation and the sterilant itself. The carrier on which they are placed can be paper, plastic material or stainless steel[18]. For isolator gaseous vapour cycle development it is generally considered spores inoculated on stainless steel carriers in a Tyvek® primary pack are a challenge that represents the hard surface decontamination in an isolator. The placement of inoculated carriers in the isolator, the concentration of spores (10^3 to 10^6) its resistance (System D value) depend upon the agent chosen and the process objective[19].

The use of the term "sterilisation" appears in the USP chapter on isolators: <1208>[17]. As the process is only a surface and atmospheric treatment[20], some regulatory bodies are reluctant to use the term "sterilisation" as is often associated with more lethal destruction of micro-organisms throughout an object. For this and other reasons, use of the terms "decontamination" or "biodecontamination" are becoming increasingly widespread.

Whatever the treatment mode or terminology, one important and essential consideration is the risk for the operators. The process should always be performed inside a "closed" isolator having a leak rate suitable for decontaminating gas containment.

The eight hour time weighted average operator exposure level (OEL) for H_2O_2, for example, is 1 ppm and the concentration inside the isolator during the process is often in the range of 400 to 1300 ppm. Monitoring of hydrogen peroxide leakage into the surrounding should be completed during cycle development to confirm safe gas containment or leaks are highly diffused so there is no compromise to the OEL[17].

For open isolators with a high leak rates may not be originally designed for gassing disinfection and in these cases (sometimes with leakage as much as 10% of the volume/hour) the operators have to leave the surroundings of the isolator during the biodecontamination cycle and the air renewal of the room has to be taken into account to assess the risk for the environment

The time required for decontamination agent removal, typically the longest part of the cycle, (by air replacement or in combination with catalysts) is an important factor of the selection of isolator system and relative treatment mode, as well as in isolator design[21].

10.4 A logical approach to isolator design

The primary functions of the isolator must be taken into account when designing it. These functions are:

- Environmental control of particulates (viable and non viable) or toxic powders
- Manipulation required in-process
- Transfer, into and out of the Isolators of materials, products and wastes
- Ventilation-filtration to meet classified conditions
- Bio-decontamination
- Isolation/containment

The end result must be a piece of equipment that is compatible with the process and current regulatory recommendations. It is important to consider the space around the isolator. It should be large enough to allow the operators and transfer items to freely circulate.

The classification of the surrounding environment is generally expected to be at least EU GMP

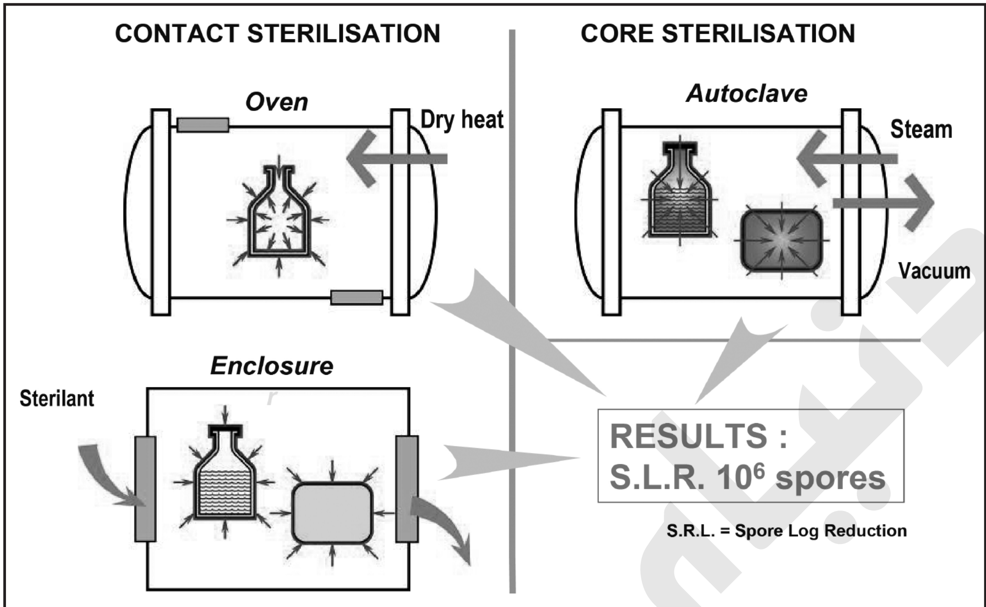


Figure 4: *Isolator sterilisation techniques (courtesy of Getinge).*

Grade D/ISO Class 8 for aseptic production purposes. This means that the exterior of the isolator must be like the interior, that is, be easily cleanable to meet the grade requirements.

Sterility testing, as a regulatory defined application, is perhaps the only widespread use of isolators for which it is possible to utilise a standard equipment design. As the operating procedures are essentially the same for all users, the isolators and appended equipments utilise the same basic principles. As sterility testing is predominantly a manual activity, then proper lighting and ergonomics for the operators are essential.

Today there are many choices of standard sterility test isolators, most use a gaseous vapour phase decontamination process, with the key difference in design and operation being the material surface decontamination method and transfer.

In some cases a batch process is used with all test materials decontaminated in a pre-test cycle, then sterility testing is completed on the batch, before opening the isolator.

In other examples rapid gassing transfer ports are used so that the sterility test isolator can be gassed for testing, held for an aseptic hold period, then secure 'just in time' delivery of test materials in achieved via the rapid gassing ports. Secure material exit can be via transfer containers, sterile bag connectors or special transfer hatched that are airflow protected from re-contamination at transfers. Another choice could be a half-suit equipped workstation with transparent flexible walls arguably provided a superior material handling capability with fewer constraints when compared to a solid wall design. With half-suit isolators the flexible walls of the isolator permit excellent sound transmission. The transparency allows the operator to feel "integrated" in the surrounding room rather than isolated from it.

Further considerations, not all positive, with the use of flexible film isolators are:

- In many installations there is a workstation isolator in which the test is performed and a transfer isolator where the outside surfaces of the samples, the media and the test

units are biodecontaminated. This batch gassing transfer isolator may suit a flexible film type.
- H_2O_2 decontamination has introduced concerns relative to adsorption/desorption on flexible walls, sleeves and half-suits. To avoid "false negatives" due to residual H_2O_2 remaining after the decontamination is complete, there is a growing tendency to use a rigid wall transfer isolator, which decreases the post biodecontamination aeration time.
- The ingress of the samples and the egress of the solid and liquid wastes can be done through appropriate rapid transfer ports (RTP), sterile containers or bags working bi-directionally while avoiding chemical and microbiological cross-contamination.

With all types of isolator, the number and the frequency of testing and the volumes of the required media, utensils and other equipment must be considered in sizing the isolators, as well as the choice of the manipulation devices (gloves and/or half-suits) and the routine processing procedures. The choice of the decontamination method (hydrogen peroxide, peracetic acid, chlorine dioxide, etc.) is an important factor affecting decontamination cycles including dwell periods and the extent/speed of aeration required.

Aside from sterility testing, most other uses for isolator are process specific to their application and require customised isolators operating in classified rooms. When designing an isolator for a particular process, the following should be considered:
- Knowledge of the process steps
- Choosing between positive and negative pressure type isolators
- Choosing between open (continuous process) or closed (batch process) isolators
- Deciding upon whether unidirectional or turbulent flow is required
- Selecting the appropriate continuous or batch transfer systems for the ingress and/or egress of components
- Fabrication of an essentially identical mock-up of the finished isolator
- Simulating the process with full consideration of fatigue factors
- Identifying the materials of construction
- Preparation of a detailed functional description includes detailed drawings

An important aspect is the selection of achievable leak tightness criteria for the isolator, consistent with process requirements and fabrication capability. While it might seem that the isolator should have a "zero" leak rate, this is certainly not possible, and the smaller the acceptable rate the more difficulty the user will have in both initial qualification and ongoing maintenance to maintain that capability. The leak tightness should be evaluated both "at rest" and "in operation" especially when moving parts are concerned, as well as during the decontamination process.

Gaskets utilised to join walls and windows can be of various profiles with either inflatable or non-inflatable designs. The chemical compatibility of these materials is as important as it is for the manipulation of components (gloves, sleeves and half-suits). The material compatibility should be considered relative to the use concentration rather than theoretical data from vendor literature.

10.5 Types of isolator application

As discussed above the use of the isolator has an important influence over its design. This section examines the different application of isolators.

API (Active Pharmaceutical Ingredient) production

The isolator for the fine chemical process is often connected permanently or semi-permanently to a process vessel. The working zone must often be explosion-proof and thus the components of the

Figure 5: *Different types of isolators (courtesy of Getinge).*

isolator must also be explosion proof. This makes aspects of the electronic components difficult to fit and to design. Any connection to a tank, reactor or to a dryer must take in account the differential working pressures of these elements and the isolator to properly install the required safety devices. The fine chemical step produces the Active Pharmaceutical Ingredient (API) that often has to be transferred and/or shipped and/or stored before the next step of the process.

Aseptic filling of containers

The aseptic process, for producing pharmaceutical products, is acceptable only where the package or product cannot tolerate terminal sterilisation[19,20]. New product developments tend to be biological in profile hence damaged by terminal sterilisation so aseptic processing is increasing. The use of an isolator and RABS barrier technology in these cases is a considerable advantage relative to conventional cleanroom technology as the operator stays outside the aseptic environment. An isolator is far preferable for the formulation of a product that cannot be membrane filtered (emulsions, suspensions) and for the filling of liquids, powders and other sterile dosage forms. When the liquid is to be freeze dried, an isolator can be placed in front of the freeze drier with automatic or manual tray loading.

Two distinct processes of filling have to be considered inside an isolator for aseptic filling: manual and semi-automatic process, where the machine is installed within the isolator on one side and the automatic process where the machine is integrated with the isolator on the other side[20,23]. The filling machine has to be supplied with empty containers, the drug to be filled and stoppers/caps in the case of vials.

For manual and semi-automatic machines, the autoclave (for stoppers and product contact fill parts) and the dry heat oven (for glass containers) can be directly connected to the isolator. The alternative is the use of a rapid transfer port equipped transfer isolator to pass the materials from an autoclave-isolator or oven-isolator. The choice of the first solution avoids an extra sterilisation of the outside of the packaging. The use of a directly connected autoclave or oven to the process isolator can also be used for the egress of materials from the isolator.

Figure 6: *An aseptic filling isolator (courtesy of Getinge, Bosch and Steriline).*

In the case of an automatic filling line of glass containers, a depyrogenation tunnel is permanently connected to the entrance of the filling isolator; the egress of the filled containers should be protected against any ingress from the external atmosphere into the isolator. When the product is toxic an external cleaning of the container is usually necessary after the sealing or capping. This part of the filling process has to be related to the removal of toxic materials and to the protection of the operators and surrounding environment.

In the case of plastic tubs of sterile empty syringes which, are ordinarily ethylene oxide sterilised, the surface outside may be biodecontaminated with an electron beam system.

Special care has to taken for installations used for the aseptic filling of toxic products. In this case the transfer port connection must be bidirectional and stay leak tight in both directions. The comparative risk assessments personnel/product can be estimated by a Hazard Analysis of the Critical Control Points (HACCP) type of method to decide whether to use containment (negative pressure isolator in a EU GMP Grade B/ISO class 7 environment) or to aseptic process (positive pressure isolator in a EU GMP Grade D/ISO Class 8 environment).

Sterility testing

The final compendial testing for sterile medications and devices includes sterility testing. This test is defined in the pharmacopoeias. A “false positive” result is to be avoided to prevent the unnecessary loss of otherwise acceptable materials. In addition to the use of isolators, the use of a closed system for the membrane sterility test has resulted in a significant decrease in the incidence of “false positive” results. Another concern can be a “false negative” result if decontamination of the sample container exterior results in penetration of the decontaminating agent into the sample container. Care must therefore be taken with the development of the isolator decontamination cycle. In such a case a growth promotion test under the conditions of the sterility test has to be provided[24,25].

Figure 7: *A sterility testing isolator (courtesy of Getinge).*

Containment

The pharmaceutical industry use more and more potent and toxic products as the active part of the drug delivered to the patient. These potent and toxic products are produced by the fine chemical industry. The industry must protect its operators and has tended to build closed loop designs including vessels for the various steps of the product developments (crystallisation, mixing, filtering, drying, etc.). At certain stages solutions or powders have to be added or extracted. Where low Operator Exposure Limit (OEL) products (<1μg/m^3) are being processed these operations should be performed in an isolator[26].

The purpose of this isolator is to protect the operators and environment so it must operate under negative pressure relative to the surrounding room. The designer must exercise caution regarding explosion proof requirements that are not always compatible with the electronic service and monitoring, and with the pressures of the vessels which have to be stabilised before the opening in isolator[27]. Negative pressure containment is used when the toxic or potent product is in its powder stage. After mixing with a solvent under negative pressure, the OEL often allows the operators to work in positive pressure isolator, if necessary, for aseptic production of injectable drug[28].

Aseptic processing

Liquids, whether membrane filtered[28] or not[29], can be transferred through a dedicated RTP container. The understanding of the monitoring is essential both for the physical measurements and the microbiological controls.

Centralised preparations in hospital pharmacies

The application of isolators for hospital pharmacies is mainly for the extemporaneous aseptic compounding of:

– A sterile solvent (physiological water or glucose in a bag)

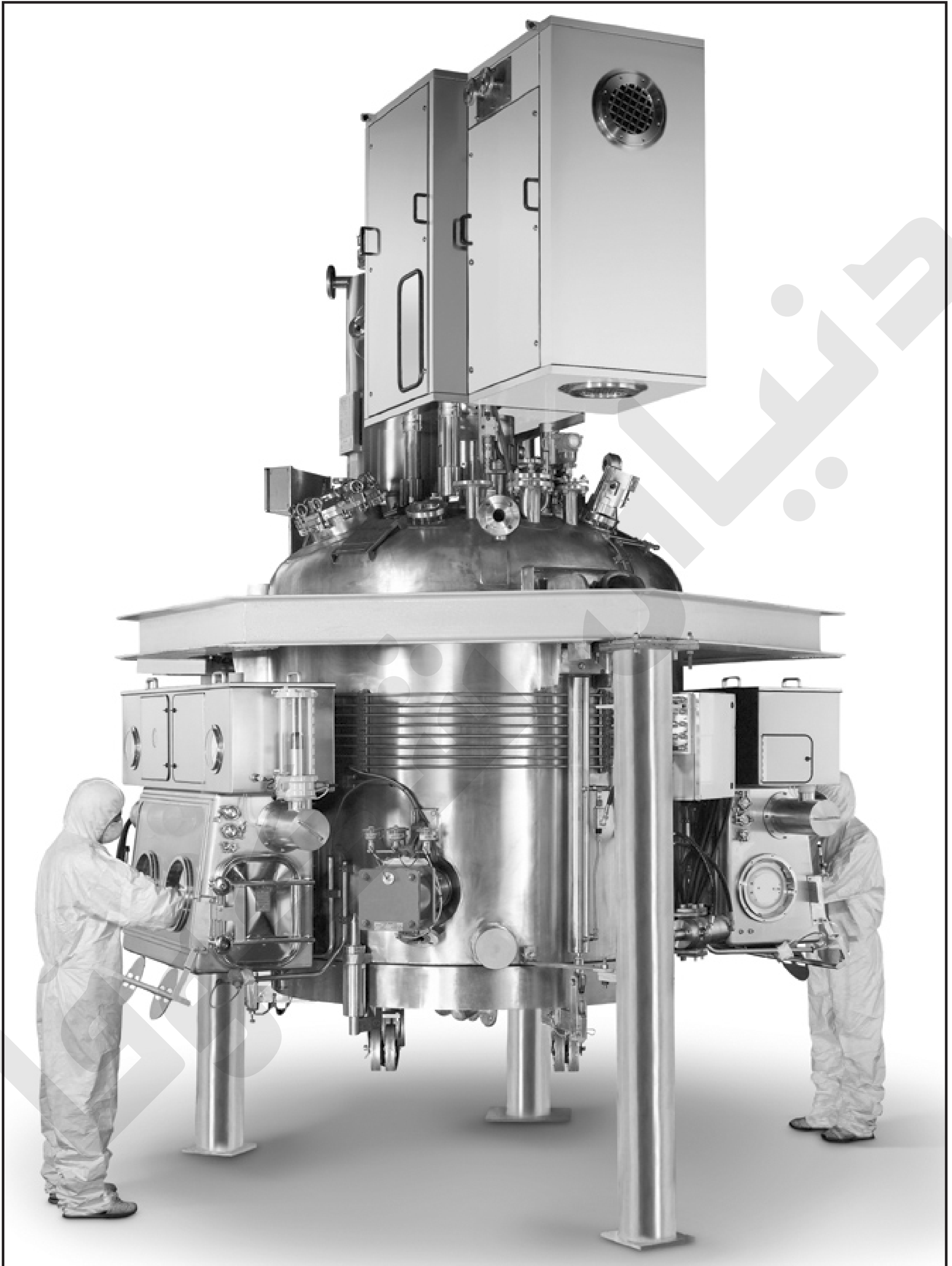


Figure 8: *A containment isolator (courtesy of Getinge).*

- A sterile non toxic or toxic drug (freeze dried or liquid drug)
- Vitamins and other additives delivered via an IV bag.

These are filled in a Pharmacy Aseptic services cleanroom with associated Pharmacy Isolators. A precise dosage is prepared from a prescription with typically no sterility testing at the end, meaning

Figure 9: *A hospital pharmacy isolator (courtesy of Getinge).*

that with regard to environmental control, the traceability of both the leak tightness of the isolator and the sterility of the isolator are essential. This type of handling is very close to what is done in personalised or regenerative medicine, i.e. handling of small quantities with a stringent control of the sterility and no time to test at the end.

10.6 A logical approach to qualification (validation)

Qualification of isolators proceeds through a series of stages: Design Qualification (DQ), Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ) and have been well defined in the literature[29]. DQ, IQ and OQ are customarily provided by the manufacturer of the isolator(s) or validation services firm in charge of the project. The PQ is usually performed by the end user sometimes with the assistance of outsiders for the cycle development/performance qualification of the decontamination process. The PQ may include a Media Fill Test (MFT). The number of containers to fill for the MFT depends upon the statistical level of confidence to be reached (2004 FDA aseptic processing guide)[30]. The expected result of the MFT should be zero contamination.

There is no standard format for the qualification documentation. Most of the DQ, IQ and OQ protocol requirements can be satisfied during either the Factory Acceptance Test (FAT) completed at the isolator manufacturer's site isolator or the Site Acceptance Test (SAT) performed at the end user's site. The logical means to organise these activities is to follow the User Requirements Specification (URS) and clearly indicate the specific design elements/operating features associated with the key points of the process.

10.7 Regulations/recommendations to follow

To guide the user in implementing isolation technology, numerous publications are today available from many sources:

- USP <1208> Sterility testing – Validation of isolator systems[17]
- USP <1116> Microbiological evaluation of clean rooms and other controlled environments (USP <1116>[31])
- European Pharmacopeia: Environment when using isolator for sterility testing[32]
- Isolators for Pharmaceutical Applications from the UK Isolator Group, available at HMSO London[33]
- ISO 10648-1 and ISO 10648-2 upon the containment enclosures
- ISO 14644-1 to 8: Cleanroom and associate controlled environments
- PIC/PICS: "Recommendations for the inspection of isolator technology"[34]
- PDA Technical Report 34[4]
- 2004 FDA aseptic processing guide
- 2009 EU Annex 1 on Sterile Medicinal Products[35]

10.8 Financial matters

Are isolators less expensive than cleanrooms? This question, often raised with each new technology, has been matter of some discussion over the last ten years[36,37].

Are these two technologies really comparable? As the isolator and operator will never be in direct contact with the product this makes for the major difference between isolators and cleanrooms. The separation of the operator from the product reduces the risk to the product. Initial investment and operating costs are the two primary criteria that form the basis for financial comparison. The capital investment for a production isolator system is equal or higher than the equivalent installation with clean room technology, while conversely the operating costs are much lower with the isolator, and with the risk of product contamination far lower, the argument for the use of isolators can be made at the financial level as well as in terms of product and patient safety.

10.9 Personnel management matters

Even if the operators are not physically operating within the isolator, they are still required to conduct the process using a glove/sleeve and/or half suit and therefore they must be properly trained. This training should include the basics of isolators, how isolators differ from cleanrooms, safety practices, aseptic technique and any other particulars of the isolator such as changing gloves without breaking containment. The completion of training can coincide with the start of Media Fill Tests for aseptic isolators, which can confirm the acceptability of the installation and to certify the operators.

10.10 Pros and cons of isolators versus cleanrooms and RABS

This chapter has set out some of the important and risk-based considerations for isolators. An alternative to an isolator or to a conventional cleanroom is a Restricted Access Barrier System (RABS)[6]. The debate between isolators and RABS is driven by several factors: patient needs, involved machine(s), personnel capabilities and finance. The major technical factor in favour of the isolator is, of course, the total segregation of the operator from the process. This separation serves to protect the product during aseptic production: the environment/operator in containment applications, and the patient, product, operator and environment when used for sterile or cytotoxic products. Isolators are sometimes more complex than RABS barriers and not all processes suit such complexity and for these reasons selection of the type of barrier technology, how it integrates with a process are key starting considerations in any new process.

10.11 Conclusion

The use of isolators and well designed barrier system technology in pharmaceutical processing helps to make the parameters of risk analysis easy to monitor and to trace. Isolator technology can be used with the initiatives of Process Analytical Technology (PAT) and the ICH Q9 guideline, as well as cGMP requirements for establishing process-based risk assessments. Such risk assessment, and the application of isolators, are applicable when handling potent/toxic powder or when a sterile process is required (for either aseptic filling or for conducting the sterility test). In most situations, the risk conclusion is that isolators represent the lowest risk option for product protection against bio-contamination and typically in most improved operator protection from biological and toxic hazards. For any given process risks, operational efficiency, complexity and robustness become key considerations and must be reviewed in all cases to assure quality.

10.12 References

[1] US Patent 2403400 James A Reyniers.

[2] Trexler PC. The development of isolators. *Postgrad Med J*. 1976; **52**: 545.

[3] Weil-Halpern F, Griscelli C. Immuno-deficient children within a «bubble». In: *Neuropsychiatrie de l'enfance et de l'adolescence* ISSN 0222-9617 CODEN NEADDF 1985; **33** (10): 427-434.

[4] PDA Technical Report 34, Design and validation of isolators systems for the manufacturing and testing of health care products, *Parenteral Drug Association*: Rockville, USA, June 2001.

[5] ISPE Baseline Guide Volume 3: Sterile Manufacturing Facilities.

[6] Pharmaceutical and Healthcare Sciences Society – PHSS Technical monograph 15; Restricted Access Barrier Systems (RABS) for Aseptic processing – Published 18 November 2011, *Pharmaceutical and Healthcare Sciences Society*: Swindon, UK, 2011.

[7] EN ISO 14644-7, Cleanrooms and assorted controlled environments – Part 7: Enhanced clean devices, ISO, Geneva, Switzerland, 2004.

[8] Akers JE. PDA Letter, June 1995.

[9] Martin P. The Use of Barrier isolator technology for a Sterile Anti-cancer Drug. In: Agalloco JP and Akers J. Advanced Aseptic Processing Technology, USA: Informa Healthcare Books, 2010.

[10] Gold PM. Maintaining containment during the processing of a highly potent potentially toxic aseptically filled drug product, PDA annual meeting Philadelphia 10-14 Nov. 1997.

[11] Manufacturing Chemist, "Evans suits itself", Sept. 1996.

[12] Lang G. On-line analysis of vapour hydrogen peroxide for isolation barrier technology, ISPE BIT conference Rockville 1-3 June 1997.

[13] Agalloco J. Barriers, isolators and microbial control, *PDA Journal of Science and Technology* 1998; **53** (1).

[14] Rivière J-M. Routine Integrity testing of isolators during their use, PDA Basel 14-16 Feb. 2000.

[15] Davenport SM. Design and use of a novel peracetic acid steriliser for absolute barrier sterility testing. *Journal of Parenteral Science & Technology* 1989; **43** (4).

[16] Eylath A. Successful sterilisation using chlorine dioxide gas, *Bio-Process International,* July 2003.

[17] USP <1208> Sterility Testing – Validation of isolator systems.

[18] Kokubo M. Resistance of common environmental spores of the genus Bacillus to vapour hydrogen peroxide, *Journal of Parenteral Science & Technology* 1998; **52** (5).

[19] Davenport SM. Sterility assurance and the use of *Bacillus stearothermophilus* as the BI for VHP decontamination of isolators, PDA annual meeting Washington DC 30 Nov. to 3 Dec. 1999.

[20] Klapes NA, Wesley D. Vapour phase hydrogen peroxide as a surface decontaminant and sterilant. *Applied and Environmental Microbiology*, Feb. 1990.

[21] Agalloco J, Akers J, Madsen R. Isolation technology regulatory highlights from PDA TR 34 USP <1208> ISO 14644-7 PICS PE-b 004-1, PDA annual meeting Washington DC 3-7 Dec. 2001.

[22] Woodworth A. Isolator for sterile filling of intravenous (IV) solutions into flexible containers, ISPE Zurich 2 Oct. 1997.

[23] Brossard J-P. Components transfer for filling isolators, ISPE Conference Rockville 1-3 June 1997.

[24] Millipore technical brief, The effect of peracetic acid on Steritest® sterility testing devices.

[25] Millipore technical brief, The effect of vaporous hydrogen peroxide on Steritest® sterility testing devices.

[26] Ryder M. Design requirements criteria for containment device selection, ISPE Amsterdam 8 & 9 Dec. 1999.

[27] Rowell R. Methodology for design procurement and testing of high containment devices, ISPE Amsterdam 8 & 9 Dec. 1999.

[28] Leblanc AJ. Compliance and regulatory issues associated with parenteral manufacturing of cytotoxic and potent compounds, PDA annual meeting Philadelphia 10-14 Nov. 1997.

[29] Rombauts R. Technical validation of isolators, AUDITS Antwerp 8 June 1999.

[30] Food and Drug Administration. Guideline on Sterile Drug Products Produced by Aseptic Processing, *Food and Drug Administration*, Rockville, MD, USA: 2004.

[31] USP <1116> Microbiological evaluation of clean rooms and other controlled environments.

[32] European Pharmacopoeia, 2.6.1 Sterility precautions against microbial contamination, *Pharmeuropa* 2000; **12** (2).

[33] Midcalf B, Lee G. *Isolators for pharmaceutical applications: practical guidelines on the design and use of isolators for the aseptic processing of pharmaceuticals*, London: HMSO edition 1994.

[34] PIC/S. Isolators used for aseptic processing and sterility testing, PE- 004-1 (Draft) 4.

[35] Delattin R. EU status of GMP for sterile products, Parenteral Society, Hinckley, 4 Nov. 1997.

[36] Brader WR. Impact of implementing barrier technology on existing aseptic fill facilities, *Pharmaceutical Engineering,* March/April 1995.

[37] Porter ME. Merck barrier isolator cost analysis revisited, ISPE Rockville Conferences, 2 June 1997.

CHAPTER 11

Validation concepts in pharmaceutical aseptic application isolators

Rajesh Thempadiyil, Manager, Technical Transfer, Dr Reddy's Laboratory, India

11.1 Introduction

Since the 1990s, the pharmaceutical industry has been subject to significant developments in relation to different technologies. In this, the most significant development is the use of barrier /isolator technologies for aseptic processing; where isolator technologies have incrementally replaced conventional cleanrooms. Currently, isolation technology and isolators are the preferred technology for any new production line for parenteral products. Aseptic processing, using isolators, separates the external cleanroom environment from the product being processed and minimises the exposure of the product to contamination (which predominantly arises from the operating personnel). Thus a well-designed positive pressure isolator, supported by adequate procedures for its operation, control, monitoring and maintenance provides substantial benefits and control over microbial contamination over conventional aseptic processing.

This chapter intends to present on overview of the essential technical information and the corresponding regulatory requirements from different angles, with emphasis on the aseptic processing specific isolators. The objective of the chapter is to understand the key aspects of design and validation of the isolator systems from the user's perspective and to provide a pragmatic approach to be followed during design and validation activities. The chapter addresses necessary user requirements and details the validation requirements for the application of isolation technology in the aseptic processing, which is an important step in increasing the level of sterility assurance of the product.

11.2 History and key definitions

Isolator systems evolved from glove-box technology in which “absolute” separation is provided between the internal and external environments. Using isolators, human manipulations within the aseptic zone can be performed without actual personnel access, through the use of sleeves and gloves and usage of half-suits. In an aseptic facility the positive pressure isolator can be used for manufacturing, filling lines, freeze dried/vial lines and transport systems – it is also used in quality control laboratories for sterility testing applications. The isolator environment can be bio-decontaminated to achieve surface sterilization (as opposed to penetrative sterilisation applied to terminally sterilised products or sanitised, as with the case of a conventional cleanroom). In addition, the exclusion of personnel and the ability to make substantial changes in the operating mode makes isolation technology more revolutionary than evolutionary, especially when compared to the conventional manned cleanroom.

The first phase of development of isolators consisted of mainly technical solutions orientated towards hardware design and construction. Then the focus of development shifted to process engineering, ergonomics, integrity, decontamination using hydrogen peroxide (H_2O_2) gassing and validation procedures. Guidance and regulatory requirements about isolator technology is now provided in all major directives including: US Food and Drug Administration (FDA), EU GMP, United States Pharmacopeia, Pharmaceutical Inspection and Co-operation Scheme (PIC/S), European Pharmacopoeia, ISO, and with the support of large pharmaceutical communities such as ISPE, PDA and PHSS.

Key definitions:

Isolator as per:

FDA: "A decontaminated unit, supplied with Class 100 (ISO 5) or higher air quality that provides uncompromised, continuous isolation of its interior from the external environment (e.g., surrounding cleanroom air and personnel)[1]."

PDA: "An isolator is sealed or is supplied with air through a microbial retentive filtration system (HEPA minimum) and may be reproducibly decontaminated[2]."

ISO: "Separative Device: Equipment utilizing constructional and dynamic means to create assured levels of separation between the inside and outside of a defined volume[3]."

11.3 Basis for positive pressure isolators

The isolators intended for aseptic processing are required to be operated under positive pressure and are subjected to a decontamination process before the start of batch processing. Isolators for handling sterile materials inside are designed according to the following basic principles:

- The air exchange between the isolator and the surrounding environment must occur only through a microbial retentive filter such as High Efficiency Particulate Air (HEPA) or Ultra Low Penetration Air (ULPA)
- The positive pressure aseptic processing isolator must be decontaminated in a reproducible and quantifiable manner to ensure the Sterility Assurance level of 10^{-6} [1,2,4]
- Entire activity/handling of materials inside an isolator shall be achieved remotely and no part of the human body can enter the isolator
- Asepsis shall be maintained for each unit operation and for material transfers. Any material entering the isolator must either be decontaminated inside the isolator or sterilised and taken inside via validated transfer devices

11.4 Validation of isolators

The isolator system must be validated before it is used in any of the manufacturing process as there has been a paradigm shift with the qualification of critical process equipment used for aseptic manufacturing, where it has become a necessity to follow a "Life Cycle" approach rather than a conventional qualification approach.

The life cycle approach for any system consists of four major phases:

1. Concept
2. Project
3. Operation
4. Retirement

At the concept phase a decision is made on whether to proceed to the project phase by considering various facts such as cost of the system, benefits of the system, etc. During this phase validation documents are not usually prepared. However, a project risk assessment can be carried out, which will reveal several facts to be considered.

The first activity during the projects phase is to prepare a user requirement specification (URS). Based on this specification, supplier selection and evaluation is performed where the specification may be subject to revision during the course of the project. This phase includes design, construction and commissioning and qualification steps required until release for operation. The validation documents shall be prepared at each step of activities such as:

- URS (User Requirement Specification)
- DQ (Design Qualification)

- FAT (Factory Acceptance Test)
- SAT (Site Acceptance Test)
- IQ (Installation Qualification)
- OQ (Operational Qualification)
- PQ (Performance Qualification).

At each stage it is also necessary to perform risk assessment[5] for the following reasons:

1. URS: By considering the product knowledge, process knowledge, regulatory requirements and company quality requirements risk assessment.
2. DQ: In addition to the above, a risk assessment at this stage will enable one to build more control parameters into the equipment/system/process.
3. Further validation steps: The assessment of equipment risks classification determines the requirements for verification and at what step in the validation process the tests should be done, i.e. FAT versus commissioning versus qualification, depending on the risk category assigned.
4. During the operation phase, the system is operated as per the defined operational procedures by appropriately trained personnel. Monitoring of the system shall be done during this phase for its continuous operation during actual production environment, with activities such as alarm trending, fine-tuning the operational parameter limits, defining acceptance criteria for the concentration of hydrogen peroxide, etc. All the changes and revalidation activities during the operational phase shall also be monitored, as part of the continuous validation strategy.

A decommissioning procedure shall be in place to handle the retirement of the system from operation. This procedure should include a detailed review of the data available, data retention, migration or destruction and management of these processes.

11.4.1 Validation life cycle approach

This life cycle model usually has following phases:

1. Planning
2. Specification
3. Construction
4. Verification and reporting
5. Routine use – continuous monitoring

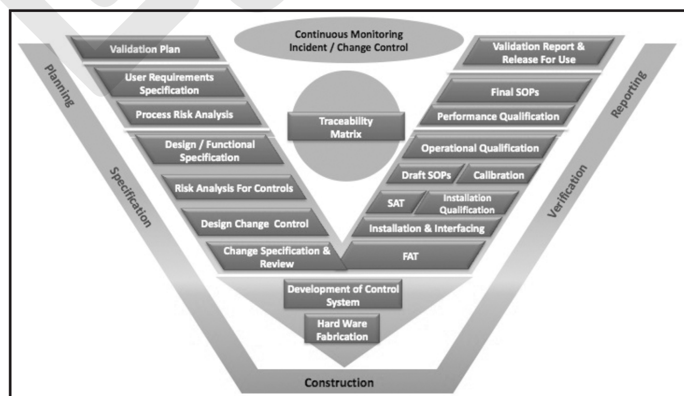


Figure 1: *Validation life cycle diagram depicts documents to be prepared and activities to be performed in chronological order by both isolator manufacturer and customer. This flow diagram can be applied for any quality critical equipment/system.*

11.5 Regulatory requirements for isolators for aseptic use

The **Good Manufacturing Practice** guidelines elaborated by the regulatory authorities of the pharmaceutical industry are devoted to ensuring product safety of the medicinal products sold in the market place. Requirements from US FDA are specified in the Aseptic Processing Guide[1], where one annex is dedicated to isolators. Critical requirements and criteria are delineated in this section of the guide. Specific stipulations for the isolators are also given in the chapter "*Isolator Technology"* of Annex 1 to the EU GMP guide – devoted to the manufacture of sterile medicinal products[6].

The principal requirements of EU GMP[6] are:

- Air classification of the isolator and background environment
- Usage of construction material that reduces puncture and leakage of isolators
- Transfer devices with sterilisation mechanisms
- Guide recognises that laminar air flow may not be present in the working zone, where high risk manipulations are performed
- For aseptic processing the background environment shall be at least Grade D
- Introduction of isolators for routine use only after appropriate validation
- During validation, critical factors shall be considered such as air classification, sanitisation of the isolator, the transfer process and isolator integrity
- Routine frequent leak testing of isolators and glove/sleeve system

The principal requirements of US FDA[1] are:

- First, the routine maintenance of the system, as it is the most important. The integrity of seal/glove/sleeve/half suits should be verified daily. Replacement frequencies shall be established for such components and transfer systems, gaskets and seals shall also be covered as part of maintenance program.
- During the airflow design, care should be taken to achieve segregation between the inner isolator and the room environment. Unidirectional airflow is required in the aseptic processing isolators.
- The isolator shall be designed with an over pressure within the range of 17.5 to 50 Pa. (Based on author's experience a limit of 30 to 35Pa is recommended in order to maintain the stipulated over pressure even in worst case conditions, e.g. hands in all gloves inserted at same time. In addition this recommended pressure limit facilitates easy operation using gloves as well as maintaining the minimum pressure). The direct interfaces, e.g. a dry heat tunnel, shall be qualified as well as the exit port or mouse hole with suitable pressure regimes to prevent ingress of surrounding room air.
- The isolator shall meet ISO class 5 air cleanliness requirements and its background should be ISO Class 8, which is equivalent to Grade C.
- Transfer devices shall be suitably selected and designed to ensure that integrity of the isolator system is maintained.
- Decontamination procedures and requirements should be discussed in an orderly manner in the section. These will be elaborated in the Performance Qualification section.

11.6 Key features of isolator design

The following sections describe the design of an isolator system for aseptic application. It covers the basic requirements to be considered and checked during the design of the isolator systems.

11.6.1 Development of the User Requirements Specification and Design Specification

The validation documents from the outset of any project, the first documentation to be developed

should be the User Requirement Specification (URS). Its basic purpose is to delineate how the isolator system will perform; it may suggest possible technical solutions and in some cases may dictate some absolute requirements. This document forms the basis for discussion with potential isolator supply companies. The successful compilation and execution of the Installation Qualification (IQ) (for installation), Operational Qualification (OQ) (for functionality and operability) and the Performance Qualification (PQ) (decontamination), is dependent on an URS containing clear, concise and testable requirements. Preparation of the URS should be the combined responsibility of subject matter experts from cross-functional functions such as manufacturing, projects/engineering, product development, validation, quality assurance and microbiology.

To commence the design of the isolator, interfaces and necessary calculations of the isolator system of a given investment object, the URS must be completed. Based on this basic specification, the design/functional specification can be developed along with the selected vendor. Interfacing/integration procedures and responsibilities shall be clearly identified and finalised during this phase.

11.6.2 Isolator – relevant GMP guidance, standards and guidelines:

GMP guidance:

- WHO Good Manufacturing Practices for Sterile Pharmaceutical Products[7]
- Annex 1: EU GMP Guide – Manufacturing of Sterile Medicinal Products[6]
- Annex 11: (Computerised systems) EU GMP Guide[8]
- Annex 15: EU GMP Guide – Qualification and Validation[9]
- PIC/S PI 014-3: Isolators Used for Aseptic Processing and Sterility Testing
- PIC/S PI 009-3: Aide-mémoire: Inspection of utilities[10]
- USFDA: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice[1]

Standards, guidelines, company-internal directives:

- ISO 10648 series of containment enclosure standards[11]
- ISO 14644 series of cleanroom standards and related guidelines as specified
- PDA 34: Design and validation of Isolator Systems for the Manufacturing and Testing of Health Care Products[2]
- EN 779[12]
- EN 1822[13]
- GAMP 5[14]
- Applicable company-internal directives and manuals.

The above editions were valid at date of publication of this chapter.

11.6.3 Design verification:

The system objective shall be clearly defined and the list of products to be manufactured in the isolator line shall also be identified. For this, the following drawings should be prepared:

a) *With respect to facility and room:*

   i. Facility layout
   ii. Facility pressure differential layout
   iii. Facility air classification layout
   iv. Facility material man movement layout

v. Air handling unit scope layout
vi. Filter cut-out layout for room
vii. Equipment layout with interface to isolators

b) *With respect to isolator*

i. General Arrangement (GA)/Isometric Drawing
ii. GA drawing in three-dimensional form
iii. Process & instrumentation diagram including decontamination process
iv. Pressure differential layout in case of series of isolators with respect to the background room
v. HEPA filter layout
vi. Location for environmental monitoring with provisions – viable and non-viable
vii. Layout with utility connection
viii. Layout with decontamination assembly system
ix. Layout with washing provision and its drainage system

c) *The following lists/specifications should be prepared:*

List of instruments/components to be installed

i. List of interlocks
ii. List of PLC inputs/outputs
iii. Electricals specification
iv. Utility specification
v. List of product contact materials
vi. List of product contact lubricants, if any

11.6.4 Environmental conditions

Air cleanliness classification

The isolator design must achieve ISO Class 5/EU GMP Grade A condition for the isolators. The author recommends targeting the design so as to achieve ISO Class 4 during the "at rest" state and ISO Class 5 (EU GMP Grade A) during the "In Operation" state. This opinion is based on the ease of achieving ISO Class 4 (>0.5μ particles and >1.0μ particles) in an isolator environment, which also illustrates the high cleanliness condition when using the isolator.

Air temperature

This is dependent upon the products to be manufactured in the isolator line.

Relative Humidity

Typical range: Range 35 to 65% RH. However, this depends on the product requirement. Options for achieving Relative Humidity are; refrigerant drying/silica-gel bed/rotary (lithium chloride) dryer (e.g. Munters Rotaire).

Pressure differentials

FDA[1] provides a range between 17.5 to 50Pa. This author recommends having a range of 30 to 35Pa when operating in a series of isolators.

11.6.5 Air handling system and airflow considerations

Unidirectional airflow

The design should enable the creation of an airflow system with direct filtered air, blown downwards in a constant stream with uniform velocity.

Airflow velocities
The regulatory figure for airflow emerging from a panel of HEPA filters is 0.45m/sec ± 20%[1,6]. However, filter face velocities less than these values are found to be adequate in an isolator environment[15]. A minimum of 0.25m/sec velocity is easily controllable and provides sufficient air changes per hour in order to sweep away particles from the system.

Air filtration – supply air
A minimum of H13, H14 HEPA[13] filters should be used in the inlet air supply. Grades of ULPA filters can also be considered as sometimes there is not much difference in the cost between ULPA and HEPA filters. Double HEPA filtration should be considered in the inlet air supply system so that even if one filter has a small leakage, the second one will assure the supply air quality.

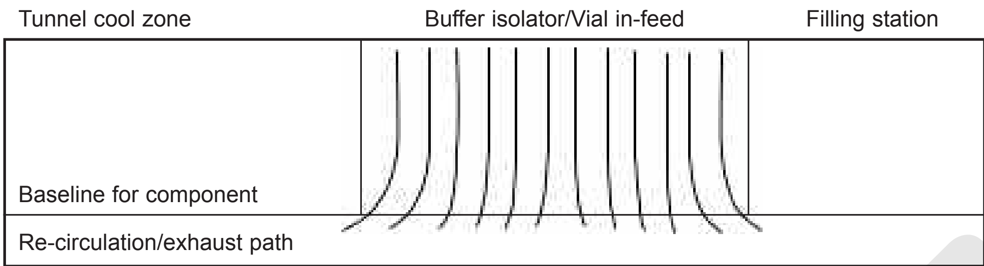
Recirculation filters
A minimum of H13, H14 HEPA[13] filters shall be used in the re-circulation system. This ensures double filtration for both inlet and exhaust filtration systems. When the isolators are used for containment applications, Bag-In-Bag-Out (BIBO) HEPA filters should be considered. When designing, care should be taken to ensure the leak-tightness of the BIBO assembly.

Exhaust filters
When isolators are used for contained aseptic applications, it is advisable to use H13 filters in the exhaust system so that the air going out into the environment is filtered. With the monitoring of pressure differential across these filters, the volume of exhausted air can be calculated.

Recovery time[6] of as low as possible shall be targeted. Usually, unidirectional positive pressure isolators should achieve the recovery time within less than one minute. Recovery time is the time taken for the air in the device to change from a "dirty" to "clean" state within a stipulated time.

For the design of the air handling unit, the following should be considered:

1. Usage of separate air handling and room air handling systems. It is advisable to use a separate air handling system to achieve fine controls during routine operations. Fluctuations due to room door opening can also be reduced by this design.
2. An airflow calculations/distribution plan should be made in such way that uniform temperature and humidity is achieved in each of the isolators.
3. Usage of a suitable mesh screen below the HEPA filters is advisable as it will improve the appearance, as well as ensuring uniformity in the distribution of air across the filter face.
4. De-humidification system – the design should enable the supply of dehumidified air during the conditioning and aeration stages of the vapour phase hydrogen peroxide (VHP) decontamination cycle.
5. Design of interface between transfer tunnel and isolators: for depyrogenation tunnels, the tunnel cool zone air intake should be considered in the airflow calculation and pressure regime. Calculation may extend to the usage of both small-size and large-size vials as airflow intake varies for both due to space between the vials.
6. When aseptic isolators are used for containment applications, the pressure differential should be designed in such a way that the interface isolator pressure is more than that of the tunnel cool zone and filling station (see illustration overleaf).
7. The scope of decontamination in isolators shall be carefully designed. The entire air re-circulation path should be considered for decontamination, along with the filtration system.
8. The position of leak-tight valves should be carefully decided to achieve the desired level of integrity of the enclosure. Usually a fan assembly is not available as leak-tight hence its position shall be outside the leak-tight valves.



9. The exhaust system can have provision to control the exhausted airflow volume. Usually, 10 to 15% of air volume is exhausted; at the same time it is compensated with fresh air. The fresh air can come from the room air handling system so that conditioned air is fed into the isolator air handling system.
10. BIBO (Bag-In-Bag-Out)/FIBO (Filter boxes from SKAN AG) filters can be considered in the exhaust and recirculation system. These filters are easily replaceable/interchangeable and are suitable for containment applications.
11. Provisions should be built in for the integrity test of the inlet, exhaust and recirculation filters, for DOP testing, to check upstream concentration and to scan downstream concentration.
12. An uninterruptible power supply for supply/recirculation fans must be considered. This will help maintain aseptic conditions once decontamination is completed, should a power failure occur.
13. Provision of non-viable monitoring mechanisms should be considered in the design as they demand a certain amount of mechanical design to be carried out. The locations should be documented. Examples of monitoring locations include: within 1m of the product filling station, near container/closure handling and at the main intervention locations.

11.6.6 Enclosure and construction material

Depending on the application and type[2] (rigid wall/flexible wall) of isolator, the following materials can be considered in the design:

a. Flexible film plastic
b. Rigid plastics
c. PVC/acrylic/polycarbonate/tempered glass for windows
d. Stainless steel

For designing the enclosure, the following selection of material for enclosure and components, should be considered[2]:

1. Chemical compatibility with materials/products to be handled in the isolator, decontamination and cleaning agents, etc.
2. The degree of structural integrity should be considered (this will relate to the isolator pressure).
3. The surface finish and cleanability. In general, a “buff” finish with a surface rugosity greater than 280 grit is effective.
4. When stainless steel is used, it is advisable to use SS 316L with high-weld quality, using either argon or tungsten inert gas.
5. Designing screws/nuts should be avoided inside the isolator. If this cannot be avoided, dome-shaped nuts should be used.
6. The isolator floor should be designed in such a way that a suitable slope for cleaning the water drain is provided.
7. For efficient closure of the doors, an inflatable gasket design could be considered.

11.6.7 Ergonomics and access devices

Isolators are designed to prevent the exchange of contamination between the enclosed work environment and the surrounding environment in which personnel are located. The ergonomics of an isolator is one of the critical factors to be considered during the isolator design. Position of the gloves should be carefully finalised, which will facilitate easy handling of operations. The following ways[2,15] of manipulating materials inside an isolator should be considered:

a. Gloves/sleeves
b. Body suits
c. Automated system such as robots/conveyor assemblies, etc.

Commonly used glove materials include Neoprene® and Hypalon®. Their material could be chosen from latex or natural rubber, polychloroprene, nitril rubber or copolymer, polyvinyl chloride and chlorosulfonated polyethylene[3]. Two different designs are available such as one- or two-piece assembly where glove and sleeves are different pieces. Two-piece design ensures a safe change of gloves in case of damage during operation. Typical lengths of gloves are 700, 750 and 800mm.

When selecting material for the enclosure and components, the following should be considered:

1. When designing the enclosure, a vertical angle of about 10 to 15° should be considered. This will enhance the ergonomics and facilitate the operations.
2. When used in an aseptic filling line, the filling machine should be designed so that the width is narrow, allowing access from only one side. A width of less than 600mm is considered suitable.
3. Sufficient numbers of ambidextrous glove/sleeves should be provided after performing a trial by modelling and/or the construction of a full-scale mock-up designed with suitable materials.
4. For cleaning the isolators, a Wet-In-Place system shall be designed in order to avoid any manipulation in the isolator environment and to achieve consistent effective cleaning. Care should be taken to avoid damage to the filtration system by the cleaning agent. Installation of suitable spray balls could be considered wherever required. However, care must be taken to ensure their sterilisation by VHP or steam sterilisation or both.

11.6.8 Transfer devices:

The loading of materials into isolators and the subsequent removal of products and waste, yet maintaining the isolator aseptic environment, is considered as one of the more complex and critical aspects of isolation technology. Generally considered techniques[2,15] for transferring materials are as follows:

a. Simple doors
b. Lock chambers
c. Product out-paths/mouse-holes
d. Waste ports (polybag with sealer)

The selection of transfer devices mainly depends on the application and classification of the background environment. Rapid Transfer ports or Double Door Transfer Port (DPTE), developed by La Calhène, are the most reliable transfer devices. The transfer ports can be used for a wide range of activities such as transfer of liquids, transfer of rubber stoppers/seals after sterilisation, transfer of samples and waste from the isolator, etc.

The aseptic and safe transfer of an aseptically filtered solution often poses some challenges. However, the usage of a liquid transfer path (DPTEs) provides a good solution when used along

with sterile-to-sterile (S2S) connectors. The S2S connector is to be used between the filtration vessel in the online filtration line, going into the filling line, to inside the isolator.

11.6.9 Glove integrity testing provisions

Glove integrity testing is one of the critical aspects of the isolators. A faulty glove or sleeve (gauntlet) assembly represents a route of contamination and is a critical breach of isolator integrity. The monitoring and maintenance program should identify and eliminate any glove lacking integrity and minimise the possibility of placing a sterile product at risk. Suitable methods and devices for performing physical integrity tests should be considered at the design stage. Various methods are available for performing glove integrity tests as follows[3]:

a. *Pressure change method*
   The system should be subjected to monitoring pressure decay per unit time after isolating the glove or containment enclosure at a particular standard positive pressure.
b. *Constant pressure method*
   The airflow rate from the pumping system needs to be measured, so as to maintain a positive pressure at constant level in the glove/isolator enclosure. This flow rate, divided by the enclosure volume, corresponds to the hourly leak rate, at the specified positive pressure.

Equipment is available to perform the integrity tests by pressure change and constant pressure methods. When selecting equipment for glove integrity tests, consideration should be given to its sensitivity[16], such are micron size at a defined pressure. The data for justifying claims made by the manufacturer should be demanded when procuring the equipment. As specified in ISO 14644-7[3], the criteria for the glove integrity test is a static reading of between ±2Pa to ±10Pa in 10 seconds when pressurised at about 1000Pa. This criterion shall be comparable with the manufacturer's specifications.

11.6.10 Decontamination system

Eliminating detectable levels of micro-organisms within the isolator is a critical user requirement specification. Provision should be made so that the isolator and its contents are decontaminated on a scheduled basis. Gassing systems are one of the proven technologies for decontamination of isolator systems. Hydrogen Peroxide Vapour (VHP) is the most advanced, reliable and widely used gaseous vapour for decontamination.

Stand alone VHP generators are available such as; Steris VHP 1000™ and BioQuell Clarus™. Many isolator manufacturers such as SKAN, Bosch, etc. integrate the gas generators within the isolator systems.

When designing the control system for a VHP cycle, the following factors should be considered:

a. The cycle should be designed in such a way that the complete airflow path is subjected to decontamination of the HEPA filters, valves, ducts, etc.
b. Functionality should be designed in such a way that all moving parts inside the isolator are exposed to gas. An intermittent can be planned during a cycle phase.
c. An aeration cycle should be designed in such a way that any residual concentration from the wrapped goods is gassed out to a safe level.
d. Provisions for holding the gloves in a proper position should be made so that the inner portion of the gloves/sleeves are exposed to the gas during the cycle, e.g. glove holder.
e. The opening and closing of the tunnel gate shall be automated as required during or after the cycle.
f. Where chemical and biological indicators are used for gas distribution and cycle efficacy studies, structured protocols are required to study key process variables in the development and qualification of robust and repeatable cycles.

g. The target total cycle time should be considered as one of the critical factors as rapid cycle techniques are available.

11.6.11 Process monitoring and control system

Data relevant for product safety and batch documentations must be transmitted to the process monitoring system of the production process and at least the following should be included:

a. Pressure differentials
b. Inlet air velocity
c. Airflow volume
d. Recycled flow rate/exhaust flow rate
e. Temperatures and relative humidity
f. Particle counts
g. Alarms and events recorded during the cycles
h. Audit trail

11.7 Qualification, monitoring and verification for continued compliance

11.7.1 Qualification Activities:

Refer to Appendix 13.A for details of the following topics:

- Design review and design qualification
- Installation Qualification (IQ)
- Operational Qualification (OQ)

Refer to Appendix 13.B for details of the following topics:

- Performance Qualification (PQ) – Decontamination cycle development

11.7.2 Process simulation studies

This is probably the aspect of environmental and procedure evaluation that is least affected by the presence of an isolator. All the standard concerns relating to the number of units, size of fill, fill speed, vial size, frequency, media choice, activities to be simulated, etc. follow the same requirements as simulations in ordinary cleanrooms. The acceptance criterion for process simulation testing or media fill in an isolator should be a contamination rate of zero, ie. no contamination should be detected in any of the filled units[17]. The absence of personnel suggests that this limit should be readily attainable in a properly designed and validated isolator-based filling system.

A failed media fill or sterility test is a serious event affecting the product being produced and supplied. In such scenarios, the investigation should involve at least the following:

a. Possible failure of the isolator to control the environment
b. The whole sterility assurance system, including components, formulated drug sterilisation, on-site and any off-site sterilisation processes, product integrity, etc.

11.7.3 Monitoring during production

Many advanced pharmaceutical production tasks – especially if microbiological risks are to be controlled – require proof that the stipulated pressure differentials and, where required, viable and non-viable particle counts or air velocities and, possibly, other parameters have been met continuously and consistently during production.

Other control parameters such as air flow rate, temperature and relative humidity are, on the other hand, indispensable for the automatic control of the air circulation system.

Thus, data have to be fed, on the one hand, into the batch control system, and on the other hand, into the process monitoring system. This last-mentioned system will have to satisfy comprehensive regulatory requirements laid down in Annex 11, Computerised Systems, of the EU GMP guide and, in the context of FDA regulations, in 21 CFR 11[18]. The computerised systems addressed in these guidance documents require validation and this should be performed according to the **GAMP 5**[14] (**GAMP** = **G**ood **A**utomated **M**anufacturing **P**ractices) specifications.

The low voltage electricity supply for both process monitoring and automatic control purposes should be connected to an interruption-free electricity supply source, as even interruptions lasting less than a second can cause them to behave erratically.

In pharmaceutical isolators, pressure differential, temperature and relative humidity are, as a rule, measured continuously. In Grade A areas, airborne particle counts and the air velocity are also monitored continuously, or at least – as specified in Annex 1 to the EU GMP Guideline and the corresponding WHO Guidance – at such a frequency that all interventions, transient events and any system deterioration would be discovered. Periodical measurements are also indicated for recording the pressure difference across HEPA filters. This pressure difference increases very slowly with time so that quarterly, half-yearly or even annual data collection with manual recording is sufficient.

Periodical measurements are again the rule for the sampling of airborne micro-organisms, except in grade A areas where frequent sampling is indicated.

Where continuous or frequent sampling is required in areas protected with unidirectional airflow, the sampling heads or sensors should be installed in locations close to the critical areas, at positions optimised during the OQ phase.

A typical batch record report shall have data for critical process parameters from the following cycles:

a. Cleaning cycle
b. Drying cycle
c. Leak testing of gloves/sleeves
d. Leak testing of enclosure
e. Decontamination cycle
f. Normal production cycle
g. Cleaning cycle

The important control of an isolator is physical; therefore, physical monitoring and testing is pre-eminent. The monitoring and testing should be organised to monitor the critical process parameters and these parameters shall be integrated with alarm systems. Alarms should be latched so that the occurrence of the alarm is still evident even though the deviation leading to the alarm being triggered has corrected itself[4].

In an isolator environment, detection of any microbiological contamination probably indicates a failure of the system. When designing the environmental monitoring programme for an isolator facility, the following should be taken into account:

a. The monitoring activities within the isolator should not interfere with zone protection.
b. In-process tests or sampling should not carry any risk for manufacturing or sterility.
c. The use of monitoring techniques such as settle plates, contact plates, swabs and the presence of sampling points for active air samplers or particle counters may add risk to the system subjected to a sporicidal process. To avoid such circumstances, consideration should be given to:

- Sampling at the end of production.
- Sampling at potentially worst case positions, e.g. in an exhaust.

The detection of any micro-organisms from environmental monitoring inside the isolator should be considered as requiring a full scale investigation. Consideration should also be given to the wisdom of releasing product that is still in-house and where the continued use of the isolator may not be appropriate.

11.7.4 Operation and maintenance

As in every GMP system, the operation should start with training the staff in each section of the organisation. It is important that isolation technology, which is very different from the conventional cleanroom process, is understood by the staff. Training can start at the very initial stage itself with proposal drawings and mock-up assemblies prepared for ergonomics trials. It is important for the operational staff to actively participate throughout the qualification activities, so that in routine operation, trouble shooting becomes much easier.

During the process, standard operating procedures (SOPs) should be developed for various activities such as operation, cleaning and maintenance of the system. The process parameters, which should be part of the Batch Record should also be finalised. In addition to routine functions, the actions to be taken in the event of any failure should also be described in the SOPs. Such events may include immediate emergencies, such as a low-pressure alarm trip or loss of a glove or failure of a leak test[15], etc.

Maintenance of the HVAC system of a isolator follows, in general terms, the procedures employed for other industrial air-conditioning systems. The only peculiarity is terminal HEPA filters: after filter replacement, an installed filter leakage test according to ISO 14644-3[19,20] is highly recommended.

The following tests and programmess should be considered as part of the routine maintenance activity:

- Leak testing
- Systematic visual examination
- Calibration
- Maintenance checks of structure as well as equipment

11.7.5 Verification for proving continued compliance

Verification of the air handling system of isolators for continued compliance, especially with ISO 14644-1[21], is covered in ISO 14644-2[22].

Air classification verification is performed in the *at-rest* and *in-operation* occupancy states and should address, according to ISO 14644-2, at least the following parameters:

- The air cleanliness class
- Pressure differences
- The air velocity

Further tests may be specified according to requirements. In air handling systems serving facilities for aseptic manufacturing, FDA and WHO require the **installed filter leak test** of HEPA filters to be performed periodically. The interval between subsequent tests has been set by FDA as at least twice a year[1], whereas WHO permits an extension to once yearly[7]. Although surprisingly not mentioned in Annex 1 to the EU GMP guide, the installed filter leak test is recommended in PIC/S PI 032-2[20].

Table 1: Verification requirements at different stages of procurement of isolator system

S.No	Activity	Factory acceptance test	Commissioning	Equipment validation
1	Engineering Documentation Verification	Verify	Verify	Refer
2	Equipment/Component Verification	Define	Verify	Refer
3	Electrical Utility Verification	Define	Verify	Refer
4	Non-Electrical Utility Verification	Define	Verify	Refer
5	Product Contact Materials of Construction	Define	Document	Verify
6	Instrumentation Verification	Define	Verify	Refer
7	SOP Identificatio	Identify	Document	Verify
8	Hardware Verification	Verify	Verify	Refer
9	Software and Application Program Verification	Define	Verify	Refer
10	Configurable Parameters/Set points Verification	Define	Fine Tune	Verify
11	HEPA Filter Installation Verification	Verify	Verify	Refer
12	Start-Up Verification	Verify	Verify	Refer
13	Preventive Maintenance Program Verification	Define	Document	Verify
14	Test/Reference Instrument List	Define	Document	Verify
15	Ergonomics verification	Verify	Verify	Refer
16	Controls, Interlocks, and Alarms Testing	Verify All critical	Verify %	Refer
17	Power Loss Testing	Verify	Verify	Refer
18	PLC Control System Testing	Verify All critical	Verify	Refer
19	Radio Frequency Interference (RFI) Testing	Verify	Verify	Refer
20	Electro-Magnetic Interference (EMI) Testing	Verify	Verify	Refer
21	Ammonia Leak Testing	Verify Enclosure	Verify	Refer
22	Glove Integrity Testing	Verify	Verify	Verify
23	Leak Testing	Verify Enclosure	Verify	Verify
24	HEPA Filter Integrity Testing	Verify	Verify	Refer
25	Pressure differences across HEPA filters, Pressure difference between isolators (in an isolator chain) during normal and, where specified, reduced operation.	Define	Fine Tune	Verify
26	Air flow uniformity (Velocity) Testing	Fine Tune	Fine Tune	Verify
27	Airflow visualization	Fine Tune	Fine Tune	Verify
28	Non-Viable Particulate Count Testing	Define	Verify %	Verify
29	Air temperature and relative humidity.	Define	Verify	Verify
30	Operational Testing	Verify representative	Verify %	Verify
31	Verification of Compliance with EU Annexure 11 and 21 CFR Pat 11	Verify	Verify	Refer
	Decontamination gassing cycle			
32	Prequalification of Isolator and Gassing System	Define	Fine Tune	Verify
33	Cycle Fractional Study	Define	Fine Tune	Verify
34	Development Cycle / Performance Qualification Cycle	Define	Fine Tune	Verify

Some operators of facilities for aseptic manufacturing extend compliance verification exercises also to the microbial limits which must, of course, be assessed in the occupancy state *in-operation*.

The decontamination cycle re-verification frequency can be set as once a year, based on the recommendation of PIC/S PI 014-3[4].

11.8 Risk based approach for qualification of an isolator system

The requirement for a risk-based approach for equipment qualification is mandated by US Food and Drug Administration, EMEA, etc. Requirements for the risk assessment are consistent with the International Conference on Harmonisation[22,23] and ASTM E2500[24]. An organisation shall have a risk-based approach which optimises the Factory Acceptance Test and Commissioning and Qualification.

The Quality Risk Management (QRM) approach to qualification[5] shall focus on verification of the critical attributes of the isolator as they relate to product performance and their relevance to quality, strength, purity, safety and efficacy. For any approach, at least a three level risk classification (High, Medium and Low) can be done which is in line with the requirements of GAMP 5 classification, applying the principles of Failure Mode and Effects Analysis (FMEA). As per FMEA, the overall risk shall be calculated by quantifying severity, likelihood of occurrence and detectability. Once this categorisation is completed then the focus of effort shall be on verifying the most critical parameters at different stages of verification/qualification. The verification requirements at different stages of procurement of the isolator system are provided in **Table 1**.

11.9 Summary

In writing this chapter, the author's intention was to address the specific peculiarities of pharmaceutical isolator systems intended mainly for aseptic processing. They serve, above all, safety objectives: to help ensure that pharmaceutical preparations are safe for the patient. Where hazardous substances are handled in the production facility, isolator systems are most suited for protection of operating personnel and the external environment against airborne hazardous particles and fumes. Essential elements for ensuring the required air cleanliness levels are air filtration, leak tightness, establishment of the desired airflow patterns and pressure differentials or other means for preventing cross-contamination into the rooms.

Each segment of isolation technology and its components can be subjected to more detailed testing. Regarding the isolation technology concept, various basic options are described.

11.10 References

1. Guidance for industry: Sterile drug products produced by aseptic processing – current Good Manufacturing Practice. U.S. Department of Health and Human Services, Food and Drug Administration (September 2004).
2. Design and validation of isolator systems for the manufacturing and testing of health care products. *PDA Journal of Pharmaceutical Science and Technology* September/October 2001. Supplement Technical Report 34, **55**(5).
3. EN ISO 14644-7, Cleanrooms and associated controlled environments – Part 7: Enhanced clean devices, ISO, Geneva, Switzerland, 2004.
4. PIC/S Recommendation of Isolators Used for Aseptic Processing and Sterility Testing, PI 014-3, 25 September 2007.
5. Ian Campbell. Applying quality risk management principles to achieve a practical verification strategy, ISPE Pharmaceutical Engineering, November/December 2009, pp 16-22.
6. EudraLex, the rules governing medicinal products in the European Union – Vol. 4: EU guidelines to Good Manufacturing Practice for medicinal products for human and veterinary use. Annex 1, Manufacture of Sterile Medicinal Products, European Commission, Brussels (frequently updated).

[7] Annex 6, WHO good manufacturing practices for sterile pharmaceutical products WHO Technical Report Series, No. 961, 2011.

[8] EudraLex, the rules governing medicinal products in the European Union – Vol. 4: EU guidelines to Good Manufacturing Practice for medicinal products for human and veterinary use. Annex 11: Computerized systems.

[9] EudraLex, the rules governing medicinal products in the European Union – Vol. 4: EU guidelines to Good Manufacturing Practice for medicinal products for human and veterinary use. Annex 15: EU GMP Guide – Qualification and Validation.

[10] PIC/S PI 009-3: Aide memoire – Inspection of utilities. Pharmaceutical Inspection Co-operation Scheme PIC/S, Pharmaceutical Inspection Convention PIC, Geneva (25 September 2007).

[11] ISO 10648-2: 1994, Containment enclosures – Part 2: Classification according to leak tightness and associated checking methods.

[12] European Standard EN 779:2002: Particulate air filters for general ventilation – Determination of the filtration performance. European Committee for Standardization CEN, Brussels (June 2002); an updated version has since been circulated as Draft European Standard prEN 779:2009 with the same title. Idem, ibid. (May 2009).

[13] EN 1822: High efficiency particulate air filters (EPA, HEPA and ULPA): Part 1 – Classification, performance testing and marking; Part 2 – Aerosol production, measuring equipment, particle counting statistics; Part 3 – Testing flat sheet filter media; Part 4 – Determining leakage of filter elements (scan method); Part 5 - Determining the efficiency of filter elements. Idem, ibid. (Nov. 2009).

[14] GAMP 5: A risk-based approach to compliant GxP computerized systems. ISPE – The International Society for Pharmaceutical Engineering – in collaboration with FDA and other regulatory authorities. Tampa, FL, USA (2008).

[15] Tim Coles, Isolation Technology, A Practical Guide, Second Edition, CRC Press, 2004.

[21] EN ISO 14644-1: Cleanrooms and associated controlled environments – Part 1: Classification of Air Cleanliness, ISO, Geneva, Switzerland, 1999.

[00] EN ISO 14644-2: Cleanrooms and associated controlled environments – Part 1: specifications for testing and monitoring to prove continued compliance with ISO 14644-1, ISO, Geneva, Switzerland , 2000.

[00] EN ISO 14644-3, Cleanrooms and associated controlled environments – Part 3: Metrology and test methods, ISO, Geneva, Switzerland, 2005.

[00] PIC/S PI 032-2: Recommendation – GMP Annex 1 Revision 2008, interpretation of most important changes for the manufacture of sterile medicinal products. Pharmaceutical Inspection Convention PIC, Pharmaceutical Inspection Co-operation Scheme PIC/S, Geneva, Switzerland, 8 January 2010.

[00] Points to Consider for Aseptic Processing – *PDA Journal of Pharmaceutical Science and Technology*: Supplement, Volume 57, 2003.

[17] Parenteral Drug Association, Technical Report No. 22, Process Simulation for Aseptically Filled Products, 2011.

[16] Gessler A, Stärk A, Sigwarth V *et al*. How risky are pinholes in gloves? A rational appeal for the integrity of gloves for isolators. *PDA Journal of Pharmaceutical Science and Technology* 2011; **65**: 227-241.

[18] 21 CFR 11: Guidance for industry – Electronic records; electronic signatures – Scope and application. U.S. Department of Health and Human Services, Food and Drug Administration, Rockville MD, USA (April 2007).

[25] Sigwarth V, Moirandat C. Development and Quantification of H_2O_2 Decontamination Cycles. *PDA Journal of Pharmaceutical Science & Technology* 2000; **54**(4): 286-304

[26] Current United States Pharmacopoeia, General Information, Chapter <1211>; “Sterilization and sterilization assurance of compendial Articles.”

[27] United States Pharmacopeia. General information, biological indicators – resistance performance tests, Chapter 55. United States Pharmacopeia 2005; 2244.

[22] ICH Q9: Quality risk management. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH, Geneva, January 2006.

[23] ICH Q10: Pharmaceutical quality system. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH, Geneva, June 2008.

[24] ASTM E2500-07: Standard Guide for Specification, Design, and Verification of Pharmaceutical and Biopharmaceutical Manufacturing Systems and Equipment, July 2007.

Appendix 11.A

Tests to be performed during the qualification of isolators

11.A.1 Design Qualification:

DQ is to verify that the system has been designed as specified in the URS (User Requirements Specification), FDS (Functional Design Specification) and relevant equipment specifications satisfying all GMP requirements. For hardware and software of the control system, verification data will be collected to prove that the system has been designed in accordance with the URS and FDS including the requirements of EU GMP, FDA GMP, 21 CFR Part 11[18] and EU Annex 11[8]. As DQ is the final step to formally review and document the proper design of the system, with all drawings and specifications, the protocol must enable the reviewers to verify that all quality-critical attributes and essential technical attributes of the system have been incorporated in the design. When the DQ report is approved, the system is ready for fabrication and construction.

Design review

During the Design Qualification a review design shall be performed. In order to perform the review, the following items should be available:

- Isolator design review plan.
- Basic specification of general requirements for isolator.
- Detailed functional drawing, isolator system, with identification of air handling units, eventual connections to other isolators and/or other equipment, active material pass-through hatches, safety work stations, airflow control elements, sensors, with specification of isolators airflow rates, air cleanliness class, pressure levels, etc.
- Technical description of isolator system including operating concept and automatic control scheme.
- Isolator risk report.
- Heat/Cooling load calculations for HVAC System with resulting air flow rates/air change rates.
- Redundancies, e.g. double fans (eventually with list of additional, so far unapproved proposals).
- Provisional layout of machine room.
- Provisional layout for supply and extract air ducts including eventual connections to active isolators and/or RABS units, active material pass-through hatches, safety work stations, etc.
- Provisional layout with unidirectional airflow protection with isolator system.
- Specifications of air handling units (including reserve requirements).
- Specification of ductwork and construction (leakage rate shall be minimal to nil)
- Specification of components such as
  - Constant and variable airflow control valves
  - Filter housings and fan filter units (FFUs)/BIBOs (Bag-In-Bag-Out) filters
  - Air filters including HEPA filters
  - Motorised and manually operated dampers including fire dampers
  - Outside air dampers
  - Supply air diffusers and extract air grilles
  - Others as required
- Proof of GMP conformity of the specifications for:
  - Room pressurisation scheme.
  - Isolator system concept

  - Airflow rates (demonstration that air flow rates are sufficient for meeting recovery test requirements and airborne micro-organism limits)
  - Airflow pattern requirements
  - Alarm functions.
  - List of parameters to be incorporated into the pharmaceutical monitoring concept including number of sampling points for each parameter
- Functional diagram and specification of the automatic control system, highlighting features for ensuring GMP compliance.-
- Functional diagram and specification of the isolator contribution to the pharmaceutical monitoring system
- Calibration plans for measuring instruments
- Logbook for system change control (if not supplied by future user)
- Change proposal, approval and assessment form (if not supplied by future user)
- Competence and training certificates for:
  - Project and qualification manager.
  - Supervisor of isolator system installation.

DQ report, prepared after successful termination of DQ, with confirmation that all errors and omissions identified during DQ have been rectified.

11.A.2 Factory Acceptance Test and Site Acceptance Test

Factory Acceptance Test (at manufacturer site) and Site Acceptance Test (commissioning at site) shall be performed for every isolator system. During these phases, optimisation of the design can be performed. Any changes occurred in the system shall be documented during these phases.

11.A.3 Installation Qualification (IQ)

The following tests should be made during the installation qualification phase of an isolator system:

1. Engineering documentation verification
   Generate a list of all documentation, submittals, manuals, drawings, parts lists, and purchase orders for the formulation isolator. Include document title/description, document number, revision number/date and the storage location of the document.

   Acceptance Criteria
   A list of the required documentation, submissions, manuals, drawings, parts lists, and purchase orders have been compiled and documented in the datasheet.
2. Equipment/component verification
   Verify and document the tag number, manufacturer, model number, serial number, and specifications, as applicable, of the critical pieces of equipment associated with the isolator, based on information from purchase orders, equipment specifications, manufacturer's manuals, vendor submissions, and/or from visual field verification. In addition, the manufacturer and model number of the major components may be documented on the datasheet. Record the method used to verify the equipment/component specifications.

   Acceptance criteria
   The critical equipment must be clearly documented as to the tag number, manufacturer, model number, serial number, and applicable specifications. The installed equipment must conform to the design specifications listed in the protocol or approved accordingly by the appropriate engineering representative.
3. Electrical utility verification
   Verify and document the power supply provided against the specification. Uninterrupted

power supply is also to be verified, wherever required. Record the method used to verify the equipment/component specifications.

4. Non-electrical utility verification
   Non-electrical utilities shall be verified as part of the IQ protocol.
5. Product contact materials of construction and product contact lubricants
   In general there are no product contact materials of construction associated with the isolator, except the clean air which is in contact with product. Hence the components in the airflow path shall be verified for its material of construction.
6. Instrumentation verification
   Verify all the instruments associated with the isolator system, including its integrated systems such as tunnel, filling machine, VHP gas system, etc.
7. SOP identification
   All applicable SOPs shall be identified during the IQ stage. If an SOP is in draft form during the execution of the protocol, a copy of the draft SOP must be attached to the protocol.
8. Hardware verification
   Confirm and document the hardware/controller configurations for all installed components, including any PID settings, Dual Inline Package (DIP) switch settings, jumper settings, and other configurable parameters for each hardware component/controller, as applicable.

   Acceptance criteria
   The hardware/controller configurations for all installed components (including any Dual Inline Package (DIP) switch settings, jumper settings, and other configurable parameters for each hardware component/controller, as applicable), are verified.
9. Software and application program verification
   The programs/source code/ladder logic (HMI) associated with the isolator is one of the critical items. Confirm and document that the critical system software has been verified as part of the SAT (name, revision, date, location, location of backup and/or other applicable information, information of the current application program(s) and/or operational database, and information on the current program and/or database). Verify and document that the software version has not changed after completion of the SAT. Reference the location of the applicable SAT documentation.

   Acceptance criteria
   The software version has not changed after completion of the SAT. The storage location of the SAT documentation must be recorded on the data sheet. Software version has not changed after completion of the SAT.
10. Configurable parameters/set points verification
    A list of all critical control/alarm parameter set points (temperature, pressure, etc.) associated with the isolator shall be developed/recorded.
11. HEPA filter installation verification
    The installed HEPA filters shall be verified against the HEPA filter installation diagram.
12. Start-up verification
    Perform the start-up activities as per the operation manual provided by the manufacturer.

    Acceptance criteria
    Document that the installation and start-up activities have been performed according to supplier recommendations, where applicable
13. Preventive maintenance program verification
    Verify and document that the isolator/systems has been entered into a preventive maintenance program. Create the PM activities based on the discussion with manufacturer and system evaluation.

Acceptance criteria
The system must be entered into a PM Program and a copy of the PM plan must be attached to the protocol.

14. Test/reference instrument list
Document all test/reference instruments, not associated with the isolator, that were used for the completion of the testing and measurements. Attach copies of the current calibration sheets, with NIST traceability information, for each applicable instrument.

Acceptance criteria
All test/reference instruments used during the qualification must be documented. Copies of the current calibration reports, with NIST traceability information, for each instrument must be attached to the protocol.

During installation qualification the following should also be ensured:

- Preliminary leakage test of air handling units, air ducts and isolator system successfully performed and documented
- Spare part list in existence, complete and approved
- Initial spare parts stock purchased and in storage

Standard operational procedures (SOPs) for OQ measurements complete and approved, e.g. for:

- Installed filter leak test (HEPA filters only).
- Differential pressure
- Room air classification
- Airflow rates
- Air velocities
- Airflow visualisation
- Recovery time
- Room air temperature and relative humidity

Pharma monitoring system: statement regarding completeness, correct installation and functionality of HVAC contribution.

Automatic control system: statement regarding completeness, correct installation and functionality plus, if applicable, correct connection to the facility's building automation system.

Change control logbook and change control forms correctly filled in, complete and up-to-date up to IQ status.

Changes requiring qualification correctly executed and qualified up to IQ status.

Training and corresponding certificates complete for:

- Commissioning personnel
- Qualification manager
- Qualification measurement team

IQ report, prepared after successful termination of IQ, with confirmation that all errors and omissions identified during the IQ stage have been rectified

11.A.4 OQ Procedures and acceptance criteria

The Operational Qualification (OQ) is intended to verify in a formal documentation process that the isolator operates according to the design specification and equipment manufacturer's

requirements. The following procedures and acceptance criteria should be used to verify the system operation:

1. **Ergonomics verification**
   The sequence of the operation of the entire system along with its integrated systems shall be verified during ergonomics verification. All the activities shall be simulated using the glove/sleeves. The position of the gloves (working height also) shall be verified for its ease of operation.

2. **Controls, interlocks, and alarms testing**
   Listed controls, interlocks and alarms shall be verified for proper functionality. The used procedure for verification shall be documented.

3. **Power loss testing**
   Verify and document the "As found" settings of the controller. Start running a cycle and then initiate a short-term power outage (~1 minute) and verify the settings of the controller again, to verify that they have not changed. This test is used to verify that the equipment will restart after an unexpected power outage.

   Acceptance criteria
   After the power outage, the "As Found after Power Loss" settings of the controller must match those settings recorded prior to power loss in the "As Found Controller Value" column. The equipment is able to restart after the power loss.

4. **PLC control system testing**
   Procedure:
   Verify the signal beginning at the connected component up to the PLC control: different signals shall be generated as mentioned below:

   Generate the individual digital input signals by activation of the connected component.

   a) Generate the individual spare digital input signals by simulation by means of a 24 Volt signal.
   b) Generate the individual analog input signals by activation of the connected component.
   c) Generate the individual spare analog input signals by simulation by means of a 4 - 20MA signal or 0-10 Volt signal (refer to the electrical diagram layout drawings).

   Verify the signal beginning at the PLC control up to the connected component:

   a) Generate the individual digital output signals by activation of the connected component.
   b) Generate the individual spare digital output by means of the connected system. Select the individual digital output to be tested, and check that the corresponding LED on the PLC switches on.

   Record the stated boundary range for each parameter, then verify that the 'Low Limit', 'Midpoint', and 'High Limit' values are accepted, and that the 'Low Out of Range' and 'High Out of Range' values are rejected.

   Acceptance criteria
   All the inputs and outputs shall be verified and comply as mentioned in the electrical drawing.

   For boundary testing, the original set-point and boundary entry range is recorded. The 'Low limit', Midpoint', and 'High limit' values must be accepted, and the 'Low out of range' and 'High out of range' values must be rejected. The set-point is returned to its original value after testing is complete.

5. **Radio Frequency Interference (RFI) testing**
   Procedure
   The main control cabinet and HMI will be checked for susceptibility to radio frequency interferences (RFI). A hand-held 2-way radio operating on the appropriate frequency will be energised at a distance of approximately 1-2 feet from the front of the cabinet/HMI.

There should be no adverse effects observed on the HMI screen or on the system, including the chart recorder, when the RFI-generating device is switched on and then off. Testing should last for approximately 2 minutes, during which time the RFI-generating device should be repetitively switched on and off.

Acceptance criteria
Radio Frequency Interference must not effect the operation of the components under evaluation. There should be no adverse effects observed on the HMI screens.

6. **Electro-Magnetic Interference (EMI) testing**
   Procedure
   The main control cabinet and HMI will be checked for susceptibility to Electro-Magnetic Interference (EMI). An EMI-generating device, such as a drill, will be energised at a distance of approximately 1-2 feet from the front of the cabinet/HMI.

   There should be no adverse effects observed on the HMI screen or on the system, when the EMI-generating device is switched on and then off. Testing should last for approximately 2 minutes, during which time the EMI-generating device should be repetitively switched on and off.

   Acceptance criteria
   Electromagnetic Interference must not affect the operation of the components under evaluation. There should be no adverse effects observed on the HMI screens.

7. **Ammonia leak testing**
   Procedure
   Perform a leak test by sealing all the possible openings of an isolator by normal operational procedures. The leak-tight valves at designated locations shall be closed before starting the test. Keep an opened bottle of ammonia inside the isolator. Pressurize the isolator at about 500Pa or less. Use yellow ammonia-sensitive cloth (bromophenol cloth) to test all areas outside of the isolator where there may be a possible leak. These cloths can locate very small leaks around gaskets and seals. Some of the check points are as below:

   - Windows and window gasketing (inflatable gaskets)
   - Glove ports and glove port gaskets
   - Gloves and sleeves
   - RTP flanges and gaskets
   - All triclamp fittings and gaskets
   - Above leak-tight valves
   - Drain valves in position and closed
   - Pneumatic line connection points
   - All electrical connectors for secure connections
   - Any interfaces with other equipment such as tunnel, lyophiliser, etc.

   Acceptance criteria
   The cloth should remain yellow during all parts of the test. This will verify that no ammonia vapours are leaking from the isolator.

8. **Glove integrity testing**
   A robust glove integrity testing and monitoring plan shall be in place for any isolator system. Different glove integrity testing instruments (e.g Skan AG, La Calhène, Ziel Germany) are available based on operating principles such as constant pressure method and pressure decay method **The sensitivity of the glove integrity testing technique** shall be clearly verified before selecting the integrity tester. Perform the test as per the operational instructions for the glove integrity tester.

Acceptance criteria
All the installed gloves shall meet the acceptance criteria for glove integrity testing.

9. Isolator leak testing:

Leaks are of a concern in isolator operations for operator and environmental safety during decontamination as well as when the containment of toxic materials is required. The isolator system should be tested against a specification for leak tightness either via pressure decay test method or constant pressure method. The leak testing cycle can be automated with the isolator system itself. Typically the cycle should have following phases;

c) Pressurising the chamber
d) Stabilisation time to maintain the pressure
e) Pressure hold or leak testing phase

This test shall be conducted before decontamination cycle using vaporised hydrogen peroxide.

Acceptance criteria
The acceptance criteria shall be decided and shall be agreed up on between the isolator manufacturer. A risk assessment shall be conducted to finalise the acceptance criteria, based on the usage of the system. The following recommendations can be followed for finalising the acceptance criteria:

a) PDA Technical Report No. 34[2] which specifies the criteria from the American Glove Box Society of a leak rate of not more than 0.5% of the isolator volume per hour.
b) ISO 10648[11] (Containment enclosures Part 2: Classification according to leak tightness and associated checking methods.) – classification procedures and leak testing techniques provided in this guidance documents can be referred to define the acceptance criteria.
c) It is observed in the industry that the acceptance criteria of leak rate of not more than 3.0% of the isolator volume per hour is widely accepted.

10. HEPA filter integrity testing
Procedure
Installed HEPA filters shall be verified as per the operating procedure for HEPA filter integrity Testing. The in-built ports shall be used for testing of the filters, with scanning the filter face for the supply filters. All the HEPA filters, such as supply, recirculation and exhaust filters shall be subjected to integrity testing.

Acceptance criteria
The leakage should be less than 0.01%[19] of upstream concentration.

11. Pressure differences across HEPA filters; pressure difference between isolators during normal and worst case operation (in an isolator chain)
Procedure
Pressure differences across the inlet, recirculation and exhaust HEPA filters shall be tested to verify the installed pressure differential. The pressure differential across the filters shall be within the limit specified by the filter manufacturer. The parameters pressure differential across HEPA filters, pressure differential between chamber and room, velocity inside the chambers are inter-related to each other. Hence the control points for these parameters shall be set properly. The pressure difference across the HEPA filter is an indication of the velocity at the filter face.

The pressure difference between isolators shall be tested during normal and worst case operation (in an isolator chain). In an isolator chain the pressure cascade shall be checked between the chambers. Pressure differences between interfaces also shall be tested. Various operational conditions such as tunnel seal opening at start of the batch, vials coming into the

line, vials filled in the line, with higher and lower sizes of vials, etc. shall be simulated during this study and the results recorded. At each of the scenarios the control system shall respond to the situations properly. Impact of the worst case such as inserting all the gloves into the system at once may be verified for any blip (negative side) of pressure in the system.

Acceptance criteria
The pressure differential across the chamber and room shall be as per the set parameters. Reference documents are listed in the isolator design stage of this chapter. The vial filling chamber shall have more pressure than other chambers.

12. Airflow uniformity (velocity) testing
Carry out the air velocity measurement inside the isolator chambers using suitable technique such as hot wire anemometer. Usually isolator manufactures install a suitable screen, where a uniform velocity can be achieved. Mesh size for such screens shall be discussed with isolator manufacturer and the installation shall be easily mountable. The hot wire anemometer can be held by the glove and sleeves at the required positions. The location for both non-viable particulate count and for velocity testing may be the same at the filter face level.

The conditions for air velocity testing shall be as follows;

a) At filter face (within 6 inches from screen face)

b) At working height i.e. within 6 inches from the bottom at container, closure and product exposure locations.

Scenarios such as with vials, without vials can be considered in order to evaluate the impact on the system.

Acceptance criteria
The results shall meet the acceptance criteria set during the design stage. Reference documents are listed in the isolator design stage of this chapter.

13. Airflow visualisation
Perform a smoke test inside the Aseptic Chamber by introducing smoke near the top of the unidirectional diffuser (via manipulation of any of the suitable technique such as smoke stick or dry ice inside the chamber using the glove ports), and observing the resulting smoke profile. The study shall be performed using the procedures given in chapter 16.

14. Non-viable particulate count testing
Procedure
Perform non-viable particle counting using calibrated particulate counters in the locations of the chambers, and measure particle levels at ≥ 0.5 and >5.0 microns/m^3. Take a minimum 1m^3 sample, three consecutive times, to obtain an average. Record the results and also attach the particle counter printouts to the datasheet. Sampling locations shall be defined in the layout and rationale for each location shall be clearly specified in the protocol. Criteria of one location per 0.5m^2 of area (Ref. PDA Technical Report No. 34[2]) shall be considered for defining the number of sampling locations. A recovery test can be performed in order to evaluate the worst case situation in an isolator environment. Typically, the non-viable particulate recovery time is observed to be is less than 2 minutes in large isolator installations.

Acceptance criteria
ISO Class 5, EU Grade A[1,6] maximum concentration limit for particle size ≥0.5 micron is 3,500 particles/m^3 and 20 particles/m^3 for particle size ≥5 microns shall be achieved in operation condition. At rest, the condition of ISO class 4 also can be set as a stringent in-house criteria.

15. Air temperature and Relative Humidity
Procedure
The temperature and relative humidity in each chamber shall be monitored continuously

using the in-built system. Alarm system shall be in place in case of any failure events. The set parameters for each of the control system shall be verified.

Acceptance criteria
The temperature and humidity readings must be as per the operational and design requirements of the isolator based on the process. The occurred alarms shall be investigated and shall be justified.

16. Operational testing
The system shall be subjected to verification of the routine operational cycles using the control system. Typically the cycles may proceed as follows:

a) Cleaning cycle (may include Wet-In-Place techniques/steam sterilisable spray guns/manual wiping) and draining the water (surface slope shall be verified)
b) Drying cycle using the HVAC system of isolator
c) Glove integrity testing
d) Chamber leak test cycle
e) Decontamination or VHP cycle
    - Conditioning phase
    - Bio-decontamination phase
    - Aeration phase
f) Normal operation cycle

Each cycle shall be verified through the HMI or control system for the process flow. Each screen of the software shall be verified for its functionality and automated operations and interfaces. The interfaces to the isolator system may include the following:

a) HVAC
b) Glove integrity tester
c) Tunnel
d) Filling/sealing machine
e) Lyophiliser
f) VHP generator
g) External de-contamination system

During the verification each and every component (such as valves, motors, blowers, doors, operational interlocks, safety interlocks, mimic displays, alarm functionalities, all interfaces, batch reports, etc.) involved in the operation shall be verified for its proper functionality. The P&ID and electrical diagram shall also be checked while this test and the observations should be recorded.

The tests to be performed during installation and operational qualification of an isolator system and acceptance criteria for the tests are mentioned in **Table 2.**

17. Verification of compliance with EU Annex 11 and 21 CFR Part 11
The installed system along with its software shall be verified for compliance with EU Annex 11 and 21 CFR Part 11 for computerised system compliance. Compliance can be verified using detailed checklists. Digital signature requirements should also be verified wherever applicable.

Upon completion of operational qualification the following should be prepared:

a) Test certificate confirming the correct functioning of the isolator and part of the pharmaceutical monitoring system, including alarm and emergency functions.

b) Test certificate confirming the correct functioning of the automatic control system plus, if applicable, correct connection to the facility's building automation system, including alarm and emergency functions.

c) Change control logbook and change control forms correctly filled in, complete and up-to-date up to OQ status.

d) Changes requiring qualification correctly executed and qualified up to OQ status.

e) OQ report stating that OQ of the isolator system is complete, with confirmation that all errors and omissions identified during OQ have been rectified.

Table 2: Tests to be performed during installation and operational qualification of an isolator system

Sr. No.	Activity	Acceptance criteria
1	Engineering Documentation Verification	A list of the required documentation, submittals, manuals, drawings, parts lists, and purchase orders have been compiled and documented in the datasheet.
2	Equipment/Component Verification	The critical equipment must be clearly documented as to the tag number, manufacturer, model number, serial number, and applicable specifications. The installed equipment must conform to the design specifications listed in the protocol or approved accordingly by the appropriate engineering representative
3	Electrical Utility Verification	Electrical utilities shall be connected and shall be available as per specification.
4	Non-Electrical Utility Verification	Non-Electrical utilities shall be connected and shall be available as per specification.
5	Product Contact Materials of Construction	The product/air supply contact materials shall meet the specification.
6	Instrumentation Verification	Instruments shall be available and connected as per the P&ID, Pneumatic and Electrical Drawings.
7	SOP Identification	SOPs to be prepared for operation, maintenance, calibration and quality control shall be identified.
8	Hardware Verification	The hardware/controller configurations for all installed components (including any Dual Inline Package (DIP) switch settings, jumper settings, and other configurable parameters for each hardware component/controller, as applicable), are verified.
9	Software and Application Program Verification	The software version has not changed after completion of the SAT. The storage location of the SAT documentation must be recorded on the datasheet. Software version has not changed after completion of the SAT.
10	Configurable Parameters/Set points Verification	The list of configurable parameters is identified.
11	HEPA Filter Installation Verification	HEPA Filters shall be installed as per the Installation Drawing.
12	Start-Up Verification	Document that installation and start-up activities performed according to supplier recommendations, where applicable.
13	Preventive Maintenance Program Verification	The system must be entered into a PM Program and a copy of the PM plan must be attached to the protocol.
14	Test/Reference Instrument List	All test/reference instruments used during the qualification must be documented. Copies of the current calibration reports, with NIST traceability information, for each instrument must be attached to the protocol.
15	Ergonomics verification	The fabrication and installation of Isolator shall facilitate ease in operation. All the components shall be easily accessible.

Table 2: Tests to be performed during installation and operational qualification of an isolator system *(continued)*

Sr. No.	Activity	Acceptance criteria
16	Controls, Interlocks, and Alarms Testing	The test results shall meet the Functional Specification.
17	Power Loss Testing	After the power outage, the “As Found after Power Loss” settings of the controller must match those settings recorded prior to power loss in the “As Found Controller Value” column. The equipment is able to restart after the power loss.
18	PLC Control System Testing	All the inputs and outputs shall be verified and it should comply as mentioned in the electrical drawing. For boundary testing, the original set-point and boundary entry range is recorded. The ‘Low Limit’, Midpoint’, and ‘High Limit’ values must be accepted, and the ‘Low Out of Range’ and ‘High Out of Range’ values must be rejected. The set-point is returned to its original value after testing is completed.
19	Radio Frequency Interference (RFI) Testing	Radio Frequency Interference must not affect the operation of the components under evaluation. There should be no adverse effects observed on the HMI screens.
20	Electro-Magnetic Interference (EMI) Testing	Electromagnetic Interference must not affect the operation of the components under evaluation. There should be no adverse effects observed on the HMI screens.
21	Ammonia Leak Testing	The cloth should remain yellow during all parts of the test. This will verify that no ammonia vapors are leaking from the isolator.
22	Glove Integrity Testing	All the installed gloves shall meet the acceptance criteria for glove integrity testing.
23	Leak Testing	The acceptance criteria shall be decided and shall be agreed by the isolator manufacturer. A risk assessment shall be conducted to finalize the acceptance criteria, based on the usage of the system. Following recommendations can be followed for finalising the acceptance criteria; a. PDA Technical report No. 342 which specifies the criteria from American Glove Box Society of leak rate of not more than 0.5% of the isolator volume per hour. b. ISO 1064812 (Containment enclosures Part 2: Classification according to leak tightness and associated checking methods.) – Classification procedures and leak testing techniques provided in this guidance documents can be referred to define the acceptance criteria. c. It is observed in the industry that, the acceptance criteria of leak rate of not more than 3.0% of the isolator volume per hour is widely accepted.
24	HEPA Filter Integrity Testing	The leakage should be less than 0.01%[19] of upstream concentration.
25	Pressure differences across HEPA filters, Pressure difference between isolators (in an isolator chain) during normal and, where specified, reduced operation.	The pressure differential across the chamber and room shall be as per the set parameters. Reference documents are listed in the isolator design stage of this chapter. The vial filling chamber shall have more pressure than compared to other chambers.
26	Air flow uniformity (Velocity) Testing	The results shall meet the acceptance criteria set during the design stage. Reference documents are listed in the isolator design stage of this chapter.
27	Airflow visualization	The air flow shall be unidirectional. Sweep away action shall be observed while testing.
28	Non-Viable Particulate Count Testing	ISO Class 5, EU Grade A[1,6] maximum concentration limit for particle size ≥0.5 micron is 3,500 particles/m^3 and 20 particles/m^3 for particle size ≥5 microns shall be achieved in operation condition. At rest condition of ISO class 4 also can be set as a stringent in-house criteria.

Table 2: Tests to be performed during installation and operational qualification of an isolator system *(continued)*

Sr. No.	Activity	Acceptance criteria
29	Air temperature and relative humidity.	The temperature and humidity readings must be as per the operational and design requirements of the isolator based on the process. The occurred alarms shall be investigated and shall be justified.
30	Operational Testing	Operational testing shall comply with Operating manual and SOP. SOP shall be available as per "As built System".
31	Verification of Compliance with EU Annex 11 and 21 CFR Part 11	The system shall meet with the requirements of both the regulations.
	Decontamination gassing cycle	
32	Prequalification of Isolator and Gassing System	• The isolator shall be in a qualified state and all involved instruments shall be in calibrated state. • The airflow shall be balanced between the gas outlets with a certain tolerance range. A total air flow the gas generator shall be achieved. • The gas outlet temperature must reach the desired limit at the end of the preparatory phase. • There should not be any significant variation between the Surface temperatures measured inside the Isolator, preferable span of 5°C.
33	Cycle Fractional Study	• Confirm that the CI colour change demonstrates an even gas distribution. • A Cycle Fractional kill time should be determined and recorded.
34	Development Cycle/Performance Qualification Cycle	a. Cycles should be developed with sufficient margin of extra kill to provide confidence in robustness of the decontamination cycle. b. As per FDA[1], a four- to six-log reduction can be justified depending on the application. The specific BI spore litre used and the selection of BI placement sites should be justified. For example, demonstration of a four-log reduction should be sufficient for controlled, very low bioburden materials introduced into a transfer isolator, including wrapped sterile supplies that are briefly exposed to the surrounding cleanroom environment.

Appendix 11.B

Hydrogen peroxide (H_2O_2) decontamination cycle development

11.B.1 Approach for gassing cycle development

Conventional approach

The conventional approach is usually known as a 'single BI' approach to sporicidal gassing cycle development, using suitable H_2O_2 gas generators. The procedure for this approach is to locate single BIs at: 'critical' locations such as gloves, stopper feed bowls, stopper feed tracks and transfer ports; potential worst case positions where gas distribution might be poor such as under open machine covers, and sampling ports; positions exhibiting extremes of temperature and/or humidity; geometric positions chosen to indicate the overall distribution of the gas, for example isolator corners, walls, ceiling and base plate.

Limitation of approach

During the development runs, inconsistent results may be observed as different locations may fail in different cycles, even though maximum lethal rate is applied to the cycle. Usually the root cause investigations will reveal inconclusive reasons for these kinds of failures. Here then, was the reason for the random underlying failures in the full-scale cycles, a low percentage of highly resistant 'Rogue's' whilst so called 'tailing' of survivors. The most likely root cause is the variability in a single lot of Biological Indicators. This variability in a single lot can be determined by performing "D Value" at multiple times.

The phenomena of variability/rogue

To expect a complex manufacturing process using a biological system to result in hundreds of thousands of product units that always react the same is hopeful to say the least. So we are therefore left in a difficult situation in that we must use an inherently variable 'sensor' to measure the effectiveness of a critical lethal process. So we must therefore call into question the wisdom of interpreting the efficiency of lethal processes based on the use of single BIs at each location.

Worst case approach[25]:

In the worst case study, the bacterial reduction is determined at positions in the chamber that are difficult to decontaminate. **Three** BIs are placed in each of the previously defined worst case positions/all identified positions.

In the first run, If at some of the locations BIs are failing and to confirm the estimates made, a second worst case study is to be carried out, but this time only positions previously found to correspond to the worst case are considered. With this iterative procedure, the duration of decontamination is adjusted to the worst case positions observed, and, at the same time increase the gassing time to achieve negative growth results and to finalize the decontamination time.

During cycle development, run a series of cycles where locations are gradually eliminated from the test as all three BIs are inactivated. The location which is seen to require the longest for inactivation of all three placed BIs is defined as the worst case location

General and worst case location Biological Indicator challenge

The isolator should be mapped and the location of BIs finalised. This should be done once the fractional study cycles are taken. BIs should be kept in triplicate in each identified location.

The transition from all three BIs surviving to all three BIs being inactivated can be observed, allowing the process lethality at each location to be quantified so the influence of a low percentage of 'Rogues' (if present) may be eliminated. The location which is seen to require the longest for inactivation of all three placed BIs is defined as the worst case location. **Table 3** below illustrates this technique.

Table 3: Location of three Bioogical Indicators (BIs) showing worst case scenario

BI locations	Cycle number		
	Cycle Time of 25 min	30 min	35 min
1	- - -		
2	- - -		
3	- - +	- - -	
4	+ + +	- - +	- - -
5	- - +	- - -	
6	+ + -	- + -	- - -
7	- - -		
8	- - -		
9	+ + -	- - -	
10	- - -		

The isolator shall be mapped in such a way that, all nooks, corners, occluded surfaces, critical container/closure/product contact parts are mapped.

Cycle fractional study

Having programmed these parameters into a cycle record (stored cycle) the BI test may be started by removing those BIs in the centre of the chamber at suitable timed intervals, say every 2 minutes, and starting at a level at which kill is not expected to occur, i.e. 50ppm measured directly in the isolator.

The fractional negative data relating to the worst case location can be used to calculate a mean process D-value at that location using the limited Spearman Karber procedure. This is unlikely to be a true D-value since the efficacy of the sanitisation cycle is hopefully increasing as it progresses over time but nevertheless it enables an estimate to be made of the process lethality delivered and quantifiable levels of safety margin to be added to the final production cycle (expressed in log reductions rather than arbitrary time or percentage units). At this stage, the cycle can be considered sufficiently defined to begin performance qualification.

General and worst case location Biological Indicator challenge

At the end of the cycle, all the BIs may be removed from the chamber for analysis. The test should be repeated making any necessary adjustments until a satisfactory result is obtained. Once the parameters have been established to achieve the required level of inactivation, this becomes the **Development Cycle.**

These parameters are then added to an overkill bio-decontamination time and the combined parameters used for Performance Qualifications as a **Secure Gassing Cycle**.

Investigational cycle approach[26]

Another approach prevailing in the industry is running an investigational cycle when a qualification cycle has failed. The approach is discussed here. If any cycle qualification study is completed with just a single BI at a defined challenge location, a resulting BI positive would have no basis for judgment of whether the cause was failure of the disinfection process or the presence of a rogue BI. The probability is low that two rogue BIs could be placed at the same location, so it is recommended that the starting position for all cycles in a qualification series would be to use multiple BIs (two or three) in each challenge location.

An investigation cycle would be necessary to evaluate a positive BI and result in high probability of a rogue BI, hence false positive, to accept the cycle. The investigation should include triplicate BIs in the positive growth location and two other reference locations (including worst case) previously demonstrating BI kill. The investigation cycle with full inactivation would result, over two cycles, in four out of five inactivated Bis at the investigation location. Such a result would be considered evidence to demonstrate adequate and repeatable lethality at the investigation location and the cycle can be accepted. Such methodology is only considered acceptable where high numbers of BIs (in the hundreds) are used in qualification studies, hence the sample number is high and the probability is high that the positive BI was a rogue.

The decision on acceptability of a cycle should be based on the results observed at each location, i.e. tabulating the results and evaluating them one by one. It is to be considered that failure of two BIs in a single location indicates the failure of the cycle. During the investigational cycle, each location (failed location, reference location or worst case locations (hottest or coolest) should be challenged with three BIs.

This kind of investigation cycle can be used in the following ways:

a) Accepting the cycle by eliminating BI false-positive results.
b) Eliminating the failed cycle itself from a study series and replacing it with a 100% kill cycle.

The iterations for investigation cycle should be decided on a case to case basis and by investigating each cycle if a failure is observed.

Cycle development methodology

Considering the presence of inherent rogues in a BI lot, it is advisable to have a robust methodology for cycle development, as follows:

1. The BIs should be subjected to pre-qualification before use with independent enumeration and System D Value resistance studies.
2. Cycle pre-qualification studies should be performed before challenging with BIs such as enclosure and cycle thermal profile, gas concentration profile and gas distribution profile with chemical indicators. Each characteristic should have a defined acceptance criteria.
3. Cycle fractional approach should be followed, to achieve a base line for minimum overkill.
4. To assure the cycle developed is robust, a full BI challenge cycle should be completed at a base line with the minimum amount of overkill. BI inactivation, together with rogue BI management at the base line, provides the basis to add overkill for PQ production cycles.
5. A minimum of three progressive cycles shall be considered for Performance Qualification at defined cycle parameters that are derived out of cycle development. Within the PQ protocol it is possible to manage BI investigation of positives if, based on the study of potential rogue BIs, the outcome of the resulting investigation were clear.
6. Annual re-qualification with BI challenges is expected unless viable environmental monitoring (plate count) results deviate.

11.B.2 Phases of gassing cycle [25,27]

a. Preparation phase

During the preparation phase, the Relative Humidity of the isolator atmosphere will be reduced to a desired level, and the gassing ductwork and evaporators heated for the subsequent introduction of the hydrogen peroxide. The phase can be controlled jointly as timed (i.e. according to the temperature) and based on a RH% limit.

The Relative Humidity of the air returning to the generator is measured and this measurement may be used to control the point at which the optimum value of RH (to be decided) has been achieved. The airflow assembly from the generator shall be made in such a way that, all the isolator chambers should be connected either inlet or in the return path. Consideration also shall be given to include the space above HEPA filters in the airflow path.

This phase is to be part of the cycle in order to maintain the uniform cycle parameters and environmental conditions during routine cycles. Studies reveals that there is no one optimum condition concerning relative humidity, hydrogen peroxide concentration and temperature against the microbial inactivation.

b. Conditioning phase

In the conditioning phase, hydrogen peroxide is introduced at a high rate to reach the desired concentration levels within the isolator. The criteria for optimisation, is to achieve a rapid increase in the gas concentration inside the chamber whilst avoiding condensation in the supply lines and any filters in the supply system. Upon injecting the gas into the chamber, a visible or sub-visible micro-condensation may be found.

c. Bio-decontamination phase

During the bio-decontamination phase, hydrogen peroxide is introduced at a reduced rate such that only the consumed hydrogen peroxide is replaced, and a constant hydrogen peroxide concentration maintained within the isolator. This phase can be divided into two phases: first to raise the concentration to the point at which condensation happens, and then to reduce the liquid evaporation rate to maintain that layer of condensation. Generally the H_2O_2 injection rate is reduced by about 40% to 50% for the second part of the gassing phase, depending on the type of isolator and the internal load. If excessive condensation is observed then the liquid flow rate is too high, the gas concentration starts to fall and then the liquid flow rate is too low.

d. Aeration

The last phase of the decontamination cycle is aeration, during which the hydrogen peroxide is removed from the system by dilution with fresh air. The size of the chamber, the level of condensation and any surface absorption are important factors. During the aeration phase, the gas concentration should be measured using suitable sensors, preferably high and low level sensors separately. The aeration phase can be set as a timed phase or based on the pre-set value of gas concentration. This set point must be set and validated to correlate with the OEL (Operator Exposure Limit) or target gas concentration level inside the chamber.

11.B.3 Gassing cycle development

Study 1: System checks, nozzle airflow balancing and nozzle temperature checks

a. Objective

- This study is performed to ensure that the isolator controls are commissioned: gas generator calibration and gas generator HEPA filter DOP tests are performed
- To verify that the chamber gassing system meets the minimum airflow and temperature requirements
- To establish and to ensure the air flow balancing between the chambers of the integrated system
- To record the temperature of the internal surfaces of the Isolator chamber to be gassed

b. Test method

Review the qualification document for isolators, instrument and equipment calibration and

relevant HEPA filter integrity tests. Gas generator, H_2O_2 sensor(s), condensation monitor(s) calibration validity also need to be ensured.

c. Airflow balancing checks

Ensure that the gas delivery pipe-work trace heating (if available) is in normal operation and continuously powered up before start of the gassing cycle. If the air-flow system is fitted with nozzle(s), ensure that the nozzles are rotating in the proper direction. If the gas delivery system is fitted with direct injection system, ensure that the air is flowing.

Run a dummy gassing cycle using deionised water, setting the parameters by giving most of the time to the preparatory phase of the cycle. Using suitable means, adjust the airflow into each of the isolators and divide the total airflow based on the volume and criticality of the isolator. Once the desired airflow volumes are set across the isolators, abort the cycle.

d. Gas exit temperature checks

Using a digital thermometer, measure and record the environmental temperature inside the isolator. Restart the cycle with abovementioned airflow balancing cycle. Monitor and record the temperature at each gas outlet throughout the preparatory phase of the gassing cycle. Abort the cycle. This heating is verified to reduce the condensation of gas inside the piping.

e. Surface temperature checks[27]

Prepare an initial BI location map for the isolator. Typically the locations should be: corners, centre of walls/windows and any device, load or equipment that may represent a thermal mass. The conditions and procedures of routine operations should be performed during the study. The normal decontamination cycle conditions and loads should be configured.

Run the cycle and using a hand-held digital infrared thermometer inside the isolator, measure and record the internal surface temperatures at the identified locations around the isolator as per BI location map. It is possible that equipment attached to a sealed chamber such as autoclaves, freeze dryers or refrigerators may affect the surface temperature differential.

f. Acceptance criteria

- The isolator shall be in a qualified state and all involved instruments shall be in a calibrated state
- The airflow shall be balanced between the gas outlets with a certain tolerance range. A total airflow for the gas generator shall be achieved
- The gas outlet temperature must reach the desired limit at the end of the preparatory phase.
- There should not be any significant variation between the surface temperatures measured inside the Isolator, preferable a span of 5°C.

Study 2: Gas distribution and cycle fraction study

a. Objective

- To establish the preparatory phase time
- To verify the gas distribution within the isolator chamber and to record gas concentrations
- To perform a cycle fractional study (the timed removal of BIs) in the isolator chambers and to establish the gassing time required to achieve a 10^6 kill of BIs

b. Test method

To perform the initial study with the Chemical Indicators (CIs) and Biological Indicators, it is necessary to set a hydrogen peroxide liquid evaporation rate and a gassing time. CIs should be placed in the corners of the sealed chamber and any part of the chamber where gas distribution

is expected to be difficult and in the centre of each face of the chamber. The time at which each of the CIs indicates the presence of gas should be noted and if there is any significant variation in the colour change span, then the gas distribution system should be adjusted in an effort to minimise these differences.

Typically, sporicidal gassing BIs are composed of grade 304 stainless steel carriers, each inoculated with a minimum of 10^6 spores of *Geobacillus stearothermophilus*, American Type Culture Collection no. 12980. The BIs, sealed in 1073B medical-grade Tyvek™, are manufactured by different manufacturers. The certified BI population should be tested and verified according to the acceptance criteria specified in the US Pharmacopeia (USP). It is advised to have all BIs used throughout the study from a single production lot and the system D-value determined independently using approved methods.

During this cycle, BIs should be placed in the positions where the CIs are placed and also at those positions where the temperatures were both the highest and lowest during the temperature mapping study. They should also be placed in the corners of the chamber and the centre of each face. It is also necessary to place a series of BIs on hangers in the centre of the chamber that may be removed and placed into sealed vials of TSB growth media to obtain a kill profile with time, i.e. a cycle fractional study. This study will verify the cycle fractional Kill Time (KT) for 10^6 reductions during the cycle. The KT time is used in assessments for determination of the second step of the bio-decontamination phase in association with setting a cycle security margin. During the first biological decontamination sporicidal gassing cycle, temperature mapping (distributed measurements) may be taken through the complete gassing cycle to assist with interpretation of results during any BI growth during development.

It is advised to monitor and record the following:

- Environmental conditions in each chamber at start and end of each phase of cycle
- If the chemical indicators can be seen, the time from start of gassing at which the first and last CIs change colour. If CIs are placed in challenge locations that cannot be seen, an alternative CI (water in a thin polythene bag) can be used to absorb a sample of hydrogen peroxide and tested at the end of the cycle with a chemical colour change-dip stick indicator.
- Record the H_2O_2 gas concentration inside the isolator chambers using a calibrated H_2O_2 gas sensor(s).
- Record the time at which visible condensation forms.

During the bio-decontamination phase, collect cycle fractional BIs into the appropriately labelled bottle of Tryptone Soya Broth (TSB) at the defined intervals of this phase. Collection consideration should be given to minimise any risk of cross contamination between BIs. Collected BIs should be incubated at 55°C to 60°C for seven days and the results recorded daily.

After aeration, the water in the CI bags should be subjected to testing for the presence of hydrogen peroxide using a chemical indicator strip. Record the observed hydrogen peroxide concentration from each Chemical Indicator.

c. System D value

After completion of the incubation period, system D values should be calculated according to the fraction-negative approach of the Limited–Spearman–Karber method. The initial spore population, certified by the manufacturer of the BIs, can be used for the calculation of the D values. This D value is the applicable test atmosphere. The D values provide a means of comparing the inactivation efficiency of different cycles:

$$D = \frac{U_{sk}}{\log N_0 + 0.2507} \qquad U_{sk} = U_k - \frac{d}{2} - \frac{d}{n} * \left(\sum_{i=1}^{k-1} ri\right)$$

U_{sk}	Mean time for sterility
U_i	The ith exposure time
U_k	The first exposure time that results in all replicate BIs showing no growth
U_1	Last exposure time where all BIs are positive just before the fractional window
U_{k-1}	Exposure time before U_k
d	Constant time interval for sample removal
n	Constant number of replicate BIs
ri	Number of BIs that show no growth out of n after an exposure time U_i
$\left(\sum_{i=1}^{k-1} ri\right)$	Number of BIs showing no growth between U_1 and U_{k-1}

d. Acceptance criteria

- Confirm that the CI colour change demonstrates an even gas distribution.
- A cycle fractional kill time should be determined and recorded.

Study 3: Minimal overkill of Biological Indicators

Based on the review of the cycle fractional data from the previous cycle, the cycle parameters for this cycle should be determined. Sufficient safety margin should be added to this cycle for the bio-decontamination phase. The cycle fractional studies may be repeated to verify the consistency and to allow comparison between the cycles. In addition to this, Biological Indicators should be placed in each of the identified locations (to cover all the chamber's nooks and corners) in triplicate and the cycle shall be taken. A risk assessment shall be performed for determining the location for Biological Indicators and shall be formally documented. The Biological Indicators should be exposed to a complete cycle and at the end of aeration these should be incubated.

Acceptance criteria for cycle

a) Cycles should be developed with a sufficient margin of extra kill to provide confidence in the robustness of the decontamination cycle.

a) As per FDA[1], a four- to six-log reduction can be justified depending on the application. The specific BI spore titre used and the selection of BI placement sites should be justified. For example, demonstration of a four-log reduction should be sufficient for controlled, very low bioburden materials introduced into a transfer isolator, including wrapped sterile supplies that are briefly exposed to the surrounding cleanroom environment.

Results: interpretation and proceedings

If at some of the locations, BIs are failing and to confirm the estimates made, a second worst case study should be carried out on the basis of the first D-value calculated, but this time only positions previously found to correspond to the worst case should be considered. With this iterative procedure, the duration of decontamination is adjusted to the worst case positions observed and, at the same time, the maximum D-value determined. The gassing time should be increased to achieve negative growth results and to finalise the decontamination time.

Performance qualification strategy (worst case approach)

The cycle duration used during PQ of our isolator systems should be calculated based on the time point when all three BIs located at the worst case location were inactivated. Additional time can be added to account for batch to batch variation in D-value and population which together influence the 'kill time'. By using three BIs, each loaded with a minimum 10^6 spores per BI, growth of one of the three BIs can be accepted because >6-log reduction has been achieved and the routine production cycles are more effective than PQ cycles due to additional safety added to the cycle. PQ can therefore continue if only a small percentage of rogues are present. It is not necessary to re-challenge every location that was evaluated in the cycle development phase. Representative numbers of BIs should be placed at a range of locations, including the 'worst case', 'critical' and 'geometric'.

Revalidation strategy

Revalidation is needed to demonstrate that the time point at which the cycle achieves a minimum 6-log reduction has not changed since initial PQ. Therefore, the reduced cycle time (rather than the routine cycle) used in PQ must be employed again.

Risk based product and occupational exposure control in multiproduct facilities

Julian Wilkins, PharmaConsult US, Inc., Bridgewater, NJ, USA

12.1 Introduction

This chapter provides a way to address regulatory concerns of cross contamination in multi product facilities. The problem is best exemplified from the following extracts from a recent FDA Warning Letter 483 (Fall 2011):

- Your firm has not established separate or defined areas or such other control systems as necessary to prevent contamination or mix-ups during drug manufacturing [21 CFR 211.42(c)].
- For example, your firm lacked an adequate assessment of the cross contamination risks posed by the manufacture of several potentially hazardous compounds
- Your facility contains shared manufacturing areas where you produce potentially hazardous compounds in multi-product equipment that are high powder generating operations
- Your firm should ensure that a documented justification and well-designed contamination prevention strategy is in place to minimise the possibility of contamination. To achieve proper product protection, sound design and control approaches must be used. Without proper separation, your firm lacks assurance that one drug does not contaminate another drug.
- Include in your risk analysis, procedures and data addressing the following potential routes for cross contamination: mix-up, retention, mechanical transfer and airborne transfer.

The chapter addresses these issues and covers the need for a risk assessment for product and occupational exposure, following the requirement of ICH Q9[1] which sets out the process to scientifically assess risk, to mitigate risk and review risk, so the risk of cross contamination and occupational exposure can be controlled to acceptable levels.

There are two primary concerns:

Cross contamination of one drug into another: Here the risk is product exposure that can lead to patient exposure to a drug that was not prescribed, and this can have negative health effects, including death.

Occupational risk: Here the purpose is to determine if the work-force is protected from the product so that the risk is reasonable. In many countries this degree of reasonableness is legislatively mandated with penalties for non-compliance.

The main product concern is with penicillin and beta-lactam antibiotics. Draft Guidance issued by the FDA places beta-lactams in the same category as penicillin, requiring segregated and dedicated facilities on a family-by-family basis[2].

The risks of cross contamination during product manufacture are now examined, focusing on patient risk, and the risks posed to personnel from occupational exposure.

12.2 What is Risk-MaPP® and why is it important?

At the June 2004 ISPE Containment seminar in Washington, DC, Edwin Melendez of the FDA was

asked to present a paper on the Agency's developing attitude to "potent compounds". At that time the FDA wanted to know how to define "potent compounds" and after surveying several pharmaceutical companies it was clear that there was no common global definition. The FDA presentation at this conference suggested that "potent compounds" should be processed in dedicated and segregated facilities; just like penicillin and shortly beta lactams.

Industry representatives were concerned with this direction and began planning the 2005 conference for ISPE with the clear purpose of educating the FDA and industry that there are safe ways to manufacture "potent compounds" and, most of all, that not all compounds in a class are equal in their hazard and not all "potent compounds" are difficult to control. The industry presentations at the 2005 ISPE Containment seminar in Washington DC highlighted first the inconsistencies of regulations across the globe in terms of "potent compounds" and "compounds of concern", as well as the use of toxicological data to indicate relative hazard of compounds and to show that there is a continuum of hazard across compounds within a class, testing and sampling methods, using toxicological data to set cleaning validation limits and the importance of exposure control to manage risk.

These presentations were the beginning of an ongoing dialogue with regulators worldwide. As a result of the conference, the FDA invited several of the presenters to provide focused presentations on handling "potent compounds". A coordinated presentation was made to approximately 40 FDA staff members in January 2006. At the conclusion of this session the FDA agreed to jointly develop Risk-MaPP[3] with ISPE.

The first planning meeting was held in NYC March 2006; Edwin Melendez on behalf of the FDA requested the guide address the following three items:

1. How to determine high hazard compounds
2. How does this fit into cleaning validation
3. Provide tools to comply with the United States 21 Code of Federal Regulation 211.42c

As the writing began, it became clear that education on the major concepts is essential for both the regulators and industry. The problem is that cross contamination provides no benefit to the patient, only risk[4].

Another important concept is that risk is a function of hazard and exposure and for the most part in the manufacture of medicines the hazard is fixed by the active ingredient so the only variable that affects the level of risk is the level of exposure. Eloquently stated by one of the guide's reviewer's "exposure itself is neither good nor bad; overexposure is not acceptable". Defining the boundary between exposure and over exposure was a major element of the guide and required the dedicated work of internationally recognised Occupational Toxicologists.

As regulators worldwide embrace the ICH documents, the logical path was to have Risk-MaPP's foundation based on ICH Q9: Quality Risk Management, which the guide closely follows.

Risk-MaPP provides a methodology to set cross contamination limits to maintain risk at acceptable levels. These limits are also used in setting cleaning validation limits and in selecting risk controls as necessary.

In November 2006 at the ISPE Annual Meeting, regulators representing FDA, EMEA and MHLW/JPMDA participated in a session where these regulators from the three ICH regions were discussing the concept of dedicated facilities for "compounds of concern". This was the beginning of ongoing dialogue on Risk-MaPP with European and Japanese regulators.

The major concepts introduced are discussed below.

1) There is a continuum of hazard: Bright lines do not exist to distinguish certain compounds as low hazard from others that are high hazard. The banding systems that are currently used for environmental health and safety categorisations should be considered a starting point for discussion and hazard evaluation but should not be used as the only determinant for the level of control needed.
2) Pharmacological and toxicological descriptions (dose-response, no-observed-adverse-effect level (NOAEL) and Allowable Daily Exposure (ADE) should be used to assess compounds instead of hazard labels. Terms such as potent, cytotoxic, cytostatic, and other product class definitions tend to induce an emotional response that might imply that these compounds are always difficult to handle and require the highest level of control.
3) Zero risk is not scientifically achievable. As stated in the Report to the FDA Commissioner from the Task Force on Risk Management, May 1999: "*Although medicinal products are required to be safe, safety does not mean zero risk. A safe product is one that has reasonable risks, given the magnitude of the benefits expected and the alternative available*." As such, quantified risk must be balanced against unmet medical needs.
4) Risk is a function of hazard and exposure. Any compound is to be considered a hazard. The severity of this hazard will depend on the type of effect that can be caused and on the dose at which this effect will appear. Exposure is the level of contact with the compound. Risk is the likelihood that a compound will produce harm under specified conditions of exposure. To minimise risk, manufacturing conditions and procedures should be established to control exposures, thus minimising the probability that a compound will produce adverse effects.
5) A consistent, robust and science risk-based approach in decision making should be made across the continuum on a case-by-case basis: this includes the derivation of science-based acceptance criteria.
6) Scientifically derived values such as acceptable daily exposure (ADE) values should be used to set limits for identification of compounds of concern, assessment of occupational exposure, the basis for cleaning validation and the determination of acceptable risk. These values are based for example on data that are submitted as required with new drug applications (NDAs) and represent a dose that is unlikely to cause an adverse effect if an individual is exposed at or below this dose every day for a lifetime.
7) Exposure, and subsequently risk, can be controlled by a variety of methods. A combination of control measures may be required to reduce risks to an acceptable level. There is not a single answer; hence a formal documented approach to risk management is required on a case-by-case basis.
8) cGxP is only one of the needs that must be met,. For example, there are also industrial hygiene and other health and safety concerns which should also be addressed. Solutions can vary and are sometimes conflicting. Effective risk management requires a shared understanding and application of assessment and control to achieve the appropriate balance between these different needs.

With the release of ISPE's Baseline Guide; Risk-Based Manufacture of Pharmaceutical Products (Risk-MaPP in September 2010[3] guidance was available for the first time on assessing the risk of product cross contamination by another product, it is important to show how this guide was developed and the impact it has and will continue to have on the pharmaceutical industry.

Risk = Hazard x Exposure

Risk

Risk has become a major regulatory topic because by controlling risk all other aspects of the manufacturing process come under control. Everything has risk, driving the car to work is a high-

risk activity yet the vast majority accept the risk. In the area of containment, risk relates to the potential for the product to cause harm to people and the environment and for other uncontrolled compounds to adulterate the product with the potential for harm to the patient.

When evaluating risk, a rational has to be formulated as to what is an appropriate level of risk. There is no such thing as zero risk and setting risk goals close to zero will result in failure. Risk has to be set to reasonable, but defensible levels.

Risk in the context of this chapter is the likelihood of harm occurring to the occupational work force and the patient. The occupational work force includes any person other than the patient. They could be operators in the pharmaceutical plant, pharmacists, care givers, nurses or anyone, anywhere that could become exposed to a hazard. The patient is the recipient by some form of dosage delivery of the pharmaceutical compound. The patient by definition (unless undergoing an elective procedure) is unwell and has reduced resistance. The safety of the drug is determined by its Allowable Daily Exposure (ADE) and is derived from toxicological data (See setting limits).

The basis of exposure to a prescribed drug is that the benefit outweighs the risk. With cross contaminates, there is no expectation of benefit. Therefore the ADE has to take this into consideration.

Excess cancers, cancers occurring a a specific exposed population above the norm in the work force, have various degrees of official governmental tolerance of between 10^{-3} to 10^{-4} excess cancers in an Occupational workforce to 10^{-4} or 10^{-5} in the Patient. In Germany, for instance, 10^{-3} excess cancers are acceptable for industries handling known carcinogens

Hazard

Hazard is the effect that is created by the pharmaceutical compound, the intermediates used in its manufacture and the chemicals used to process the compound (these may be more hazardous than the pharmaceutical compound).

The hazard for a pharmaceutical compound is normally defined by its Allowable Daily Exposure (ADE). Other properties are important such as carcinogenicity, mutagenicity, sensitisation, etc. The hazard for a compound does not vary. However, with dilution with excipients, varying process steps may increase or decrease the risk of exposure. The limit for occupational exposure is expressed conventionally in the form OEL (Occupational Exposure Level), or other company specific descriptor, as micrograms per cubic metre per eight hours (mcg/m^3/8 hours). This is the safe limit at which a person may be exposed for eight hours a day, 5 days per week for 40 years and is based on inhalation. The ADE is the allowable daily intake for 40 years with no observable adverse effect.

The effects of pharmaceutical compounds and, to a far lesser extent, intermediates are assessed by a variety of methods, the chief of which is a full *in vivo* toxicological study. This is a costly and time consuming business. Many early stage compounds have not undergone this level of scrutiny, so review is based on similar substances and on the use of correction factors.

Much has been made of potent compounds and various authorities have taken a starting point for potent compounds at 10mcg/m^3/8 hours. Therefore a pharmaceutical compound with an OEL of 11mcg/m^3/8 hours is treated differently to one of 9mcg/m^3/8 hours. This is patently nonsense, particularly bearing in mind the variability factors used in the calculations. The truth is that there are no bright lines, merely a continuum of hazard from compounds with low activity and no undesirable "…gens" (mutagen, teratagen, carcinogen, etc.) to those with higher activity and some …"gens" and/or sensitisation.

Categorisation and banding

Most companies have adopted a method of characterisation, which was initiated in the early 1990s. These characterisations were meant to reflect performances of various exposure control technologies, but led to some significant anomalies. Today a base ten approach is almost universally adopted. The diagram below indicates commonly adopted bandings or characterisations.

Occupational Exposure Limit (OEL)

>1,000µg/m^3	1,000µg/m^3	100g/m^3	10g/m^3	1g/m^3	<1g/m^3
>10,000µg/day	10,000µg/day	1,000µg/day	100g/day	10g/day	<10g/day

Acceptable Daily Exposure (ADE)

***Figure 1:** Diagram showing the exposure bandings and the difference between the occupational exposure levels and the ADE.*

Such bandings are useful as a short- hand description of a compound. For instance, a Category 5 indicates the highest level of activity and requires fully contained processing, whereas a Category 1 requires little control. However, cross contamination of a Category 5 with a Category 1 would possibly permit a far greater weight of the cross contaminant than the category 5 compound, whereas the cross contamination of a Category 1 with a Category 5 will result in significant issues.

Exposure

Exposure is created by an emission, ie. a release of the hazard from its contained boundary into a space that can result in exposure. Exposure occurs when an emission enters, by any suitable route: the occupational workforce, the product and ultimately the patient. Emission does not mean exposure has occurred, but for an exposure to occur an emission has to take place first. This is an important distinction because some industrial hygiene data records exposure for the occupational workforce but only emission for product cross contamination.

Exposure is the only variable in the equation.

12.3 Routes of exposure

Why Product Exposure is very different from Occupational Exposure

Cross contamination is the contamination of one pharmaceutical active by another active. The routes by which this can occur are**:**

a. **Mix up** of ingredients, actives, clean and dirty equipment, etc. so that one product is adulterated by another. The optimal state is to have all transactions into and out of processes and rooms involving, ingredients, components, packaging, labelling, people, clean and dirty equipment recorded by electronic means with minimal reliance on manual data entry. Line clearance is also a significant issue.

b. **Retention**
An important question is: "Is the level of API retained on product contact parts after cleaning?" ISPE's Risk-MaPP Baseline Guide uses a health-based limit for this retention. The use of a health-based limit is protective of the patient whilst a dose-based limit is not always. 1/1,000 of the lowest therapeutic dose may provide a limit that is higher than the ADE for compounds in categories OHC 4 and above, but the ADE is lower for some compounds in OHC categories below 4. A significant issue is having a validated process with routine verification which is statistically justifiable. A significant issue is the use of visual inspection for release when the cleaning limit is below the visual threshold.

c. **Mechanical transfer:** This is the movement of particulate from one place to another caused by contact between contaminated and non-contaminated equipment, gowning, people, etc. allowing transfer into or onto another product. The risk at batch scale is lower than at the individual dosage level, but it can be an issue where high hazard compounds meet highly vulnerable batches. An example is a high hazard contaminating a small batch of antacid which has a very large tablet and which can be taken in large numbers (high maximum daily dose). At single dosage level, such as tablets or capsules and particularly uncoated tablets, the ADE is the limit on as many unit doses comprising the maximum daily dose (MDD). In some cases this is a significant number, but up to 12 doses in a daily dose is common.

d. **Airborne:** Once thought to be a major cause of cross contamination but through evaluation and data has been shown to be a very small risk, other than where high concentrations can sediment onto product and product contact surfaces. An example is the cleaning of a Fluid Bed Dryer where data show that high concentrations remain when the cleaning is complete, with the risk of sedimentation onto the clean equipment. Airborne concentration can only impact the product if it sediments into another product otherwise it is captured in the air-handling system.

Routes of exposure to the occupational workforce:

e. **Inhalation:** This is the standard route of occupational exposure on the basis that the operator has to breathe and therefore the limit is based on the concentration in mcg/m^3/8 hours that is protective of health and if protected adequately by suitable respiratory protective equipment, the concentration can be increased proportionally to the efficacy of the protection.

f. **Ingestion:** This is a shared route with inhalation, but of lesser concern since the operator should not be ingesting anything in a pharmaceutical environment. Risk exists where bad practices and failure of hygiene allow ingestion to take place via mechanical transfer from hands and gowning, for instance in the canteen.

g. **Dermal:** Sensitisers may cause immediate effects through skin contact and may possess transdermal characteristics, particularly with certain solvents. It should be a basic principle of gowning that dermal areas are not exposed in the pharmaceutical environment by the use of gown and gloves. Faces are often left exposed and the human behaviour of touching the face is a risk.

h. **Mucosal:** Eyes and mouth (buccal mucosa) and lower region mucosal areas which can be contaminated during the use of the toilet, as a result of a failure to wash the hands before and after using the toilet is a route of exposure.

i. **Puncture wound:** allowing a product to interact with blood.

Route of exposure to the patient:

j. This is by the route of administration, oral, nasal, dermal, parenteral, topical, anal and vaginal.

12.4 Factors to consider in conducting a Quality Risk Management Plan

Risk assessment

This is the data gathering stage of a Quality Risk Management Plan. This stage has three elements. Risk Identification, for Risk-MaPP this is the setting of the Allowable Daily Exposure (ADE) which is the cornerstone of the Risk-MaPP Guidance. The Risk Analysis is the collection of data on the products, processes and facility that constitutes the risk management plan. The steps required are:

Risk identification

Setting hazard limits. With OEL versus ADE the questions to answer are: what are the critical differences? and why you should keep them separate even though they are based on the same data?

To answer these, Risk-MaPP lays down a method to determine the Allowable Daily Exposure. The basic formula is identical to the one that can be used to set Occupational Exposure Limits. But there are differences that have to be understood.

The ADE is a quality derived value that has to have full traceability and documentation. It requires a written procedure and protocol, has to be set by a toxicologist, requires references and peer review and is a regulated document. An OEL is the company's responsibility, set to afford worker safety within the local laws. The ADE has to pass regulatory scrutiny by every regulatory body to which product from the facility is sent: the OEL is of no regulatory (quality) concern.

Regulators are not bound by Guidance, even their own, and the c in cGMP means current, and current has no real definition. The advantage of guidance is that it provides a reference framework to negotiate with the regulator and can be used in a robust and scientifically-based rebuttal of observations.

It is not the purpose of this section to do more than provide the non-toxicologist with sufficient information to understand why it is imperative that a qualified toxicologist set the ADE.
Hazard is defined in ICH Q9 as a potential source of harm. ICH Q9 also includes the severity of harm in the definition of risk. Hazard describes the inherent property of a compound to produce adverse effects, e.g., in patients that may be exposed to the compound as a trace contaminant in another pharmaceutical product. The compounds typically considered include:

- APIs
- Excipients
- Solvents
- Process intermediates
- Other compounds such as impurities, by-products and degradants.

Health-based limits, such as the ADEs, can span several orders of magnitude and should cover all pharmaceutical compounds. Controls may be needed to maintain exposure at or below acceptable levels. As part of an overall risk management approach, it is useful to have a qualitative tool to help determine the need for and to prioritise comprehensive quantitative risk assessments of the compounds handled.

The foundation of a risk-based approach for manufacturing pharmaceutical products comprises:

- identification of hazards
- assessment of dose-response relationships
- establishment of health-based limits

The identification of hazards and assessment of dose-response relationships are collectively referred to as hazard characterisation.

The ADE represents a dose that is unlikely to cause an adverse effect if an individual is exposed, by any route, at or below this dose every day for a lifetime.

The Risk-MaPP guide contains numerous references to peer-reviewed articles on setting health-based exposure limits.

For hazard identification, a formal review of all available animal and human data should be performed for each compound. Where innovator companies employ third party and Contract Manufacturing Contract Research Organisations there is a duty for both parties to take full

responsibility for the communication of data on the compounds. Regulators will see both parties as being equally responsible.

Identification of the critical effect

The purpose of a hazard evaluation is to identify all possible hazards associated with a compound and to rank hazards according to their severity. When combined with a dose-response assessment, the critical effect can be defined. This is typically the first significant adverse effect observed as the dose increases. Due to differences in the route of exposure, the toxicological profile of the compound in question and the potential for local effects at the site of contact, the critical effect may be different for patients and operators. In addition, the ADE Monograph is a regulatory document requiring greater detail; references to supporting documentation; method justification and the qualification, published articles and curriculum vitae of the qualified and certified toxicologist setting the limit. The OEL document is far simpler and does not need regulatory scrutiny. Even though they may be derived from the same data they must be separate.

For some endpoints, such as cancer due to direct damage to DNA, it is often assumed that a threshold does not exist and some effects (eg mutations) can be expected at low doses. In this case it is necessary to determine the "acceptable" level of response for the effect (e.g., a 1/100,000 excess cancer risk).

The formula below is for illustration only and should not be used by unqualified persons. Risk-MaPP has a far more extensive discussion on this topic because it was used to show regulators that a science-based assessment of a safe limit for potential cross contamination was possible.

Calculation of the Acceptable Daily Exposure (ADE) value

This is undertaken to the following formula:

$$\text{ADE (mg/day)} = \frac{\text{NOAEL x BW}}{UF_C \text{ x MF x PK}}$$

Where:

ADE	Acceptable Daily Exposure	(mg/day)
NOAEL	No-Observed-Adverse-Effect Level	(mg/kg/day) or
LOAEL	Lowest-Observed-Adverse-Effect Level	
BW	Body Weight	(kg)
UF_C	Composite Uncertainty Factor	
MF	Modifying Factor	
PK	Pharmacokinetic Adjustment(s)	

Toxicological expertise is required to determine the appropriate uncertainty factor(s) to apply.

Addressing sources of uncertainty using science-based adjustment factors

a) Intraspecies differences (interindividual variability) (UF_H). This uncertainty factor reflects the variability that exists in the subpopulation of interest. A default uncertainty factor of 10 is typically used in the absence of specific data.

b) Interspecies differences (UF_A). This uncertainty factor is used to extrapolate a NOAEL (or LOAEL) from animal studies to a human sub-population The FDA guidance document on setting doses in initial clinical trials includes specific species extrapolation factors (FDA, 2005) (Section 16.1, reference 19).

c) Subchronic-to-chronic extrapolation (UF_S) – when the duration of the study used to identify the critical effect is different from the actual exposure scenario.

d) LOAEL to NOAEL extrapolation (UF_L)

e) Database completeness (UF_D) – An additional uncertainty factor may be required if the overall toxicity database is incomplete

f) Modifying Factor (MF) – A modifying factor may also be considered if there is a need to address residual uncertainties not covered by the other factors.

g) Pharmacokinetic adjustments (PK) – Route-to-route extrapolation is required in cases where the ADE is derived from a study conducted by a route (eg oral) that is different from the potential route of exposure (e.g. parenteral or inhalation). Similar adjustments may be necessary when setting occupational exposure limits. The ADE as defined in Risk-MaPP is protective by all routes of administration.

h) Additional factors may be needed to adjust health-based limits such as ADEs because of the potential for bioaccumulation with repeated exposure. This adjustment is not necessary if the ADE is established using studies of sufficient length where the steady state at the maintenance dose is known, since any accumulation has already been taken into account in this situation.

Derivation of ADEs for compounds with limited data

Dolan *et al* published a paper with recommendations on how the "Threshold of Toxicological Concern" concept could be applied to relatively unstudied compounds encountered in the pharmaceutical industry[5]. It builds on the approach used by the FDA (termed "Threshold of Regulation"). Dolan *et al* expanded this concept to include three categories of compounds with limited or no toxicity data:

1. Compounds likely to be carcinogenic
2. Compounds likely to be potent or toxic
3. Compounds not likely to be potent, toxic, or carcinogenic

The corresponding ADEs recommended for these three categories are 1, 10, and 100mcg/day, respectively. These ADEs are intended to be applied when justification is needed to support cleaning validation or extraneous matter evaluations for compounds with limited or no information. The same estimated ADEs can be used for early development compounds based on limited data.

Extrapolation to an acceptable level of risk for genotoxic compounds

For certain categories of compounds (i.e. genotoxic compounds) the dose-response curve is assumed to be linear at low dosages and the assumption is that there is no threshold below which adverse effects would not be expected. This is based on the theory that a single change in DNA could lead to a mutation and possibly cancer. It is not in the scope of this chapter and a qualified toxicologist is required to make this assessment.

Setting acceptable levels of exposure for sensitisers

Sensitisation should be included in the toxicological endpoints considered when setting an ADE. The underlying mechanisms of sensitisation reactions, which are immunologically-based responses, are understood and it is known that there is a dose-response for both induction and elicitation of allergic reactions. Whilst thresholds are believed to exist for both induction and elicitation, these can be very low in highly sensitive individuals, and the challenge is defining a level of exposure that is not expected to elicit a reaction in all but the most sensitive individuals.

Setting Occupational Exposure Limits (OELS)

The safe level of exposure for patients may be different to that established for workers. The critical effect used to derive the limits may be different due to differences in the anticipated route of exposure and, even where this is the same, the choice of uncertainty factors may differ. Workers are generally healthy individuals, whereas patients may be children or elderly and, by definition, have at least one active medical condition. Adjustments may be necessary to address route-to-

route extrapolation, even if the same critical effect is used. For example, an OEL derived from the ADE may need to be adjusted for differences in bioavailability between the route used to identify the critical effect (eg. oral) and the anticipated route of exposure (eg. inhalation).

If the same assumptions apply regarding the critical effect and susceptible subpopulations, an Occupational Exposure Limit (OEL) can be calculated directly from the ADE by dividing the dose allowed by the volume of air breathed by a worker, typically $10m^3$, engaged in light work for 8 hours. Likewise, surface target values can also be derived by dividing the ADE by a standard surface area (eg, $100cm^2$), but this is often viewed as a convenient, if not arbitrary, approach for assessing exterior equipment and facility surfaces for worker protection purposes.

Setting health based cleaning limits

Cleaning validation programmes require the establishment of acceptance limits. Currently, arbitrary limits such as 1/1,000 of the lowest clinical dose or 10ppm in a batch are commonly used. These limits ignore toxicological data and are either too restrictive or not sufficiently restrictive. In many cases, overly restrictive limits severely limit the handling of products in multi-use facilities or result in partial or totally dedicated facilities. Additionally, items that present very low risk of cross-contamination, such as utensils for pre-weighing operations, are dedicated or made disposable without any real merit.

Using the ADE has the benefit that it is based on data that regulators have already accepted in the drug filing or are readily available on reputable internet sites. The ADE is toxicologically derived and not simply based on dosage.

The ADE is expressed in mg/day and is the limit protective of all populations. Can be used to identify the maximum carry-over at both bulk and single dosage scales by considering the number of maximum daily doses in a batch, or the number of doses in a maximum daily dose at single dosage scale, normally taken as the point at which blending to uniformity is complete. It is also used to set cleaning limits.

The current standards of 1/1,000 LCD and 10ppm for setting cleaning validation limits do not always provide adequate protection, especially for compounds within the classes of hazardous drugs such as hormones and antineoplastic agents.

The basic calculations for cleaning validation swab and rinse samples would change in one way. Instead of the step:

$$\frac{\text{Lowest marketed dose}}{1{,}000} \times \frac{\text{Batch size}}{\text{Maximum daily dose}} = \text{Maximum allowable carryover}$$

The following step is used:

$$\text{ADE} \times \frac{\text{Batch Size}}{\text{Maximum daily dose}} = \text{Safe Threshold Value (STV)}$$

This change allows the calculation of limits that are derived from actual safety data. A comparison of residue data with product ADE values, will therefore provide the appropriate level of assurance that compound residues, or related degradant levels, on equipment product contact surfaces pose no significant risk to patients.

In addition, calculating ADE-derived STV, and subsequently capturing and trending residue data against the ADE value, can assist in quantifiably determining the product cleaning profile as either

suitable for manufacture within a multi-use production facility or requiring dedicated equipment and spatial separation.

Calculating safe threshold values for swab and rinse samples follow the typical calculations currently used:

$$\text{Swab STV (mg/swab)} = \frac{\text{ADE x SB x TA x RF}}{\text{MDD x SSA}}$$

$$\text{Rinse STV (mg/L)} = \frac{\text{ADE x SB x RA}}{\text{V x MDD x SSA}}$$

Where:

ADE	Acceptable daily exposure	(mg/day)
MDD	Maximum daily dose	(mg/day)
PK	Pharmacokinetic adjustment(s)	(cm^2)
RA	Rinsed area	
RF	Recovery factor	
SB	Smallest batch size	(mg)
SSA	Shared surface area	(cm^2)
TA	Test area	(cm^2)
V	Volume of rinse	(L)

Issues with the use of 1/1000 of the clinical dose, LD50 values, and the 10ppm specification
The use of arbitrary non-health-based limits is not scientifically justified if sufficient data are available to derive ADEs and compound-specific, scientifically-derived health-based values.

An example is when the allowable residue level is calculated by dividing the low clinical dose by 1,000. For most APIs, this approach yields a value that is overprotective compared to the science-based ADE for the majority of APIs. Conversely, 1/1,000th of the low clinical dose for highly hazardous drugs may not be sufficiently protective. Whilst the 1/1,000 of the lowest therapeutic dose provides a conservative cleaning limit for the majority of non-high hazard compounds, it does not do so for some high hazard compounds.

In many companies cleaning occurs based on a worst case, artificially created compound that is then subject to three validation runs and annual randomised verification. (in which failures may occur). For a manually cleaned system to be released on a campaign basis, a visual inspection is used (about 4mcg/cm^2 or lower if a visual cleaning study has been performed). In many cases the visual limit is above the cleaning limit required[6].

The use of median lethal dose (LD_{50}) values to derive cleaning limits has been recommended for equipment cleaning agents (eg detergents). A factor of 0.5×10^{-4} is often used to extrapolate from an LD_{50} value to a "safe" residue level. Use of LD_{50} values is not recommended for risk assessment, even where this is the only value available. Another non-science-based approach for setting cleaning limits is the use of the “10ppm” specification[7].

Risk analysis
This is the collection of data on the facility under review[8]. The following is a typical list. These data have to be captured in a meaningful way. The larger the facility the more significant the ability to manage this data will be. Proprietary systems to manage this data and the entire process are available.

1. Products made
   a) Amount of API
   b) ADE

   c) Dosage size
   d) Maximum daily dose
   e) Batch size etc:
2. Batches made
   a) How many?
   b) When (day, month, year)?
3. Equipment used
   a) What equipment is used?
   b) Where is it located?
   c) What risk of emission does it represent?
   d) (Much more data can be collected to aid in cleaning evaluation, spatial and temporal separation)
4. Facility
   a) Finishes
   b) Room access control
      i. Manual
      ii. Electronic card reader
   c) Room access type
      i. Door
      ii. Airlock
   d) HVAC
5. Risk ranking for risk and vulnerability

This information is required to aid in the risk evaluation.

Risk evaluation

A number of risk assessment tools exist and any one of them can be used. However, be very careful to understand that the regulator expects an evaluation based on science, not based on the perception of the team. Statements have to be based on data, and/or peer reviewed articles. Supporting documentation is required so that statements made can be supported in greater detail.

The FMEA is a well-supported methodology and many articles and books cover its use. However, it is essential to clearly define the scoring system for severity (typically based on the ADE of the compound in review), occurrence and detection, and to clearly identify the outcome of the resulting Risk Priority Number (RPN) in terms of Acceptable without review, Acceptable with review, Requiring review and possible remediation in the near term and Cease operation until remedied. The scoring system also has to be identified. (1, 2, 3; Green, Yellow, Red; 1-10; and the author's preference 1, 3, 5, 7, 10). With this system, the lowest score is 1 and the greatest 1,000.

For this system the following could be considered.

- 1-30 Acceptable without review
- 60-99 Acceptable with review
- 100-342 Requiring review
- 343-1,000 Cease until remedied

For each line item, a comments column should contain narrative as to how the results were arrived at and any other notation so that in subsequent reviews the thought process used on the assessment is captured for future reviewers.

Risk control

Once the evaluation has identified the areas of concern, the next phase is to implement controls. These can be procedural, engineered and administrative in nature. A careful review is required to understand if the solution proposed meets the need. Too often the control is based on perception and presumption of performance. Routine verification of performance is required by the regulator to make sure the controls are effective and to allow risk acceptance to take place.

Risk reduction

The route to reduction is emission control. Most engineering controls for emission are ineffective for a wide range of reasons. The risk evaluation phase should identify where gaps exist between the actual and required performance. Both occupational and product exposure have to be evaluated. For occupational exposure the inhalation and dermal routes are the key routes, whereas retention (failure to clean to the appropriate limit), airborne sedimentation and mechanical transfer are the chief routes of product exposure.

Applying the performance requirement to an actual process

Actual performance has to be proven for any system; a wide range of variables makes it impossible for a generic or parametric performance to be stated and case-by-case performance validation and monitoring is therefore required.

Actual performance as opposed to the exposure level (the not to be exceeded limit) is expressed as a Time Weighted Average (TWA). It has to take account of the following challenges to the system:

- Material characteristics
  - Specific gravity
  - Particle size distribution
  - Electrostatic properties
  - Flow characteristic
- Equipment issues
  - Late in the maintenance cycle
  - Equipment wear and damage
  - Distortion of high accuracy components
  - Equipment malfunction
  - Operability
  - Ergonomics
- Iterations
  - How many tasks are performed in a shift
  - What type of task is performed at each iteration
- Operators
  - Operator fatigue
  - Operator technique
  - Operator error
- Utility failure

Since each active liberating event is subject to so many factors the actual liberation at each event will vary.

Figure 2 shows the liberation levels of active material from a containment system over a sequence of repeated events. As can be predicted, the results vary over a range of exposure levels. The control and containment system must consider the worst-case liberation. It also shows how

important constant monitoring and proper evaluation is. Most systems are challenge-tested with inappropriate materials, with too little iteration, under ideal circumstances. This cannot truly reflect real, not to be exceeded, performance.

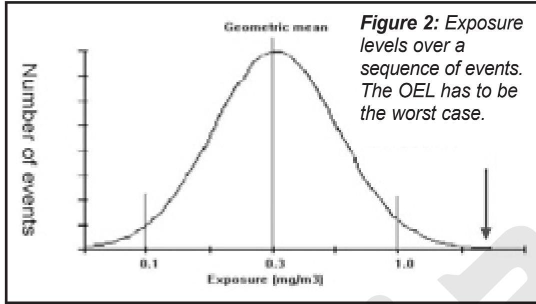


Figure 2: *Exposure levels over a sequence of events. The OEL has to be the worst case.*

Wet	Dry size
Large >100 micron particle	Small <10 micron
Dense	Light
Closed	Open
No Energy/Velocity	High Energy/Velocity
No Technique Required	Highly Technique Dependent
Low Δp	High Δp
No Transfer	Multiple Transfer
Well Maintained	Poorly Maintained
No Explosion Risk	High Explosion Risk
One Iteration	Multiple Iterations

Exposure will vary from iteration to iteration and from operator to operator. The greater the dependence on technique and air entrainment as the mechanism of exposure control, the greater the maximum exposure and the variability.

A fully contained system where issues of energy, pressure and technique have been holistically addressed provides one surprising and one obvious effect. Concentrations inside fully contained processes appear to be significantly lower than the open process concentrations. Whereas figures up to 32,000 mcg/m^3/event duration are seen for open operations such as scoop and dump, only 6,000 mcg/m^3/event (60+ minutes) has been seen on the same open operations inside an isolator and this figure reduces to 1,000 mcg/m^3/event in very low airflow glove bags. The reason is simple – control. Isolator environments are controlled within very precise parameters; the airflow inside an isolator is simply not intended to support aerosols while that is exactly what open processing requires to convey exposure to the nearest boundary filters.

Pressure

There is another major factor and that is the differential pressure between the contained environment and its surroundings. To protect people, a negative pressure is desirable, where protection of the patient is the concern, then the pressure gradient should be reversed, or so goes the current wisdom. Most failures of isolators and transfer valves are directly attributable to over pressurisation. This can be as simple as a powder mass descending during blending imposing a piston effect and therefore pressurisation. The over pressure can result in significant discharge through imperfect seals.

Actual performance testing

It is usually unwise to test a system using the active; often the active is too expensive or does not exist in sufficient quantities for repetitive testing, but surrogate testing has its challenges too.

The criteria that have to be considered in developing a testing protocol are:

Does the surrogate represent the worst case in terms of particle size, particle shape and electrostatic properties, etc?

Surrogate handling

The test compound has to be loaded into its initiating container without risk of liberating detectable levels:

- The test environment has to be checked for the presence of the compound before any test is performed

- Personnel handling the target compound can have no part in the operation recovery or testing of the surrogate
- The surrogate must have similar specific gravity and size distribution to the active, this is to ensure that the dwell time in the air is similar too and similar in concentration to the active
- The surrogate must be easily detected in the concentrations encountered and be in the mid range of detection for the equipment.

Iterations

- The worst case number of shift iterations must be replicated

Operators

- The operators should as far as possible try to ensure a reasonable level of poor technique performance, this is best done by giving minimal training to the operators before testing
- Some predictable fumble factors should be induced to give a real feel to the test, remember the OEL is a not to be exceeded limit

Equipment

- Should undergo a number of repetitive uses to represent end of maintenance cycle conditions
- Where malfunctions can be predicted they should be induced on some of the cycles

Cycles

- The whole test sequence has to be repeated a number of times with some cycles being run exactly as events dictate and some identified cycles being run with induced worst case events.
- A range of operator skill and dexterity models should be applied

Air sampling

- Both full shift sampling and short term 15 minute samples should be performed, with the short term sampling occurring at potential liberation events to demonstrate if that event is a cause of emission and if so what is its scale.

Risk acceptance

The company has to document that it is acceptable to allow manufacture to proceed and be compliant. This is not as simple as it sounds since the FDA is looking for metrics that allow the statement to be made that cross contamination is controlled below the ADE limit. These are some of the methods that could be used:

1. <u>Mix-up Incident reports</u>. More than one verification per item and manual and electronic verification
2. <u>Retention</u> 100% or statistically relevant verification by analytical method and visual observation with a visual observation program
3. <u>Airborne Data</u> closed process or data showing the rate of sedimentation and airborne concentration is not leading to cross contamination
4. Mechanical Transfer Procedures and Methods. To decontaminate anything passing into or out of a process room, whether open or closed processing is used.

Risk acceptance must be based on sound scientific principles and be recorded in compliance with quality standards.

Risk review

This is not a process to be placed on a shelf and left. The documentation has to reflect the current products, processes and methods used and every new introduction has to be vetted to see if it represents a new worst case or a new vulnerability as well as being checked for other issues. The

whole documentation group should be reviewed annually and signed off. All changes should be kept in a version control so that all the changes are transparent. Deviations and incidents should be reviewed at the same time and feed-back incorporated in the process so that the system is constantly improved.

Risk communication

The process and its outcomes should be communicated to all who need to know. Documented training with an element of testing participants and re-training if they have unsatisfactory grades should be implemented and routinely reinforced. This includes suppliers of API and third party suppliers.

12.5 What is the current regulatory position on cross contamination and Risk-MaPP?

FDA

The FDA has been a member of the team since day one.

The document was 18 months with the regulator in a line-by-line review. The term ADE was at the request of the FDA to prevent any mix-up with the term ADE that some associate with the oral route.

CFR 21.211.42 Subpart C – Buildings and Facilities Design and Construction Features

There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mix-ups during the course of the following procedures

Warning letters

Risk-MaPP® was published in September 2010 the same month the FDA issued a 483 to a major manufacturer outside the USA. By January this was a warning letter. The failure to have a risk assessment for cross contamination was one of the citations. The letter (redacted) can be found on the FDA site under warning letters.

> *"Your firm should ensure that a documented justification and well-designed contamination prevention strategy is in place to minimise the possibility of contamination. Include in your risk analysis, procedures and data addressing the following potential routes for cross-contamination: mix-up, retention, mechanical transfer, and airborne transfer."*

The FDA have issued a number of such letters in the past to companies that are much smaller.

> *"…your firm lacked an adequate assessment of the cross-contamination risks posed by the manufacture of several potent compounds…"*
>
> "…*During this meeting please be prepared to provide the following: …a detailed risk assessment of all your potent compound drug products…"*
>
> *"your firm lacked an adequate assessment of the cross-contamination risks posed by the manufacture of several potentially hazardous compounds (e.g.,* ***(b)(4)****) at your facility. Your facility contains shared manufacturing areas where you produce potentially hazardous compounds in multi-product equipment that are high powder generating operations"*

The FDA's next step for non-compliance after a warning letter is a consent decree. In a presentation a few years ago after a spate of well publicised consent decrees there was a presentation on what a consent decree means. The first slide was "be prepared to spend $1,000,000,000".

EMA

After years of work by the shared facilities working group the uncertainty of the word "certain" in Chapter 3.6 is going to be replaced. It is a move from prescriptive to risk-based GMP's and is to be applauded.

The Eudra Lex, the rules governing Medicinal Products in the EU Volume 4 Part 1 chapters 3 and 5 have been completely rewritten and are available for comment until July 2013. The rewrite basically follows ICH Q9 in that a science based risk assessment is required for every compound

introduced into a shared facility. The term certain has been removed as have the categories such as hormones. The new wording has to be read in conjunction with the draft for comment “EMA Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities” This document uses the PDE first seen in ICH Q3 as a means to set the health hazard of residual solvents, rather than the FDA suggested ADE as used in Risk-MaPP®. Actually PDE, ADE and ADI are all calculated in the same way.

The new wording corrects some preconceptions in the industry. Hormones do not have to be made in dedicated facilities so long as one compound does not cross contaminate another above the limit set by a health based limit. One hormone is just as hazardous to another hormone, as cross contamination by a hormone in a non-hormone product. The same is true for all the other emotive products such as cytotoxic, Mutagenic etc.

The use of dose based or arbitrary cleaning limits is no longer acceptable, cleaning limits have to be health based. Risk- MaPP® provides a sound basis for understanding the issues.

Anvisa (National Health Agency of Brazil)

Art 125. “Should be segregated and have dedicated facilities used for the production of certain drugs, such as certain biological preparations (e.g live micro-organisms) and the highly sensitising materials (e.g. penicillins, cephalosporins, carbapenems and other beta-lactam derivatives) in order to minimise the risk of serious damage to health due to contamination. …”

§ 2 The production of certain highly actives such as some antibiotics, certain hormones and cytotoxic substances should be held in segregated areas.

§ 3 In exceptional cases, such as accidents (fire, flood, etc.) or emergency situations (war etc.), the principle of campaign work on the same premises can be accepted provided that specific precautions are taken and that necessary validations (including cleaning validation) are completed

Article 255

§ 1 The risk of accidental cross contamination arises from the uncontrolled release of dust, gases, vapour, aerosols, or organisms from materials and products in process, residual on equipment, insects, clothes of the operators, their skin, etc.

§ 2 The significance of the risk varies with the type of contaminant and the product which was contaminated.

Article 256

The occurrence of cross-contamination must be prevented through appropriate technical and organisational measures, such as:

- Production in exclusive areas and closed (e.g. penicillins, cephalosporins, carbapenems, other beta-lactam derivatives, preparations with biological organisms, certain hormones, cytotoxic substances and other materials that are highly active)
- Production in campaign (separation by time) followed by appropriate cleaning according to a validated procedure
- Use of chambers, pressure differential and supply air and systems of exhaustion
- Reduction of the contamination risk caused by recirculation or not treated air re-entry or inadequately treated;
- Use of validated procedures for cleaning and decontamination
- Use of "closed system" production

WHO Annex 3 to TRS 957, 2010

“Not all products containing hazardous substances are equally potent and risk assessments should be carried out to determine the potential hazards to operators and the environment”.

- Annex 3 of Technical Report Series No. 957, 2010 Primary focus is on HVAC with some guidance on personnel protection and use risk assessments to determine applicability

PIC/S

- PIC/S mission is "to lead the international development, implementation and maintenance of harmonised Good Manufacturing Practice (GMP) standards and quality systems of inspectorates in the field of medicinal products."
- New GMPs issued in the Autumn of 2007 with no real change for cross contamination issues (dedicated facilities)

Ministry of Health Labour and Welfare (Japan)

A rare statement of support was issued for Risk-MaPP; normally, MHLW makes no comments on guidance by non-regulatory bodies.

- (*5) In the case where the products, etc. are easily dispersed and cause hypersensitive reactions in a minute amount or could cross-contaminate and seriously affect other products, it must be ensured that the work rooms are exclusively used for such products, etc and their air-handling system is separated from those used for other products Clarifications from Enforcement Regulations*
    - *Penicillins and other compounds with strong physiological activity should be in a dedicated room with dedicated air-handling*
    - *Article 9(5) is not applicable where conditions are such that the material cannot disperse or cross-contaminate*
- *Comment by MHLW is that products which dedicated facilities are to be required should conform with those in ICH Q7 not only for bulk facilities but also for pharmaceutical facilities.*

MHLW has announced the issue of notice for dedicated facilities. However, such a guideline has not been issued yet.

ICH Q7A, Section IV.D Containment (4.41)

Materials of an infectious nature or high pharmacological activity or toxicity

> *"...dedicated production areas should...be considered… unless validated inactivation and/or cleaning procedures are established and maintained*

Occupational Safety and Health Administration (OSHA)

OSHA recently fined a pharmaceutical tablet repackaging operation a considerable sum for worker exposure. The case is noteworthy because:

1. OSHA asked for the compounds in tablet form being repackaged
2. OSHA knew the OEL for the compound
3. OSHA put in their own IH to take the samples

12.6 Conclusions

It is clear that the major agencies are getting increasingly tough over cross contamination. It is also clear that they are quite ready to take on anyone. One only has to look at the news to see that the penalty elements are capable of causing significant loss, a loss easily avoided by being pro-active and transparent. It is also true that the agencies need some education to fully understand the real risks involved.

In reviewing large numbers of pharmaceutical companies both big and small, it is staggering to see the deficiencies that lead to 483 Warning Letters which can be simply cured. There seems to be a

sense of infallibility, that the regulator will not spot the deficiency and will accept explanations that have not a vestige of science in them.

What to watch out for:

What inspectors think you should know about how to make drugs and what they will discover undertaking a properly conducted QRMP will be of surprise. This helps to understand why the regulators have concerns. There are two major reasons for this:

"Cannot see the wood for the trees"

When people see the same plant every day they become blind to the idiosyncrasies and mistakes that go on all the time. People think they know the SOPs and they think they know what best practice is, but often they do not. People rarely, if ever, visit other facilities so they do not know how they compare with others. If they do visit another manufacturer's site, often they will never see what is really going on. It is advisable to employ a specialist who spends his life visiting clients facilities and who will see your facility for what it is, good or bad.

Silos

Companies can live in silos, without much interaction at production level. Departments live in silos jealously guarding their property from others in the organisation that may use it to their advantage. When Risk MaPP was being written, we had all the team members together. But the cleaning specialist had never talked to a toxicologist before.

12.7 References

1. ICH (2005). Quality Risk Management: Q9: International Conference on Harmonisation Technical Requirements for Registration of Pharmaceuticals for Human Use.
2. FDA Draft Guidance, Non-Penicillin Beta-Lactam Risk Assessment: A cGMP Framework, March 2011. www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM246958.pdf
3. ISPE (2010). Volume 7 Baseline® Guide Series: Risk Based Manufacture of Pharmaceutical Products (Risk-MaPP).
4. http://www.fda.gov/Safety/SafetyofSpecificProducts/ucm180580.htm
5. Dolan DG, Naumann BD, Sargent EV, Maier A, Dourson M. Application of the threshold of toxicological concern concept to pharmaceutical manufacturing operations. *Regul Tox Pharm* 2005; **43**:1-9
6. Walsh A. (2011) Cleaning acceptance limits for APIs, Pharmaceutical Engineering Part 1 July/August 2011, Part 2 September/ October 2011.
7. Forsyth R J, Van Nostrand V, Martin G P. Visible residue limit for cleaning validation and its potential application in a pharmaceutical research facility. *Pharm Technol* 2004; **28**(10): 58-72.
8. Wilkins J J. (2010) A Qualitative Risk Assessment of Cross Contamination. *Pharmaceutical Engineering* 2010; Jan/Feb 2011.

Future of aseptic processing

James L Drinkwater, Process Director, Bioquell, UK

13.1 Introduction: The future of aseptic processing

There are a number of key developments in technologies and regulatory initiatives that have and will impact on the future of aseptically processed medicinal products, as a counterpart to terminally sterilised products.

Together with the individual developments and improved process knowledge, the interaction between critical quality attributes becomes critical to a risk-based approach in bio-contamination control and aseptic operations.

Aseptic processing[1] has a primary focus on risks to patients prescribed the medicinal product, so risks are considered at every step of the process that may impact on product quality, efficacy or patient safety.

Developments and initiatives impacting on aseptic processing include:

- The changes in product profiles with development of more biologicals that cannot be terminally sterilised hence require processing aseptically.
- The development of risk-based initiatives[2,3] such as Quality Risk Management (QRM), Quality systems and Quality by Design (QbD) and application in control of critical quality attributes and monitoring technologies detecting deviation from a defined control state.
- The understanding of the risks associated with microbiological contamination from gowned operators[4,5] in conventional 'open processing' and the need to employ barrier technology[6,7] to separate processes and operators to assure bio-contamination control.
- The use of more assured and possible to validate biological decontamination processes[8,9] to complement assured sterilisation processes – less reliance on end-point testing.
- Development of monitoring technologies, including real time and in-process, to monitor parameters that form part of critical quality attributes in an aseptic process, for direct process monitoring and in related surrounding environment.
- The development of pre-sterilised disposable technologies replacing tradition stainless steel processing vessels and product lines.
- Process and validation efficiency improvements together with considerations to energy saving and sustainability.

By understanding a process you can then understand the associated risks that may impact on product quality, efficacy and patient safety.

The complex nature of aseptic processing has risks in bio-contamination at every stage of the process but in particular at interactions and interventions with operating personnel.

A risk-based approach is required for every aspect of an aseptic process, including; specification, design, control of quality attributes, qualification, operation, operator training, critical parameter monitoring and on-going qualification and through any process change or improvement. This

chapter examines the changes taking place in relation to aseptically filled products and the technological changes required to meet manufacturing and product requirements. This includes a review of isolators and RABS, decontamination processes and the latest regulatory thinking in relation to aseptic processing.

13.2 Changes in product profiles

Pharmaceutical and Bio-pharmaceutical companies are changing product profiles as conventional large molecule chemical entities are being replaced with more biologically derived products.

Biological products are typically damaged by the heat and stress imposed by conventional termination sterilisation so, with an increase in the manufacturing of such products, aseptic processing is becoming a critical production and operation methodology. With more biological protein based products there is also an increase in lyophilisation – freeze drying to provide necessary product stability over shelf life. This further processing step adds another level of bio-contamination risks with in-process transfer of partly open and filled product containers.

By adopting aseptic processing it should not be underestimated that there are significant challenges in bio-contamination control and very specific methodologies and technologies are required. In particular the use of conventional 'open process' cleanroom operations where gowned operators are in close proximity to sterile product, open product containers or related sterile equipment or surfaces is now considered not to be best practice in cGMP[10,11].

The use of Isolator or Restricted Access Barrier System (RABS)[12] technology that provides operator to critical process sterile product separation has become a regulatory expectation. It should be understood that barrier technology alone will not provide the necessary risk reduction in bio-contamination control but good operating methodology and practice are also critical.

13.3 Development of risk-based initiatives such as Quality Risk Management (QRM) and Quality by Design (QbD)

Quality Risk Management (QRM) and Quality by Design (QbD) are key regulatory initiatives that introduce a risk-based approach to all aspects of a defined process with consideration towards compromise of product quality, efficacy and patient safety.

A risk-based approach to regulatory inspections will have a focus on all areas of risk and in particular will consider changes, including changes in control, procedure, personnel, processes and facilities.

Microbiological deviations from classified conditions[8,9,13,14], as defined in regulatory documents, or in the state of control and deviation from an established trend, should be one area of on-going assessment and will be subject to regulatory scrutiny.

In particular, how such bio-contamination deviations were investigated, to identify a root cause(or provide a high probability of root cause) via a Root Cause Analysis (RCA), and a Corrective and Preventative Action (CAPA) to assure deviations do not reoccur, will be subjects of inspection audits. These processes need to be thorough and closed out in a timely manner and are often subject of regulatory citation.

Quality by Design changes the focus from end-point testing, such as: in validation; the 'three repeat PQ tests or cycles'; in Performance Qualification studies and in operation: media fill simulations and routine Sterility testing to assuring quality by using process knowledge and designing in control and monitoring so any deviation in critical quality attributes is detected, reported and recorded.

Defining critical quality attributes may require supporting experimental work to ascertain acceptable deviation limits or limitations in a design space that other parameters or critical quality attributes operate. Such experimental work would typically need a Design of Experiment (DoE) to thoroughly challenge the process and target parameters or control state with any related interaction.

Risk assessments are tools[13] to assist critical decision making, define key requirements or critical parameters that would put a process, product, process operatives or patient at risk. Selecting the correct risk assessment tool for the task is important and may include a number of risk assessment models together to suit a particular process. Whatever the number of risk models, the assessment comes down to whether the risk is low, medium or high. The key to risk management is then how you mitigate such risks to acceptable and manageable levels.

13.4 Understanding risks associated with microbiological bio-contamination

Unlike particles that are not viable, e.g. dust or metallic particles, micro-organisms proliferate and grow under suitable conditions. The typical definition of a colony-forming unit, cfu, indicates the ability to proliferate as two micro-organisms become four, four become eight and so on. Such a viable state of growth has the ability to become clinically significant: organisms that may impact on a medicinal product quality or potentially harm, or cause death, to patients by infection. Therefore, any micro-organisms introduced into a process at any stage, may proliferate and grow to harmful levels through processing and subsequent holding (shelf life).

Actual medicinal products are sterilised as part of the process by conventional pharmacopoeial[4,5] methods. The challenge in aseptic process is not to compromise this sterility at any stage of processing, during transfers or administration to patients.

Microbiological monitoring sampling plans[14] and tools are known to cover just a fraction of the process and typically provide poor recovery so detected cfu contamination would normally be far less than actually in the environment or on critical surfaces.

One example given of this detectable scale of bio-contamination is that if you detect one cfu this may be a representation of a village of micro-organisms and if you detect ten cfu this may indicate a city. An environmental monitoring result of 0 cfu does not actually mean the environment is sterile and free from micro-organisms – contamination will probably be present (for a discussion of the limitations of environmental monitoring, refer to Chapter 21).

Knowing that monitoring and detection may be a poor indication of actual bio-contamination levels, the emphasis needs to shift to designing good bio-decontamination processes and operations that eliminate (or restrict to a minimum) introduction of bio-contamination throughout the process. Microbiological monitoring results are typically after the process event. Real time detection of viable bio-contamination during processing is in development and may later improve process control when a more complete picture of the control state will be possible.

It will still be necessary to monitor for bio-contamination and assurance of the state of control even with good quality design. Processes often involve operators and transfers between controlled areas[15] and few are fully automatic from beginning to end. By characterisation of the control state then monitoring the trend of microbial contamination deviations (Incidence or Hit rates) can be used to detect changes in the control state and instigate thorough investigations.

Sterility testing would detect a contaminated batch but as far as detecting contaminated units the process is scientifically challenged and the results statistically insignificant. In aseptic processing there are more chances of contaminating product units because of process interactions and

possible interventions. Some interventions that lead to risk include the process of completing environmental monitoring to check the control state.

There are a number of key processes that combine to produce a sterile medicinal product by aseptic processing. These include, but are not limited to:

- Sterilisation of a bulk medicinal product by one of the pharmacopoeial referenced processes.
- Create an aseptic environment where the product can be processed aseptically without compromise to product sterility. Typically for GMP this would be inside an isolator or RABS barrier controlled by engineered filtration systems to meet a classified environment (EU Annex 1 Grade A or ISO 5/4.8), together with a validated bio-decontamination process.
- Transfer the sterile product from bulk through various process stages into the final closure container without compromise to sterility during in-process transfers.
- Prevent bio-contamination of the aseptic environment by excluding people by physical barriers (in the case of isolators) and combined barriers (physical and aerodynamic) in the case of RABS) and in advanced aseptic processing with no open door operator access to the critical process zone after the last process bio-decontamination step.
- Prevent bio-contamination ingress into the aseptic environment of the barrier during all material transfers into and out of the barrier.
- Monitoring inside the barrier to confirm state of control, real time total particle counting, and supporting microbiological monitoring.
- Procedural controls that cover any trained operator, QC or maintenance intervention.

It is clear that isolator and RABS barrier technologies will play a significant part in future aseptic processing following the significant risk reduction step of separating the most contaminating source, people-operatives, from the aseptic process.

Once a barrier is selected or defined, key considerations of risk assessments include:

- The methodology for interaction between sterilisation and bio-decontamination (disinfection) technologies and processes.
- The in-process transfer methods or devices to transfer the sterile product into and out of the barrier together with all necessary sterile closure-container parts, process support parts and environment monitoring materials.
- How the barrier is operated regarding set-up, processing, inherent interventions, e.g. stopper charging; also monitoring, any necessary line clearance and deviation control.

13.5 A risk-based methodology and key considerations for selecting a barrier technology (isolator or RABS) for aseptic processing

Isolators and RABS are now well-defined technologies and both are suitable for aseptic processing. The choice between the types of barrier technology should be the subject of a risk assessment with reference to a given process and requirements.

How the barrier is operated is critical to meeting requirements of aseptic processing and reliance should not be placed on the fact a physical barrier is present. Considering the engineering aspect, the isolator barrier technology provides the highest assurance of barrier separation. As the barrier is physical it is possible to seal for leak integrity testing and possible to control at positive or negative differential pressure in respect of the surrounding environment. Applications are wider than that for RABS. An aseptic-toxic process may be more appropriate for an isolator than RABS.

The higher physical barrier integrity of isolators also facilitates operating in a background environment classification ISO 8 (equivalent to EU Grade D (minimum)) and often installed in an EU GMP Grade C, in order to safeguard against future standards that may require additional levels of protection.

Restricted Access Barrier Systems (RABS) use a combination of physical and aerodynamic/airflow protection around the critical process zone and at barrier transfers. This less integral barrier has a minimum background environment of ISO 7 (equivalent in operation to EU Grade B).

If the application is to retrofit a barrier technology to existing process equipment, e.g. as a filler, then such engineering interfacing does not suit an isolator. Floor-mounted RABS barriers, having very little mechanical interface to the process equipment, may suit this requirement better.

The Pharmaceutical & Healthcare Sciences Society (PHSS) have produced a detailed RABS Technical Monograph[12] that includes FDA and MHRA regulatory review. This comprehensive guidance document details two main RABS definition types based on open or closed operation or open and closed airflow design format, two operating methodologies regarding interaction between sterilisation and disinfection, three risk-based intervention groups and three transfer device groups.

When commencing a new aseptic processing facility, at the project stages of initial specification, setting user requirements and the integration with the filling/process equipment consideration is required on a RABS design and air management format, together with the specific operational methodology to be employed. Evaluation of how operational methodology is supported by the chosen design format is an important step when defining appropriate barrier technology for any given process.

The PHSS have defined **Open and Closed RABS** as a definition of the hierarchy of contamination risk by decreasing containment ability. There are risks in operations if there are open barrier door interventions potentially exposing gowned operators to an aseptic process and sterile product. There is risk in RABS barrier design format as the more reliance on airflow protection than physical barrier, the higher the risk.

In drawing up the definition it became necessary to separate the definition of Open and Closed into two categories, one relating to operation (whether barrier doors are opened or closed in aseptic processing) and one for design (whether the barrier design has a greater (open) or lesser (closed) extent of air flow overspill to the surrounding environment as part of aerodynamic protection.

The following PHSS definition hierarchy applies to all RABS types as defined in the PHSS monograph:

Closed operation RABS – this means in-operation RABS barrier doors remain closed for defined process steps.

Open operation RABS – this means the operation has an open door operator intervention during aseptic processing.

Each RABS operational type can be used with the following RABS design type.

Closed design RABS – this means only overspill air is at transfer devices and the critical process barrier is of a closed design, like an isolator.

Open design RABS – this means there is air overspill, for protection of the critical zone, around the barrier and at transfer devices.

Table 1: Different types of transfer devices

A	A transfer device that maintains protection from contamination between remote aseptic zone(s) through to the RABS process zone(s). Protection in transfer is maintained through physical containment or mobile Grade A/ISO 5 airflow carts.
B	A pre-sterilised transfer bag or container that may include aseptically prepared or sterile components and interfaces with the RABS barrier and provides the necessary level of risk reduction in preventing compromise of sterility during the transfer procedure.
C	A transfer device or procedure that includes a decontamination step at the interface with the RABS barrier. Transfer decontamination may include a sterilisation, gaseous decontamination or manual disinfection step.

Transfer devices for RABS barriers

In principle there are three types of transfer device or procedure used in RABS barriers (see **Table 1**).

The hierarchy of contamination risk associated with Open barrier door interventions

Limitation or elimination of exposure to potential bio-contamination as a result of 'Open' barrier doors during each phase of RABS operations can be ranked in a hierarchy of risk reduction. Although every stage of an aseptic process is open to bio-contamination risk, there is a step change of increased risk as the process advances to exposure of product containers/ closures and aseptically prepared product.

Three types of intervention with their associated levels of potential contamination risks are possible in RABS:

Type 1: *Open barrier process interventions: Highest contamination risk interventions*

Open barrier door operator interventions, completed after set-up, sterilisation or aseptic assembly of pre-sterilised parts and the last bio-decontamination step, are the highest risk interventions since open product containers or prepared products may be exposed.

Operator interventions, through Open RABS barrier doors during aseptic processing, would be subject to scrutiny from a GMP compliance perspective.

Type 2: *Open barrier set-up interventions*

Where there is no facility to Clean and Sterilise-in-Place (CIP/SIP) the product contact parts, or to surface sterilise the indirect product contact parts in-place, the line set-up has to include aseptic transfer and assembly of pre-sterilised parts, together with the necessary environmental monitoring.

If, after line set-up and aseptic assembly, there is no further open barrier door intervention during aseptic processing (where open product containers and prepared products may be exposed) there is a reduction in contamination risk compared to that present during a type (1) process intervention.

Large items such as component feeder bowls may not be possible to clean and surface sterilise in-place particularly if manual disinfection is used for the RABS barrier and non-product contact parts, so aseptic transfer and assembly may be necessary. With relatively long periods of open barrier doors, required for access in aseptic transfer and assembly activities, there is higher potential risk of bio-contamination exposure from related personnel and the surrounding environment. Such activities should be risk assessed and documented with defined monitoring during the procedure for viable (microbiological) and non viable (particle) contamination.

***Type 3:** Closed barrier 'inherent and corrective' interventions done via barrier glove-sleeves or a transfer device*
During aseptic processing there are accepted 'inherent and corrective' interventions that are completed in the closed barrier door state, maintaining the closed operation RABS status.

Inherent interventions would be charging, via aseptic transfer devices, of stoppers, over-seals or caps, etc., into pre-sterilised/surface-sterilised feeder bowls located inside a decontaminated RABS barrier.

Corrective interventions may be the correction of machine or container indexing faults occurring during product container processing or components feeds, and are affected using barrier gloved hands for access and manipulation and not via an open barrier door. Good aseptic practices should be maintained during corrective interventions so that barrier gloves do not contact direct or indirect product contact parts.

13.5.1 More assured and possible ways to validate biological decontamination processes

In aseptic processing it is necessary to have assured biological decontamination processes. Assurance comes from and understanding of the science and microbiological inactivation mechanism of the bio-decontamination process together with the ability to control, monitor and validate the efficacy of the process with biological challenges[16] (via highly resistant biological indicators).

Primarily there are three types of bio-decontamination processes, for surface treatments, that complement the tradition and higher level penetrative sterilisation processes, typically:

Gaseous Vapour Phase (GVP) decontamination, disinfection and sanitisation. GVP and disinfection would be validated with a biological challenge whilst sanitisation may use a laboratory-validated disinfectant agent but the process is just monitored for bio-decontamination impact by environmental monitoring.

Manual disinfection is a widely used process for material transfer surface decontamination between controlled areas. It is accepted such manual disinfection processes have challenges with process variation (operator dependent) and repeatable efficacy. Risk assessments should therefore be used to verify limitations and are acceptable for the application and make the risks of residual bio-contamination manageable.

More and more pre-packaged sterile disposables are used in aseptic processes and it should be confirmed that outer packaging is suitable (or possible) to bio-decontaminate during transfers.

Gaseous Vapour Phase (GVP) is the highest level of surface bio-decontamination processes (not equivalent to penetrative sterilisation) that is capable of being validated to achieve conditions of surface sterility and typically used for a Gassing-in-place (GIP) process for indirect product contact parts such as filling line feeder stopper-hopper bowls, trackways and specified barrier gloves.

Validation of GVP decontamination includes pre-cleaning studies to confirm normalised starting bioburden levels. Also, studies are completed to confirm there are no unacceptable levels of post-cleaning agent residuals that could compound with hydrogen peroxide agent and mechanically transfer to a product (via product closure stopper contact), impacting on product quality and, if a biological, possibly efficacy.

Disinfection processes are also applied as gassing processes[15] which provide a much higher level of automated control, repeatability and assured efficacy. The benchmark process for surface decontamination in bio-contamination control, including viruses, used in the pharmaceutical and

biopharmaceutical industries is hydrogen peroxide vapour. As a sporicidal surface treatment process, hydrogen peroxide vapour is possible to validate with biological indicator challenges to achieved 4-log to 6-log efficacy. For reference, 1-log reduction is 90% reduction in spore population at each log step of inactivation. With six steps of log reduction (6-log), together with a margin of overkill, it is possible to achieve, under specified conditions, surface sterilisation.

Sanitisation, often used for cleanroom and support areas (to critical zones) is a process that delivers a validated agent to target surfaces, often manually by mop and bucket for large areas, or spray and wipe, or use of impregnated wipes for smaller areas.

Typically, residues will be left after such sanitisation processes and if there is a potential impact on a process, either a rinse-step will be required or the use of alcohol-based agents that would evaporate off a surface and physically remove residues.

It should be noted that some sanitisation agents may have long contact times to achieve biological efficacy. This is different from a laboratory, where an agent may be validated, the airflow conditions in an operational environment may dry off the sanitisation agent hence reduce efficacy. In these circumstances re-wetting may be required.

13.5.2 Comparison between vapour and atomised decontamination agent generation and distribution processes

Disinfection processes that spray (manual) or atomise agents (fogger variants) have significantly different scientific mechanisms of delivery and distribution to target surfaces than that of vapour processes.

Hydrogen peroxide vaporised molecules are significantly (orders of magnitude) smaller than atomised molecules from spray or fogger type delivery systems. In addition, vaporised processes are dynamic because at the optimum conditions of saturated vapour pressure there are as many molecules arriving on the surface as leaving (equilibrium state), hence there is a need for constant replenishment of the agent.

Replenishment is important to overcome any natural breakdown of the agent including catalytic effects of contact surfaces or catalase-positive effects of micro-organisms. Such negative affects need to be overwhelmed by the decontamination agent.

The overkill effect of vaporised processes is completed both by higher concentration agent than that of manually applied or atomised agents and the dynamic surface replenishment process. For hydrogen peroxide vapour, the starting agent is typically ~30-35% w/w and as low as 6% for manually applied, sprayed or atomised agents.

In contrast, a manually applied, sprayed or atomised agent is a one-way delivery system and whatever reaches the surface is then static and subject to degradation breakdown before natural evaporation or bio-degradation.

The scientific difference between the dynamic vapour process and the static disinfection process is significant in relation to process efficiency, efficacy and the control of residuals.

Typically, if high concentration vaporised disinfectant agents are used, the resulting effect via free radical attack, achieves micro-organism destruction. With such an impact, only one sporicidal agent is required in a disinfection program, hence no agent rotation is required.

Conversely, if manually applied agents are used in low concentration and the disinfection process

is subject to a number of process variables, the impact may be of marginal efficacy and it may be useful to rotate disinfection agents (one being sporicidal) to provide a multi-faceted attack.

It should also be noted there is considerable improved material compatibility with vaporised agents that are administered in a process of controlled delivery to surfaces at invisible levels and controlled removal as part of the process so there are no residues that may cause corrosion. Manually applied disinfection agents or delivery by atomiser/fogger can leave residues that rely on the slow process of bio-degradation and as a result the residues may have time to cause corrosion.

13.5.3 Hydrogen peroxide vapour cycle development – key requirements for cycle optimisation

The rationale required for cycle development of hydrogen peroxide vapour decontamination processes is based on characterisation studies. Characterisation includes collective and iterative studies that combine to study process variables and the transition between sub-lethal and lethal efficacies.

A hydrogen peroxide decontamination process needs integrating into any application with a special focus on system configuration that is suitable for vapour containment and exposure of all target surfaces, together with delivery and removal of the decontaminating agent. Ideally, such integration is completed at the initial full system design phase and not as an add-on after a containment system isolator, RABS or room is built. Retrofits are possible but are more challenging to integrate and often do not yield optimum bio-decontamination cycles.

In advanced aseptic processing, automation is used to configure any system for bio-decontamination as well as communicate with any associated hydrogen peroxide vapour generator such that the transitions between cycle phases are controlled without operator intervention.

Hydrogen peroxide vapour is not a ‘true gas’ that diffuses readily but instead is subject to hydrogen bonding with characteristics of poor passive diffusion. As a result there is a need to impact distribution with kinetic energy, either in the form of distribution nozzles (creating high velocity jets for vapour turbulence and mixing) or distribution fans.

This poor diffusion characteristic is good for safety as any leakage tends to hang in the locality without motive force so vapour containment and restriction of exposure to surrounding operatives is much easier. A key point is that hydrogen peroxide vapour is evaporated by flash evaporation and is a condensable vapour. In this case, delivery losses are common by the reduction in temperature of the high concentration vapour stream resulting in condensation losses before the target areas, which leads to a common cause of cycle inefficiency.

Also, some surfaces are highly absorbent, including HEPA filter media, and such impacts need to be considered in cycle development or avoided as the primary delivery route for the high concentration agent to the target areas. Once in the target areas, circulation of the mixed vapour (at lower temperature and concentration conditions) through all associated HEPA filters, return air-ducts, etc. reduces the impact and potential of hydrogen peroxide absorption and saturation of material surfaces.

Scientific study[17] has confirmed that the optimum process for bio-decontamination using hydrogen peroxide is to achieve micro-condensation of all surfaces in the target areas but at an invisible level (2-6 micron layer deposition). Any visible condensation does little for additional efficacy and adds to aeration (residual removal) time. It has to be accepted where there is a relatively high thermal gradient, across a window etc. From inside a target area to the surround, there may be some visible (preferential) condensation. However, inside the target area on all other surfaces, micro-condensation should not be visible.

As reaching dew point is critical, there is little point dehumidifying the environment to low and dry conditions before vapour injection. Study confirms that even at 75% relative humidity starting conditions, there is more than enough efficacy at saturated vapour conditions. Thus a 50% relative humidity starting condition may be considered suitable for most applications.

At saturated vapour conditions, past dew point, the environmental gas concentration reaches a 'plateau' condition indicating that saturated vapour conditions have been achieved. Under these conditions, hydrogen peroxide molecules are delivered to target surfaces where they bond and replicate by the simple process of micro-condensation.

Gas concentration profile studies should be studied as part of cycle development and such profiles, compared cycle to cycle, indicate repeatability of the decontamination process and that the process is under control and not impacted further by variables. The cycle parameters of peroxide injection rates and phase times are developed initially to ascertain the required gas concentration profile. The optimum is a rapid rise to plateau conditions followed by near plateau conditions (after transitions peaks as injection rates may change).

Having studied gas-vapour delivery and concentration profiles, the next iterative study is gas distribution by use of chemical indicators (CIs). By placing colour change or 'water bag' chemical indicators around different challenging locations, the understanding of the rate and local concentration development can be studied.

Colour change indicators should be compared as a first to last with change colour indicating different rates of vapour concentration development (manufacturers can provide a time span expectation but for optimum cycles below 5 minutes in the first to last colour change is expected). Where CIs and resulting colour change cannot be seen, the typical approach is to place a small (2ml) polybag of water in a challenge location. The thin polythene layer of the bag permits hydrogen peroxide to permeate and pass into the water, which has an affinity for hydrogen peroxide, and will hold and retain the sample. After the cycle, a colour change dip-stick indicator can be placed in the water-peroxide sample when different colour gradients indicate the effectiveness of gas distribution.

Once cycle parameters are developed that yield saturated vapour conditions and demonstrate acceptable gas distribution, the efficacy challenge studies can be completed with biological indicators.

For hydrogen peroxide vapour, the biological indicator (BI)[18] of choice (highly resistant and more resistant than naturally found flora) is *Geobacillus stearothermophilus.* This BI is spore-inoculated and dried on a stainless steel coupon and enclosed in a Tyvek® (filter paper) primary pack. Typically, inoculations are around 2 million spores so that even with 50% recovery in enumeration checks, the resulting inactivation (kill) can prove 6-log efficacies (1 million to 1 spore in six log steps and full inactivation with a little overkill).

It is recommended that pre-study system D-value tests are completed on BIs to control potential poor quality BIs. Such poor quality is a result of poor manufacture and uncontrolled contamination leading to significant resistance variability, hence a poor indicator of cycle efficacy and control repeatability.

Following a review of BI location placement, selected for critical and worst case locations, BI challenge studies can then be completed.

Initially, an expected overkill – over time cycle is used to confirm full 100% BI inactivation together with a rationale to manage any potential rogue BIs[19]. Rogues are highly resistant and out of trend (in a batch lot) BIs and expected in low numbers, e.g. 1 in 300.

Following the full inactivation BI studies, further studies should reduce cycle parameters (injection rates or times) to transition from full kill into the fractional field where BIs are partly inactivated and partly survive – showing turbid growth in a suitable growth media broth tube, such as tryptone soya broth (TSB).

Once the transition kill to no kill phase is characterised, judgements can be made on setting additional overkill to the full kill cycle for cycle security. With the final kill, parameter developed studies can then advance to aeration studies as the time to remove residuals (micro-condensate and gaseous vapour) down to the target end point, which is typically set to one part per million, being the operator exposure level (OEL). Lower target concentrations may be set if the product to be processed is a biological and impacted by very low concentrations of hydrogen peroxide as an oxidising agent.

Fluorescence detection chemistry using co-enzyme based horseradish peroxidase methods can now be used to assess residuals down to parts per billion levels in solutions.

Test solutions may include water to simulate a biological product exposure and take up of residual gaseous concentrations in cycles or at typical aeration end point targets that may impact the product via the oxidation process.

13.6 Process optimisation and improved efficiency with consideration to reduced process down times

Process optimisation includes automation to limit or eliminate manual process interventions and assure continuous in-process transitions. One other, and major, consideration is down time or process turnaround time. Time spent on set up, changing parts, disinfection processes and aseptic assembly is all non-productive time and any process should include a review on how to optimise down time.

Aseptic assembly, associated monitoring, should be reduced to a minimum as such processes add risk and time in set up. Decontamination processes should be efficient and optimised. In addition, it may be possible to overlap respective sterilisation and disinfection processes.

For example if a gaseous vapour phase decontamination process is used, the ‘Kill’ part of the process cannot be completed as the same time as a Clean and Sterilisation-in-place (CIP-SIP) process because associated heat will impact the gaseous process. However, in the aeration phase where gaseous residuals are removed (the longest part of the cycle) then CIP-SIP processes can be started, hence overlapping cycles and saving overall time.

13.7 Development of monitoring technologies and interaction with biological decontamination processes

Microbial (viable) and total particulate (viable and non-viable) monitoring technologies are developing to provide more real time information and data processing for trend analysis.

Continuous particle monitoring systems[20], now required for critical processes in Grade A/ISO 5 environments, can be characterised for a given process so trend deviations can be detected, reported and alarmed.

Continuous particle sensors (laser light scattering technology) are sensitive devices and can be damaged or their optics coated by disinfection agents or residuals. It is recommended to protect these sensitive instruments during any decontamination or cleaning process. Consideration has to be given to the method of protection of the sensors from the decontamination agent.

A simple cap on an iso-kinetic cone (a sample cone used to provide balanced velocity of air-path in sampling) will occlude surfaces from any decontamination or cleaning process.

By design, a particle monitoring sensor by-pass line with divert valve may be used to gas the sample cone and first section of the sample line in-place while by-passing the sensor. Such systems are typically automatic and by-pass is at selection of the gaseous decontamination process and switch back to on-line particle counting after the decontamination cycle is complete.

Active air samplers typically take a $1m^3$ sample volume with adequate sample capture velocities as opposed to 'trickle feed' air samplers that may not have enough impact velocity (known as a D50[21] rate) to capture micro-organisms on a growth culture media plate. The air sample path should also be decontaminated and if a remote air sampler (outside the barrier) is used, there may be dead legs where gaseous vapour cannot reach. One solution is to use an active air sampler with an integrated impeller so only a cable to a drive-power unit passes through any isolator/RABS barrier wall.

13.7.2 Single use product bag and integrated delivery line systems

With the development of new biological and toxic products, typically of a smaller batch size than conventional chemical entities, there may be an advantage not to use conventional stainless steel process vessels and delivery lines and instead use pre-sterilised single use disposable bag-line systems. The saving here is elimination of cleaning and sterilisation validation processes associated with stainless steel vessels.

By use of disposable polymer type bag systems, consideration has to be given to extractables and leachables that may contaminate the product. Manufacturers will provide information but further product compatibility and stability studies may be required. Another advantage of disposable bag-line technology is the pre-sterilised assembly of the product bag, delivery lines and associated product filters so that no further aseptic assembly is required in-place during process set up.

13.8 The development of pre-sterilised disposable technologies

More and more pre-sterilised sterile consumables are now used in aseptic processing to improve process efficiencies and reduce requirements for sterilisation facilities on site. The challenge with such consumables and disposables is that the sterile packaging, often a Tyvek bio-barrier and plastic outer pack, are difficult to surface decontaminate during in-process transfers between different grades of room or barrier classification.

Spray and wipe disinfection of disposable packs is challenged because of the Tyvek type paper filter material; instead, gaseous vapour phase processes are now considered. Gassed material transfers need special chambers with interlocked and sealed doors together with necessary control communication with an associated decontamination agent vapour generator.

13.9 Process and validation efficiency improvements together with considerations of energy saving

The pharmaceutical and bio-pharmaceutical industries have initiatives to improve validation efficiency by integrating more process equipment at vendor and process equipment manufacturing factories before using more reduced and targeted function and performance validation studies on-site in the final location. A risk-based approach to validation is part of Quality Risk Management (QRM) with acceptance criteria defined by risk assessment if not a defined parameter requirement from regulatory, standards or guidance documents.

Factory Acceptance Tests (FAT) have become an important step in reducing equipment integration risk and eliminating the need to complete the same tests over and over again during different phases of the qualification process. There is significant more risk if process equipment is assembled together (integrated) at the user site for the first time. Validation efficiency starts with a clear User Requirement Specification (URS) with clear specification, definitions, and performance expectations together with GMP compliance requirements.

A critical fundamental of Quality by Design (QbD) is process knowledge. Such knowledge is important in risk assessments, setting of acceptance criteria for studies and associated control and alarm (permissible deviation) set points. In some cases the interaction between control or alarm points will need experimental study to verify or set a 'Design space' in which safe and effective operation is possible. A Design of Experiment (DoE) process may be required to structure studies in establishing the design space.

The traditional approach of three repeat validation studies is changing to more continuous validation studies with constant trend review of the state of control using more and more in-process monitoring and control. Parametric Analytical Technology (PAT) initiatives focus on more in-process control and monitoring and less reliance on end point testing.

13.9.2 Considerations in energy saving in bio-contamination control

Bio-contamination control can consume significant amounts of energy, being inefficient, poor for the environment and costly.

Operational Cleanrooms that use high qualities of conditioned air (heated and humidity controlled) may have opportunities in energy saving through set-back in flow conditions during silent hours or between product campaigns. Shut down and restart of HVAC may be considered if the product or process is only completed on a seasonal bases, e.g. production of vaccines.

Validation studies will be required to confirm recovery to the control state after set back, but without occupancy in silent hours or shut down periods such recovery has proven possible.

There are also energy saving in design as over-rated HEPA filters (with high pressure drop) or over-specified fan ventilators can waste significant energy. In system design, the increased use of recirculation rather than significant fresh air exchanges in ventilated and conditioned air can also make savings.

New standards and guidance are developing in the area of energy saving and such initiatives should be considered in the initial User Requirement Specification (URS) stage or as part of a continuing improvement process.

13.10 Summary: The future of aseptic processing

Aseptic processing is increasing with the development of new biological profile products that cannot be terminally sterilised due to potential or real product damage/modification effects. People-to-process separations are the first critical steps in reducing risk of potential bio-contamination of aseptically prepared medicinal products. In addition, process knowledge provides clearer understanding of associated risks so they can be defined, controlled or managed. What is also required is less reliance on operator tasks or interventions by use of automation and integrated systems in order to reduce the risk from human error.

It can be seen that bio-contamination control assurance has moved from reliance on monitoring to confirm compliance to meeting control state, to designing-in quality with processes that have a high degree of process control and in-process monitoring. Monitoring technologies are developing to provide more real time feedback with trend analysis and reporting.

There is far less emphasis on acceptance by end point testing, such as sterility testing that is known to be scientifically and statistically challenged. Instead, continuous process validation and trend monitoring processes are used. As bio-contamination risks are present at every step of aseptic process, more emphasis on improved decontamination processes is required at in-process transfer steps where materials or products move between different sterile or aseptic zones or equipment.

13.11 References

1 Advanced Aseptic Processing. Presented by Rick Friedman, FDA, at International Society of Pharmaceutical Engineers (ISPE), Washington Barrier Conference, Washington DC, USA. June 2005.

2 ICH Q10: Pharmaceutical quality system. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH, Geneva (June 2008); see also [14], Part III.

3 ICH Q9: Quality risk management. Idem, ibid. (January 2006). See also [14], Part III.

4 European Pharmacopoeia (Eu Ph) 3rd edition.

5 United States Pharmacopeia (USP): 2005.

6 ICH Q10: Pharmaceutical quality system. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH, Geneva (June 2008); see also [14], Part III.

7 ICH Q9: Quality risk management. *Idem, ibid.*(January 2006); see also [14], Part III.

8 ISO 14698-1: Under rewrite in 2012. Cleanrooms and associated controlled environments – Biocontamination control – Part 1: General principles and methods. *Idem, ibid*. (September 2003).

9 ISO 14698-2: Cleanrooms and associated controlled environments – Biocontamination control – Part 2: Evaluation and interpretation of biocontamination data. *Idem, ibid*. (September 2003).

10 Eudralex. The Rules Governing Medicinal Products in the European Community, Annex 1, published by the European Commission, Brussels: Belgium, 2009.

11 FDA. "Guidance for Industry. Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice," (FDA, Rockville, MD, USA, August 2004).

12 Pharmaceutical and Healthcare Sciences Society – PHSS Technical Monograph 15: Restricted Access Barrier Systems (RABS) for Aseptic processing – Published 18 November 2011. *Pharmaceutical and Healthcare Sciences Society,* Swindon, UK.

13 PHSS Technical Monograph 14: Risk Management of Contamination (RMC) During Manufacturing Operations in Cleanrooms. *Pharmaceutical and Healthcare Sciences Society*, Swindon, UK.

14 PHSS Technical Monograph 2: Bio-contamination classification and monitoring in controlled areas. *Pharmaceutical and Healthcare Sciences Society*, Swindon, UK.

15 Watling D *et al*. Theoretical analysis of the condensation of hydrogen peroxide gas and water vapour as used in surface decontamination. *PDA J Pharm Sci* 2002; **56**(6):291-9. (See also: Watling D. Theory and practice of hydrogen peroxide vapour. Online URL: www.pharmaceutical-int.com/categories/biodecontamination-using hydrogen peroxide vapour/theory-and-practice-of-hydrogen-peroxide-vapour.asp (status 18.02.2008):2002).

16 Kokuro M, Inoue T and Akers J. Resistance of common environmental spores of the genus bacillus to hydrogen peroxide vapour. *PDA J Pharm Sci* 1998; **52**: 228-231.

17 Unger-Bimczoz B, Kottke V, Hertel C and Rauschnabel J. The influence of humidity, hydrogen peroxide concentration and condensation on the inactivation of *Geobacillus stearothermophilus* spores with hydrogen peroxide vapor. *J Pharm Innov* 2008;**3**:123-133.

18 Biological indicators for sporicidal gassing processes. Parenteral Drug Association Technical Report TR51, PDA: Bethesda, MD, USA.

19 Drinkwater J, Chewins J and Steele G. Biological indicators don't lie, but in sporicidal gassing cycles do they sometimes confuse the truth? *European Journal of Parenteral and Pharmaceutical Sciences*: 2009; **14**(1):5-11.

20 PHSS Technical Monograph 16: Best practice on particle monitoring in pharmaceutical facilities. *Pharmaceutical and Healthcare Sciences Society*, Swindon, UK.

21 Ljungqvist B and Reinmüller B. Monitoring of air in clean environments – a comparative study with instantaneous microbial detection. *European Journal of Parenteral and Pharmaceutical Sciences* 2011; **16**(2):49-53.

Aseptic process simulations/media fills

MARCO BUDINI, CONSULTANT, PHARMACEUTICAL TECHNOLOGY & COMPLIANCE, ITALY

14.1 Introduction

The aseptic process simulation (also known as media fill when it includes the filling step) is a tool for assessing the capability of an aseptic process to produce sterile products. The principle is to replace the actual product with a microbial growth medium and to simulate all the process steps, starting from the point where the product is rendered sterile up to the closure of the final container. After a suitable incubation of the final containers, it is possible to visually detect any product contamination that occurred during the process.

The use of the process simulation in the pharmaceutical industry started in the early 1980s and soon became a regulatory expectation/requirement, both for FDA (*Guideline on Sterile Drug Products Produced by Aseptic Processing, 1987*)[1] and for the EU GMP (*Good Manufacturing Practice for Medicinal Products, 1992*)[2].

Presently, the requirements for performing aseptic process simulations are described in the 2004 FDA guidance on *Sterile Drug Products Produced by Aseptic Processing (Aseptic Processing Guidance)*[3] and in the EU GMP Annex 1[4]. Both regulations are now harmonised in terms of number of units to be filled and related acceptance criteria. Very similar requirements are also reported in other regulations/guidance documents (i.e. PIC/S).

The main requirements for performing aseptic process simulations have become more and more demanding during the last 20 years, but the technologies used for aseptic compounding and filling have improved at a faster pace. Since personnel are unanimously recognised as the main source of contamination, the increased use of isolators, restricted access barrier systems (RABS), cleaning in place/sterilised in place (CIP/SIP) systems and the recent introduction of “intrinsically safe” aseptic connectors combined with single use technologies (such as bags and filter assemblies) have reduced drastically the possibility of accidental contamination during aseptic operations. Consequently, the relevance of the aseptic process simulation/media fill might be less valuable today compared to the period it was firstly introduced[5]. This is indirectly confirmed by a 2003 Product Quality Research Institute (PQRI) survey[6], where it is reported that 91% of the media fills resulted in no contamination. If repeated today, the survey could lead to even higher results.

However, even if the process simulation is now less powerful in discriminating an excellently designed aseptic process from a mediocre one, it remains the most useful tool currently available for assessing the aseptic process capability, since the other two principal types of assessment: sterility testing and environmental monitoring, have even greater weaknesses in terms of determining the probability of microbial contamination.

- Sterility testing, performed according to compendial requirements, has well known statistical and analytical limitations and it is able to detect only gross contamination.
- Environmental monitoring, even if performed using an intensive sampling plan, both in terms of number of sampling point and sampling duration, can detect only a very limited fraction of the microbial contamination present in the environment where aseptic

operations are performed. Moreover, correlating the environmental monitoring results with the probability of contamination of the product is arbitrary.

The aseptic process simulation, even if it is only a point in time demonstration of the capability of the process to produce sterile products, is still very useful as part of the initial validation of new or modified facilities/processes and as a routine qualification and monitoring tool thereafter. It is not rare in cases of new "state of the art" aseptic filling lines (equipped with isolator, CIP/SIP) to fail the initial media fills due to hidden equipment flaws not identified during qualification. Moreover, the aseptic process simulation is still a fundamental regulatory requirement and it is always included in the list of main topics to be reviewed during inspections.

Considering that the main principles of aseptic process simulation are now well described in the above mentioned regulations and in the recently revised PDA Technical Report No 22 "Process Simulation for Aseptically Filled Products" (2011)[7], the main purpose of this chapter is not to repeat well known concepts, but to comment on some aspects of this quite complex activity taking advantage of the author's experience in sterile products manufacturing and control. Please note that some considerations made in this chapter are based on the author's personal experience and opinions and might not reflect regulatory expectations.

14.2 Documentation

A process simulation study is a complex and challenging activity requiring the definition of several parameters and an accurate planning of all the steps. Therefore a clear and detailed documentation of both the activities to be performed and the results obtained is mandatory for a successful outcome of the study and its validity from a GMP point of view.

Since process simulation is often considered a validation activity, the overall approach to be used is generally described in the Site Validation Master Plan (or a policy) and in one or more SOPs (**Figure 1**).

Once the general principles have been established, the detailed operations and parameters necessary to design and execute a simulation study are described in specific protocols and batch records for each process to be validated (and monitored thereafter).

The use of this hierarchical approach for process simulation documentation, with increasing level of details, prevents the risk of having different approaches in multiple departments of the same facility, an event not so infrequent in the past, and difficult to defend during GMP inspections.

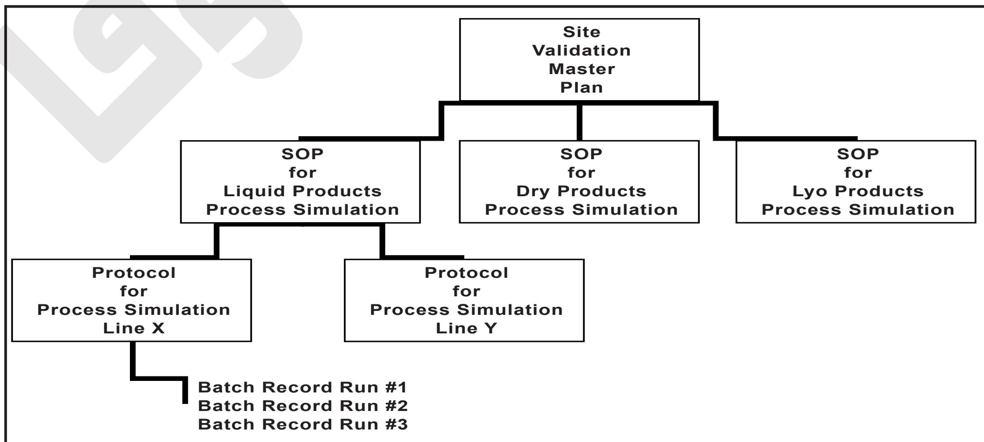


Figure 1: *Example of SOPs for liquid and dry products.*

Some companies don't use protocols for documenting the process simulations/media fills results, but only the batch records of the executed runs. Even if a batch record may contain all the information necessary for performing a process simulation study, the use of a protocol with one (or more, in case of multiple runs) attached executed batch record(s) allows a higher flexibility in assembling the validation package and increases the overall clarity of the documentation, considering that the batch record format is sometimes not easily readable by persons not familiar with the production process.

The process simulation protocol can also be used for documenting the assessment of the contamination risk associated with the process to be validated, if not performed on a separate document. The contamination risk assessment is the basis for supporting the design of the process simulation study to be used for that specific process (see also section 3 of this chapter).

A typical (but not exhaustive) list of sections to be included in a process simulation protocol is reported below:

- Detailed description of the commercial process to be simulated, including room(s) identified, equipment identified, product path, connections, etc. A drawing and/or a flow diagram is generally included
- Reason for the qualification (initial qualification, routine re-qualification, or for a specific cause such as a significant modification to a filling line)
- Risk assessment (if not performed in a separate document) to identify and evaluate the process variables that can adversely affect the sterility assurance of the product and thus to justify the design of the process simulation study to be performed
- Media selected and process used to sterilise it
- Size and number of runs
- Duration of runs
- Line stoppages
- Personnel organisation during simulation (number of shifts, shift change overs, maximum number of people to be present simultaneously, etc.)
- Line speed
- Container size
- Closure type
- Line configuration selected for the process simulation (product path, number of aseptic connections, etc.)
- Fill volume
- List, description and frequency of interventions to be performed/simulated and related line clearance prescriptions (if required)
- Other worst case scenarios to be included in the study (if any)
- Environmental and personnel monitoring plan to be performed
- Cross reference to the batch record to be used (to be attached to the report upon completion of the simulation)
- Instructions for the inspection of the filled units
- Instructions for incubation (temperature, duration)
- Instructions for the inspection of the incubated units (or cross reference to the related SOP)
- Accountability requirements after each relevant step
- Growth Promotion Testing requirements
- Acceptance criteria
- Cross reference to personnel training
- Instructions for media destruction

At the end of the study, a report is written summarising the results, discussing deviations from the protocol/batch record requirements (if any) and assessing if the study met or not the acceptance criteria. Follow-up actions, if any, should also be included.

More details on the above points are described in the following sections of this chapter.

14.3 Risk assessment

An evaluation of the risks of contamination associated with aseptic operations has often been performed in the past years by the technicians involved in designing and/or improving a specific aseptic process, even if not in a formal and documented way. Minimising these risks has been the driver that allowed the aseptic processing technology to evolve so rapidly in the last two decades.

Nowadays, a more formal and structured risk assessment is expected. The advantages of performing this exercise are multiple, both for new and for existing processes, and some of them go beyond the scope of process simulation. A structured risk assessment allows one to:

- Identify and evaluate the steps and interventions that can potentially compromise the sterility of the product
- Define possible corrective actions to mitigate the risks identified above
- Determine and justify the "worst case" scenario (line speed, container size, duration of fill, number and type of interventions, etc.) to be used during process simulation
- Identify the critical process parameters and thus the most suitable control/monitoring plan and related acceptance criteria
- Improve the overall process understanding and thus the decision making process in case of deviations

Several risk assessment methods specific for aseptic processes have been recently developed[8,9,10,11,12]. The author found the simplified Akers-Agalloco[9] method very straightforward and particularly suitable for the identification and ranking of the steps and parameters to be included in the "worst case" scenario to be used for a specific aseptic process simulation study (See section 14).

14.4 Media selection

The growth medium to be used for aseptic process simulation should combine several attributes. It should:

- Support the growth of a wide range of micro-organisms, with particular attention to those that are most likely present in the proximity of the process to be assessed (as resulting from personnel, such as Micrococci and Staphylococci, and other micro-organisms representive of the microflora detected from the environmental monitoring programme)
- Be suitable to mimic as much as possible the actual process (example: easily filterable for sterile filtration and liquid fill, easily fillable for powder fill, etc.)
- Be clear after dissolution to allow an easy and reliable detection of any potential contamination, after incubation
- Be easily cleanable in order to not expose the equipment and the surrounding environment to risks of adventitious contamination

For the majority of aseptic process simulations, Tryptic Soy Broth (TSB) is considered the best compromise. TSB (also called Soybean Digest Casein Medium, SCDM) is a relatively rich media (in terms of variety and concentration of nutrients) that comes from enzymatic digestion of proteins (from animal or plant origin). TSB shows a good capacity of supporting the growth of aerobic micro-organisms commonly associated with human-borne contamination. Good recoveries of yeast and moulds are also obtainable in TSB.

Furthermore, the medium can be used also for simulating processes normally performed in anaerobic conditions, if during simulation it is possible to replace the inert gas with compressed air, without altering the other relevant parameters. A typical example is represented by lyophilised products, where inert gases are often used for breaking the vacuum in the chamber at the end of the lyophilisation. Replacing the inert gas with compressed air for process simulation is generally not problematic. Only when the simulation needs to be performed in strict anaerobic conditions, the use of specific media as the Fluid Thioglycollate Medium (FTM) is required. This might be necessary in case of repeated isolations of anaerobic organisms in environmental monitoring (EM) samples and/or sterility test failures (example: when the contamination is found only in the FTM medium). In these cases, performing the aseptic process simulation in the actual anaerobic conditions (thus without replacing the inert gas with compressed air and using a media fill volume close to the maximum nominal volume) should facilitate the recovery of these organisms.

In most cases TSB is used in solution, normally at the concentration recommended by the supplier. Only when it is necessary to simulate operations requiring the use of sterile dry products (some aseptic compounding, dry products filling) it can be used in the powder form as a replacement of the actual product and reconstituted afterwards before incubation (see more details in section 11). Other special cases are represented by sterile cream/ointment products in tubes, which could require the addition of a thickener to increase the viscosity of the medium.

Obviously, TSB must be sterilised before it is used for simulating a sterile product.

When the media is used in the liquid form, it is usually sterilised by heat or by filtration. Considering the relatively large amount of media required by the current regulations, sterilisation by heat in autoclave might be not practical. Moreover, the sterilisation cycle to be used should strictly observe the recommendations of the media supplier in terms of heat exposure to avoid an impact on the growth promotion properties. Designing a suitable autoclave cycle meeting the above requirements might not be an easy task when a large amount of liquid is involved.

Sterilisation by filtration is thus the preferred method. Where possible, it can be performed using the same filtration apparatus (compounding vessel, filtration system, collecting vessel and/or in-line filling) employed for the product to be simulated, thus becoming part of the entire process simulation assessment, unless the actual process requires assessment of these steps separately (example: when the formulation/sterile filtration step and the filling step are performed in different facilities). The nature of the media could require a type of sterilising filter (or even a pre-filter) different from that used with the actual product, but this is not a problem, since the scope of the aseptic process simulation does not include the sterilising filter validation, which should be assessed separately, with different procedures. For the purpose of aseptic simulation, it is crucial that the media follow exactly the same process path of the actual product starting from the point where they are rendered sterile. Thus it is important that the sterilising filter holder has the same characteristics of the actual one and it is assembled, sterilised and tested in the same way; independently of the type of membrane installed inside.

There are some concerns related to TSB media sterilised by filtration regarding the presence of *Mycoplasma* contamination, which cannot be eliminated by 0.22µm filtration and therefore could represent a potential harm for the challenged aseptic line. As well as for Bovine Spongiform Encephalopathy/Transmissible Spongiform Encephalophty (BSE/TSE) where the use of certified, or vegetable sourced media (as Vegetable Peptone Broth) is required, the use of TSB media certified for absence of *Mycoplasma* and/or the implementation of pre-treatment steps (i.e. irradiation of the dehydrated media) is advisable. These steps also have the benefit of inactivating the inherent microbial contamination of the TSB powder, which can represent an issue for the compounding area environment and when the filtration is not performed immediately after reconstitution of the media in WFI. Some

companies perform a bioburden test on the prepared bulk media prior to filtration and/or reinforced EM sampling in the formulation area to monitor this potential risk. Based on all these considerations, the use of TSB especially designed for media fill trials with all the above mentioned properties (rather than "conventional" laboratory grade) is now increasing among pharmaceutical companies.

When the medium is used in the powder form it is generally sterilised by radiation using a validated process. If possible, it must be packed in the same container as the actual product to be simulated in order to mimic exactly the same manipulations during the process.

Whichever is the process used to sterilise the media, suitable controls should be executed to assure that, before use, the medium is sterile and growth promoting. This is not always possible when compounding, sterile filtration and filling are executed in-line. In these cases, representative samples of the sterile filtered media are collected and analysed concurrently with the execution and incubation of the media filled units.

14.5 Frequency and number of runs

Since the publication of the 1987 FDA Guidance on aseptic processing, "at least three consecutive separate successful runs" are necessary for the qualification of a new process/filling line. This requirement is still present in the 2004 FDA Guidance and in the 2008 EU GMP Annex 1. It is applicable also for revalidation following major changes to the equipment/process, or whenever there are doubts about the ability of the aseptic process to exclude contamination (extended shutdowns, anomalous trends in environmental monitoring, sterility test failures, etc.).

For routine re-qualification, one run per process/line twice a year is recommended. Where there is a combination of different vial sizes and product presentations, a risk-based approach is often undertaken in order to select representative or 'worst case' combinations, as discussed below.

14.6 Size of runs

As already stated in the introduction, the recent progresses in the aseptic filling technology reduced considerably the risks of microbial contamination. This implies that the size of the media simulation, to be meaningful, should be big enough for detecting very low contamination rates as expected today (if present). Ideally, the commercial production process in terms of batch size and total duration of aseptic operations should be used. In practice, considering that production batch sizes can easily reach values of several hundreds of thousand units, it is permissable to use other simulation models (see also section 7), provided that they are addressing all the potential risks of contamination occurring during commercial production.

The 2004 FDA Guidance states that "a generally acceptable starting point for run size is in the range of 5,000 to 10,000 units". In practice, for commercial products with lot sizes usually much bigger than 10,000 units it could be difficult to justify that a simulation run smaller that 10,000 units can be considered representative of the actual process. Moreover, the run size must be big enough for allowing the simulation of all the manipulations and interventions occurring during the commercial process.

In conclusion, there is not a clear regulatory requirement for the run size to be used for a specific process simulation/media fill. Some companies use a fixed percentage of the commercial batch size (for example: 20%) while others prefer to fill the complete batch. As a general reference, it could be interesting to note that the already mentioned 2003 PQRI survey[6] reported an average media fill run size of 12,981 units. For commercial batch sizes > 10,000 units, the author's suggestion would be to use the following empiric rule: 10,000 + 0.1 x (commercial batch size). This approach has the advantage of progressively reducing the ratio between the media fill size and the commercial batch size while the latter increases, as reported in the examples listed in **Table 1**:

Table 1: The factor 0.1 can be replaced by other values, according to the outcome of the risk assessment of the process and the complexity of the operations to be simulated.

Commercial batch size (units)	Calculated media fill size (units)	Ratio (%)
20,000	12,000	0.6 (60%)
50,000	15,000	0.3 (30%)
100,000	20,000	0.2 (20%)
200,000	30,000	0.15 (15%)

14.7 Duration of runs

In this case the rules are clearer since regulatory (at least FDA) expectation is for a duration that encompasses the longest filling process performed on the line. This is mainly true for conventional filling lines more exposed to contamination originated by personnel, where the risk is usually higher at the end of filling, due to potential environmental bioburden build-up during operation and operator's fatigue.

Some companies, during process simulation, add an "extra time" to the longest process time used for commercial products as a "worst case". This allows also the generation of data for supporting any deviation implying extended process time for commercial batches (example: in case of equipment breakdown).

From the practical point of view, unless the decision is to use for the simulation the entire commercial batch size, the problem is how to combine a duration equal or longer than the longest commercial process with a media fill run size usually much smaller than the product batch size. Assuming that the filling line is generally not designed to run much slower than its "standard" speed (in any case it would be not representative of the actual filling conditions – see also the following section), there are several options for combining a long duration with a small batch size. Example:

- Fill the containers alternatively with sterile media and WFI (or just run empty units in place of those filled with WFI). Media fill should happen at the beginning of filling operations (immediately after the line set-up), during/after manipulations/interventions, at the end of filling.
- Perform the process simulation run at the end of a production fill operation, with a new set-up of the line, but without performing any room/line cleaning/sanitisation operation in between and with the same personnel shift (worst case). This is an effective way to measure operator fatigue.
- Perform the process simulation running the line intermittently (example 10 minutes every hour up to the total duration of the commercial process) taking care to include line set-up, all the planned interventions, all the shift changes, etc. During the idle time the personnel remain in the filling suite.

This last option is simpler and it is used by some major companies.

14.8 Line speed

The FDA Aseptic Process Guidance is quite clear regarding line speed. The aseptic process simulation plan should adequately address the range of line speeds employed during production, but evaluate a single line speed on each run, with appropriate justification. In theory (and often also in practice) the slowest line speed represents the worst case, since the

exposure of the opened containers to the environment is the longest, thus increasing the risk of contamination. In some cases, mainly when small (thus potentially instable) containers are filled on high speed lines, the highest speed can be the worst case, because it can generate more line stoppages, due to containers breakages/blockages, and thus more operator's interventions. For these reasons, when the line to be validated is designed to fill more than one container size, it is worthwhile to evaluate and justify the most appropriate line speed for media fill together with the container size to be used. For example, one option could be to fill the biggest container size at the slowest available speed and the smallest container at the highest speed. During the fill it is also important that representative line stoppages take place in order to represent filling line breakdowns.

14.9 Container size

Regarding the container size itself, the most critical factor is the mouth opening. It is evident that the bigger the opening the higher the risk of collecting microbial contamination from the environment.

Since container size and line speed are linked together, in the case of initial validation/re-validation of a line designed to fill multiple container sizes, a possible approach, after a thorough evaluation of all the process parameters involved, could be:

- 3 runs with the biggest container size/mouth opening at slowest speed
- 1 run for each intermediate container size at slowest speed
- 3 runs with the smaller container size at the highest speed

However, based on the author's experience, some agencies could not accept bracketing container sizes during initial validation, thus it is recommended to check the approach to be followed, before designing the validation study.

For routine revalidation, a rotation of the above combinations can be applied during the bi-annual simulations, taking care of running the identified worst case (if any) at least once a year. If a clear worst case combination is not identified, the most frequently used container size should be tested at least once a year.

14.10 Closure type

This variable is rarely considered when designing a media fill study. With the same container size and mouth opening, the performance of the filling process and thus the risk of contamination can be influenced by the characteristics of the closure. In fact, the composition/physical properties of the elastomer used, combined with the treatment required by the process (steam sterilisation, gamma radiation, vacuum packaging, etc.), can have a negative impact on the machinability of the closures, generating more line stoppages/interventions. In case of more than one closure type being used with the same container size/mouth opening, the worst case closure (if any) should be used for the media fill runs.

14.11 Processes/line configuration and set-up

It is quite common that more than one product is processed on the same line and in some cases this implies different set-up configurations. For example, a particular product could require the addition of a recirculation loop for adequately feeding the dosing pumps, or to perform more aseptic connections compared to another product. In these cases, a thorough evaluation of all the possible combinations must be performed.

Applying the already mentioned "Simplified Method" it is possible to calculate the individual risk associated to each single step and thus evaluate if there is a combination of them representing the worst case configuration to be used during the process simulation/media fill.

This is a very basic example of the application of the method to the set-up step.
The same filling line is used for two products:

- Product A is a sterile solution contained in a tank; it requires a filling line set-up lasting 60 minutes and includes an additional in-line sterilising filter placed just before the filling machine
- Product B is a sterile suspension contained in a tank; it requires a filling line set-up lasting 50 minutes, but it cannot (obviously) be filtered in-line

According to the “Simplified Method” the aseptic set-up risk contribution is obtained by multiplying four factors: set-up time x complexity factor x product delivery factor x novelty factor.

Using the factors reported in Table II of the “Simplified Method” and assuming that both the complexity factor and the novelty factor are equal to one for the two examples above, we obtain:

- Product A: 60 x 0.75 = 45
- Product B: 50 x 1.0 = 50

This means that the product B gives a higher set-up risk contribution. For the process simulation it would be possible to combine the two processes using the set-up of product A (60 minutes) and removing the in-line filter as for product B (factor 1.0), obtaining an overall set-up risk of 60 (worst case), thus encompassing both processes.

In case it is not possible to combine the worst case steps for creating a single configuration encompassing all the individual processes, each configuration should be assessed independently.

In some cases it is not possible to mimic exactly the commercial process configuration, due to intrinsic limitations of the media fill versus the actual process. Two typical examples are:

- Sterile powders
- Lyophilized products

Sterile powders (Figure 2)*:* when a sterile powder filling has to be simulated, the actual product is replaced with a sterile placebo powder (example: irradiated lactose or mannitol). After dosing the placebo in the vial, it is necessary to add the sterile liquid medium to allow the powder dissolution before the incubation starts. Alternatively the sterile dehydrated medium itself can be used as a replacement of the product. In this case, WFI is added to reconstitute the medium before the incubation step.

In both cases it is necessary to drastically modify the commercial process to include a liquid dosing device and related piping, in order to perform the liquid addition immediately after the powder dosing and before closing the filled unit. The modern powder filling machines are designed to accommodate a liquid dosing station when the process simulation needs to be performed, while older machines need to be retrofitted with peristaltic pumps or other extemporaneous solutions, with additional risks of malfunctioning and contamination, which are not present in the commercial process.

Lyophilized products: in this case the modifications are mainly related to the lyophilisation step parameters, in order to minimise the risks of compromising the growing properties of the medium, while simulating all the critical steps that can be related to contamination risks. Among several possible options for simulating the freeze-drying step, the author’s preference is for exposing the media filled units to the entire duration of the commercial lyophilisation cycle, but performed at room temperature and with reduced vacuum (enough to avoid boiling of the medium). In this case all the potential risks of contamination, such as duration of the cycle and vacuum (even if reduced), are maintained.

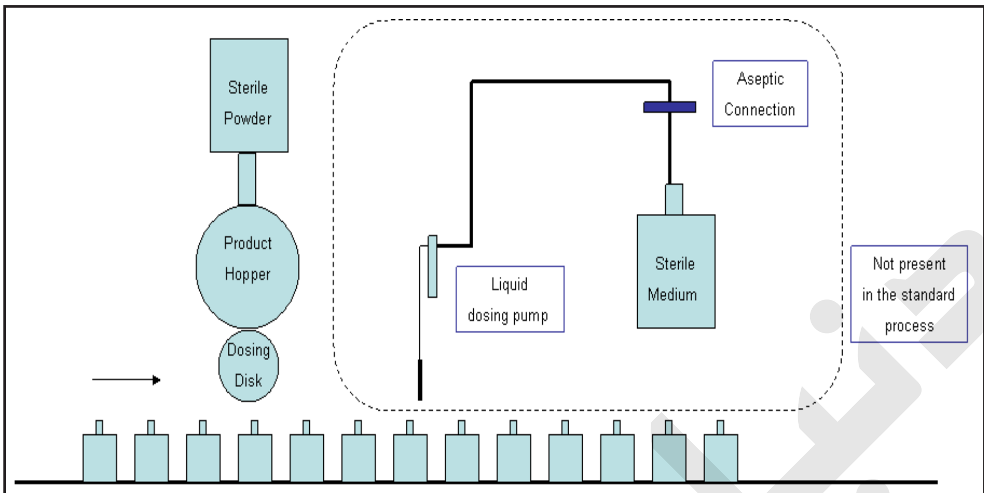


Figure 2: *Example of a schematic sterile powder filling configuration for media fills.*

14.12 Fill volume

The quantity of liquid media to be dosed in each container should be enough to contact the entire inner surface of the vial/ampoule (including the closure system) when the container is appropriately inverted and swirled before starting the incubation and to allow an easy visual detection of the microbial contamination after incubation.

Generally, a filling volume equal to 50% of the container nominal volume is considered adequate and suitable for recovering aerobic micro-organisms, but smaller volumes might also be acceptable if evidence is given that the swirling operation is sufficient to put the media in contact with the entire container-closure inner surface.

Considering the criticality of the swirling/inverting step (before incubation and at the final read), it should be described in details in the media fill batch record or in a specific SOP.

14.13 Number of persons and activities

Process simulation design should consider the maximum number of persons allowed (by site SOPs) to be simultaneously present in the area where the aseptic process takes place. For example, if the SOP allows the simultaneous presence of two production operators plus one environmental monitoring operator and one maintenance technician during aseptic operations, the same situation must be repeated during process simulation. In order to be fully representative of the actual process, the above persons should be not only present, but they should perform/simulate the same activities as in the commercial process.

For processes running on multiple personnel shifts, the above considerations should include also the situations occurring during the change of shift. Often this implies that the two production teams are simultaneously present in the filling area, in order to avoid line stoppages during shift change. In other cases the change of shift could imply an overlapping of the two teams in the gowning/de-gowning rooms. These potential worst cases must be simulated during media fill.

14.14 Interventions

The term intervention includes all the activities that imply a perturbation of the critical aseptic area (ISO Class 5 or EU Grade A). Since personnel are the greatest source of microbial

contamination, each manual intervention inside the critical aseptic areas exposes the product to a risk of contamination.

Evaluating the risk associated with each type of intervention is not easy, also because even the most trained operator cannnot perform a specified activity exactly the same way each time. For minimising this variability, it is essential that each type of intervention is clearly described in specific SOP(s) or in the batch record and that each operator involved in their execution is effectively trained. Again, the Akers-Agalloco Simplified Method represents a useful tool for the determination of intervention risks. The evaluation is based on a criticality factor (from 1 to 5) linked to the "difficulty" of the operation, multiplied by a weighing factor (from 1 to 3) associated to the proximity of the intervention to the open containers and to other critical contact parts. The score obtained by each intervention, further weighted for its number/frequency during the process, may be combined with the scores of the other interventions for obtaining the overall score of that process step. This method can be used for several scopes, as optimising the aseptic process itself (thus trying to minimise the overall score), or defining the worst case approach to be used for aseptic process simulation (in this case, by identifying the combination of interventions leading to the maximum score). The Simplified Method could also be further adjusted case by case, taking into consideration other weighing factors based, for example, on the outcome of the air flow pattern (smoke test) studies.

Independently from the associated risk, the interventions are generally divided in two main categories:

- Intrinsic to the process (or normal interventions according to FDA Guidance)
- Extrinsic to the process (or non-routine interventions according to FDA Guidance)

The first category includes all the interventions necessary for running the process as it is designed. The list of these interventions depends on the type and design of the aseptic process. The list can be quite long for older processes based on manually intensive operations, while it can approach zero for fully automatic, closed systems with Cleaning In Place/Sterilising In Place (CIP/SIP). Some examples are:

- Line set-up
- Aseptic connection(s)
- Containers/closures feeding
- Product sampling
- Lyophilizer loading/unloading
- Weight checks
- Environmental Monitoring

These routine activities are usually well standardised and need to be executed during process simulation according to the established procedure/batch record and with a frequency (according to FDA Aseptic Process Guidance) that is representative of the standard process. The meaning of "representative" in this context needs some further considerations. For example: the batch size of the process to be simulated is 100,000 units and the simulation run size is 20,000 units. The stoppers to be used are supplied in bags containing 5,000 stoppers each. The normal process requires theoretically 20 additions (in reality they can be a little more, depending on the line start-up and the wastes during filling), while the simulation run is only 4-5. Is this frequency representative? Performing 20 additions required by the normal process during the simulation could be not realistic and, in some cases, difficult to manage. An acceptable compromise could be to increment the number of the theoretical additions necessary during process simulation by a worst case factor (example + 50%). With the above

example, the number of stopper additions during process simulation would raise from 4-5 to 6-7, This should be considered enough for adequately challenging the process and can be easily obtained by adding only a portion of the stoppers contained in each bag without overloading the stopper bowl.

Due to the fact that the type and number of intrinsic interventions are inherent to the process to be simulated, their inclusion in the process simulation protocol/batch record is usually not complex.

The second category includes all the other interventions that are not strictly necessary for running the process, but that can occur due to expected or unexpected problems during operations. Some examples are:

- Removal of jammed containers/closures
- Removal of broken containers/product residues
- Replacement of a dosing device
- Adjustments to the line set-up (guides, stars, etc.)
- Breakdowns (sensors, switches, belts, etc.)

Planning the type and frequency of these activities during process simulation is more difficult, since their occurrence in normal production is not predictable and can vary from batch to batch. Some rare events, like a sensor breakdown, can happen less than once in a year. In order to assure that also rare events are represented during media fill, it is suggested that a thorough evaluation of the historical data of the process to be validated is performed. This exercise is usually executed on a yearly basis as part of the periodic performance evaluation of the main pieces of equipment. Some modern filling lines and ancillary systems are capable of maintaining detailed electronic records of all the stoppages and the related reason (where applicable). This feature facilitates the collection of the data necessary to plan the type and frequency of extrinsic interventions to be included in the process simulation. Very rare events do not need to be included in each biannual run, but can be executed/simulated less frequently. For new filling lines, where there is no historical data available, the type and frequency of these types of interventions can be extrapolated from similar processes already in place.

A list of permissible interventions should be created and retained to ensure that only interventions conducted during media fills are allowed to be performed during production runs. It is common practice to list all allowable interventions for each production fill line/process based on the media fill intervention data. A process for ensuring that newly discovered intervention(s) during production runs are properly evaluated to determine the contamination risk of the intervention should be implemented and incorporated into the media fill program.

14.15 Line clearance

When an intervention occurs during filling of a commercial product, there should be a very clear and detailed SOP defining what happens to the product, containers and closures present on the line. Depending on the type, duration and overall risk of microbial contamination related to the intervention, the SOP may, or may not require the removal of the opened containers (and closures, if applicable) present on the line. The line clearance SOP must be exactly applied during process simulation. Removing more units during process simulation would obviously be considered not acceptable. Some modern filling lines perform automatically the line clearance, removing always a pre-established number of units, when necessary. When line clearance is performed manually, there should be a system (counting the units, or marking them in some way) to assure that the right amount is removed in a reproducible way. Another method used by some companies is to have defined zones specifying the area where the units are to be removed

(example in-feed to closure station). This proves to be more beneficial that culling out the number of units since the size of the units will vary per fill line per process. The same concept should be applied to the line set-up (one of the most critical operations for microbial contamination risk). The number of units to be discarded in this phase before the beginning of the production of "good" units must be the same as during normal operations.

Some companies incubate the media filled units discarded during line clearance, evaluating them separately from the other units in the run. This is not a requirement, provided that the line clearance SOP is clearly defined and correctly applied during media simulation. Moreover, although this information could be useful from a process understanding point of view, the opportunity of segregating and incubating normally discarded units is still debated, since it represents a significant variation in respect of the routine process which media fill should simulate as closely as possible.

14.16 Environmental monitoring

The environmental monitoring SOP(s) to be used during process simulation is generally the same as for normal production.

Some companies use an extended environmental monitoring (EM) plan during process simulation but this is not adding much value to the exercise:

- If the result of the process simulation run is "pass", even in presence of EM excursions, this result cannot be utilised to justify similar excursions during routine production
- If the result of the process simulation is "fail", it is rare that the extended sampling plan can significantly increase the probability to identify the root cause. Obviously, the fact to have increased the number/frequency of the samples (and thus the number of interventions) cannot be used as a justification for the failure.

On the other hand, since taking EM samples inside the critical area is considered one of the most risky interventions, it is not allowed to reduce the EM sampling plan during process simulation.

14.17 Personnel monitoring

As far as personnel monitoring is concerned, it is quite common to use extended sampling plans during process simulation. In fact, the process simulation represents an excellent opportunity for assessing/re-assessing the ability of each operator to perform aseptic operations (including critical interventions) in a correct way.

Moreover, since personnel represent the greater source of contamination, increasing the number of sampling locations on the operator's gown during process simulation, in respect of routine production, can help in identifying the source of contamination in case of failure.

Active participation in a successful process simulation, by executing/simulating the operations described in the protocol/batch record, is a requirement for personnel operating in critical areas and it usually represents the final step of their qualification programme. After the initial qualification, active participation to at least one successful process simulation per year is required to maintain the qualification status.

14.18 Other worst case scenarios

Process simulation may be an opportunity for challenging the aseptic process with a series of other worst case scenarios, in addition to those already discussed above, with the aim of assessing these activities from the microbial contamination risk point of view.

Some examples are:

- Use sterile material (bulk product/media, containers, closures, product contact parts) at or beyond the expiration date established by related SOPs
- Perform the simulation at or beyond the maximum time window allowed from the last room sanitisation

This approach may be acceptable in principle, but it presents several drawbacks:

- Adds a lot of complexity to the already complicated process of media simulation
- Makes the planning of a simulation run a nightmare
- In case of failure, the investigation and the identification of the root cause is much more complex

Balancing the pros and cons, the author would not recommend using this approach, at least for routine re-qualification. Some of the above situations could be included as part of the initial validation of a new process/filling line, but where applicable, it is preferable to validate these conditions separately, with specifically designed studies (for example: sterile bulk holding time studies).

It is to be noted that FDA Guidance requires avoiding "best case" scenarios in terms of environmental conditions ("making the process appear cleaner than it actually is"), but this does not imply to combine all the possible worst cases, resulting in an unrealistic situation.

Generally speaking, the inclusion of a particular procedure or practice in a media fill process could give useful information about its impact on the contamination risk associated with the process, helping also in the identification and removal of unacceptable practices. Of course the opposite is not always true, so including a practice in a successful media fill does not *per se* mean that the risk associated with this practice can be accepted from a sterility assurance point of view. Again, the FDA Guidance is clear on this point: *"Media fills should not be used to justify practices that pose unnecessary contamination risks".*

14.19 Considerations regarding isolators

The FDA Aseptic Guidance states *"...a process conducted in an isolator can have a low risk of contamination because of the lack of direct human intervention and can be simulated with a lower number of units as a proportion of the overall operation".* This seems to be a little bit contradictory, since a lower potential contamination rate would require an increased number of media filled units for a meaningful assessment of the aseptic process performance. In practice, for isolators the same media fill size/commercial batch size ratio is used as for conventional filling lines. Since isolators are often used in combination with highly sophisticated and fast filling lines, the actual number of the media filled units per run can reach quite high values.

14.20 Cleaning after process simulation

The environment and the equipment involved in a process simulation must be effectively cleaned after the completion of the simulation run(s), in particular, all the product contact parts which have been in contact with the culture media. Media residues can significantly increase the risks of microbial growth inside the aseptic area if not thoroughly removed. Moreover, the medium itself represents a foreign material and the risk of its carryover in the following batch must be minimised. Therefore, it is common practice to include TSB (or other media used) among the products to be evaluated in the cleaning validation plan of the involved equipment.

14.21 Incubation of filled units

As already stated at the beginning of this chapter, an aseptic process simulation starts at the point where the product/media is rendered sterile and ends when the final container is sealed. Then the

filled units are put under controlled temperature conditions to promote microbial growth before the final visual inspection.

Before the incubation starts, it is advisable to visually inspect the filled units in order to:

- remove those units that are not perfectly sealed
- invert and swirl the units to wet the entire inner surface of the container, including the closure

Only the units presenting integrity breaches should be removed. All the other defects that are not compromising the container/closure integrity (example: particulate matter, cosmetic defects) should be incubated. In any case, all the units removed should be accurately counted, similarly to those removed as part of the line clearance (see section 15 above).

A very detailed accountability of the filled units and discarded units is required in order to get 100% reconciliation with the number of integral units to be put in incubation. This number will be checked again during the visual inspection after incubation when 100% reconciliation is expected.

It is important to keep the chronological filling sequence while collecting the filled units and during the pre-incubation inspection (example: by using progressively numbered boxes). These data are crucial during the investigation, in case of contaminations found after incubation.

The incubation should be performed in qualified thermostatic chambers able to reliably and homogeneously maintain the temperature inside the selected range (± 2.5°C from the set point). A printout of the temperature profile during the incubation is usually part of the process simulation documentation.

Incubation conditions (duration and temperature) must be suitably selected for maximising the chance of growth of the most typical organisms that could be present within the aseptic area, on personnel or in the product pre-sterile stages. Based on the normal bacterial flora usually recovered in clean rooms, international guidance documents recommend a temperature range between 20°C and 35°C and a duration not shorter than 14 days.

If two temperature ranges (typically 20-25°C and 30-35°C) are selected on the basis of the organisms normally isolated, 7 days per each range are applied and usually units are incubated first at the lower temperature range. The two temperatures approach is generally considered the most appropriate for maximising the recovery of the cleanroom microflora. Regardless of the temperature range chosen, it is the responsibility of the manufacturer to generate data in support of the selected incubation conditions.

14.22 Visual inspection after incubation

At the end of the incubation period, the filled units are 100% visually inspected by appropriately trained people for detecting any evidence of microbial growth. In addition to the final inspection, intermediate observations of incubated units may be performed, especially if preliminary results are important (example: initial qualification of a new aseptic process/filling line). Typically, an intermediate observation is performed after the first half of the 14 days incubation period when the units are moved from the lower to the upper temperature range. When such observation takes place, the time needed for the inspection (usually some hours) should not be accounted as incubation time, unless it can be demonstrated that media filled units are kept under controlled temperature conditions throughout the whole observation process.

Typically QC microbiology staff is the most appropriate group to put in charge of this activity, but also production personnel can perform this task, provided that they are appropriately trained. The availability of examples of sterile and contaminated units (real or by picture), during staff training and/or

actual visual inspection performance, is highly advisable. In any case, all doubtful units must be put under the attention of a microbiologist who can assess the presence or absence of microbial growth.

Any contaminated unit detected during post-incubation inspection must be carefully documented and the reason for contamination thoroughly investigated (see the following section 24 for more details).

At the end of the visual inspection a complete accountability of the inspected units should be performed. As already mentioned, 100% reconciliation with the number of units put in incubation is expected. Any deviation should be investigated and could potentially lead to the invalidation of the process simulation run.

Any media filled unit to be used for ancillary activities (such as growth promotion test, media suitability studies or container closure integrity studies) must be first fully incubated, observed and accounted for media fill result: once this has been completed and documented, units can be withdrawn and used/stored for validation or other purposes. Generally, it is not acceptable to take away any unit from the media fill population before completion of the incubation and inspection pathway.

Some sterile products are aseptically filled in opaque containers. Examples are some eye drops and sterile creams/ointments in plastic/metal tubes. They are subject to aseptic process simulation, but the visual inspection after incubation cannot be performed by examination of the filled containers, as described above. In these cases, the most common option is to transfer the whole content of each single unit to a transparent container and to perform the inspection as described above. For semi-transparent tubes, other options may be available (example: use of medium which changes colour in the presence of contamination), but only after an appropriate validation.

14.23 Growth promotion test (GPT)

The medium used for process simulation should demonstrate its ability to support the growth of a low number of micro-organisms as those potentially present in the surrounding environment of the aseptic operations under scrutiny.

An inoculum of <100 CFU for each filled unit is considered as adequately “low” in regulatory documents (PIC/S); however an even lower number could simulate more closely the actual contamination (potentially coming from a single bacterial cell) which can fall into a media fill unit.

Organisms selected for media simulation/media fills GPT should include representative strains from the microbial flora associated with the manufacturing environment. Usually the panel for the Sterility Test set, as detailed in United States Pharmacopeia (USP) <71>/European Pharmacopoeia (EP) 2.6.1. These indicator organisms, together with one or more isolates directly recovered in the production area, are used (as recommended by FDA). It is a sound practice to periodically review the list of organisms selected (particularly the environmental isolates), based on the identification data and trends, in order to ensure that they are still representative of the real microbial risks challenging the process.

The GPT is usually performed at the end of the incubation period, directly inoculating a representative sample of the units filled during the process simulation/media fill. This allows challenging the media after it has been exposed to the whole process and thus also to any inhibition factor potentially present in the process itself (example: leachables from the container/closure).

The inoculated units should be incubated at the same temperature(s) used for the filled units, but for a much shorter period, not longer than 3 days for bacteria and 5 days for fungi, since the inoculated organisms are expected to growth much faster than the stressed organisms that could be potentially recovered during the simulation.

If a GPT fails it means that the medium used in the process simulation was not capable of supporting microbial growth. In such cases the reason for the failure must be investigated; the simulation run must be invalidated and repeated, once the root cause of the failure has been discovered and corrected. Nevertheless, if any contamination is detected in a simulation run that failed the GPT, this cannot be any longer considered invalid and thus must be evaluated according to the acceptance criteria reported in the study protocol/SOP.

14.24 Interpretation of results – acceptance criteria

This long debated topic in the 1980s and 1990s is now clearly described and harmonised in the FDA Guidance, in the EU Annex 1 and in other requirements/recommendations (as the PIC/S PI 007-6) including the consideration that the target of each process simulation is zero contamination, independently from the number of units filled. Since these acceptance criteria are well known and easily accessible they will not be repeated here. However, regulations make no distinction between initial validation and routine re-qualification, leaving some uncertainties on this aspect. For example, considering the case where the simulation run is > 10,000 units, the above mentioned regulations state:

- One contaminated unit should result in an investigation
- Two contaminated units are considered cause for revalidation, following investigation

How to apply the above acceptance criteria to a validation study based on 3 runs x 10,000 units? In theory, there could be several possibilities:

1. The most restrictive approach would require that all 3 runs have zero contaminated units. Justification: since it is an initial validation (ideal conditions) no contamination is expected.
2. At the other extreme, the 3 runs could be considered as totally independent, thus accepting a maximum of 1 contaminated unit per run, provided that the investigations justify the acceptability of each run.
3. The author's option is to consider the 3 runs as a whole, as far as the acceptance criteria are concerned. This would mean that 1 contaminated unit over 30,000 can be acceptable, if appropriately investigated, while a total of 2 contaminated units (in the same run or in separate runs) are cause for revalidation, after investigation.

However, it is the manufacturer's responsibility to select the appropriate approach.

The option 2 should be considered only theoretical and would be hardly accepted, considering what is reported in the FDA Guidance *"…recurring incidents of contaminated units in media fills for an individual line, regardless of acceptance criteria, would be a signal of an adverse trend on the aseptic processing line that should lead to problem identification, correction, and revalidation."*

14.25 Invalidation and abortion of process simulation runs

In special circumstances, a process simulation run might not be completed or fail for reasons that are extrinsic to the process being simulated. Some typical examples:

- Breakdown of one of the machines composing the line, not reparable in time for completing the run
- Electrical power shutdown
- Failure of an HVAC system
- Medium contaminated at the source
- Vent filter integrity failed

In the first three examples above, the run is considered aborted since it cannot be completed as planned and the target number of units to be filled is not reached. Since the breakdown of one

piece of equipment does not necessarily implies a risk of contamination of the units filled before the problem occurred, it is important to manage the media filled units in the same way the actual product would be, in similar circumstances. If the actual product filled before the breakdown/shutdown would be considered acceptable, the media filled units should go ahead with all the remaining steps (incubation, inspection, evaluation) using acceptance criteria consistent with the number of units filled. In case the type of breakdown/shutdown occurred would imply rejection of the product, the simulation run can also be rejected, without incubation.

In the last two examples, the problem could be detected only after the end of filling and in some cases after some incubation days, resulting in a broad contamination of multiple (if not all) the units. In this case the run can be considered invalid, provided that there is a clear evidence that the root cause is extrinsic to the actual process (media contaminated at the source) or can be 100% detected by the in process controls (vent filter integrity).

In any case, the invalidation of a process simulation should be a rare event and it should be supported by a very robust justification.

14.26 Investigation of process simulation contaminations – failures

A process simulation/media fill failure should be a very infrequent occurrence and thus represents a relevant shock for the affected facility and it usually requires the involvement of a multidisciplinary team for investigating the root cause(s), evaluating the impact on the product(s) manufactured since the last acceptable simulation (if any) and implementing the necessary corrective/preventive actions before repeating the study. Even when a single contaminated unit is found, a deep investigation is required before considering the impacted run as “pass”, when the media fill size is greater than 10,000 units.

The investigation team usually includes experts from QA, QC – Microbiology, Production, Engineering, Validation and Maintenance. It should be able to review and compare many different documents and data, finding out possible correlations and abnormal events which could be related to the contamination.

Generally an investigation for a media fill contamination should include:

- Identification (up to species, if technically possible) of the contaminant(s) and of all the organisms recovered in the critical and ancillary areas and on personnel during the process simulation and particularly during the production phase when the contamination occurred
- Viable and non-viable EM data and trends (last 6-12 months) review
- Personnel monitoring data and trends (last 6-12 months) review (all operators involved in the process, including people dedicated to cleaning)
- Review of the production records with a particular focus on interventions within the critical area and any abnormal event (should include operators’ interview)
- Review of any deviation from standard procedures occurred during the media fill
- Review of sterilisation processes and their qualification status
- Review of the decontamination processes of non sterilised materials (i.e. materials introduced in the area by UV pass-boxes) and their qualification status
- Review of the data from HVAC system, particularly related to the maintenance of the desired pressure differential between different areas
- Review of data from HEPA filters integrity test, considering the possibility of a repetition of the test
- Review of the calibration/qualification status of the critical equipments and instruments used in the process
- Review of the cleaning and disinfection procedures and how they have been executed (by check-lists, logbooks, operators’ interview)

- Review of post filter integrity test data
- Review of training files of all involved operators (including people dedicated to cleaning)
- Review of any change in the process or in the manufacturing area that occurred since the last successful media fill

The investigation should also take into consideration the potential impact of the failure on other aseptic processes performed at the same facility, should the root cause be linked to systems/procedures shared by multiple processes/filling lines. In case of doubt, the operations on these lines could be put on hold up to the end of the investigation.

It is good practice to have a QA person observing the media fill execution with a “witness” function: in case of failure, the review of the witness’s notes, and an interview, can be of great help. Some companies use also video recording for the same purpose, but a full coverage of all the critical areas involved in the simulation could imply the use of multiple cameras, resulting in a complex set-up of the video recording system.

For the same reason, keeping the filled units in a well identified chronological order up to the end of the incubation can allow correlation of the contaminated unit(s) with a defined time window, facilitating the investigation.

Although the investigation team must be multidisciplinary, it should be considered that media fills are process simulations designed to assess the microbiological contamination risk associated with a given process. Therefore, in case of failure the role of a microbiologist is obviously highly relevant, as the following considerations will underline.

Crucial information comes from the identification of the contaminant(s) recovered in the media fill unit(s). Although the contamination origin can be also obtained by simple microbiological and biochemical techniques such as Gram staining and oxydase/catalase test, identification up to species level is required in case of media fills contaminants.

Obtaining this information can allow the investigation team to get data about the organism’s typical habitat (human skin, water, soil, etc.) and about some important characteristics such as pathogenicity and resistance to sanitising agents or to other chemicals. Moreover, knowing the species of the media fill contaminants it is possible to make correlations versus environmental isolates recovered during media fill execution or frequently found in the area. The increasing use of genotypic identification systems can improve the accuracy of these correlations that could be crucial in identifying and/or confirming the source of the contamination.

In order to ensure that the investigation exercise provides the highest probability of success, it is also very important to preserve as much as possible the “evidence” of all the possible routes of contamination. As already discussed at the beginning of this section, a media fill failure represents a major issue in the management of a production facility, with a potential risk of prolonged filling line stoppages, disruption of the stock and delays in product delivery. It is not infrequent that the investigation team is exposed to pressure for finding the root cause and implementing the related corrective actions as soon as possible. Sometimes, this approach implies that some remediation activities, such as: non-routine sanitisation/fumigation of the environment, re-balancing of the differential pressures, etc., are implemented before having the time to identify (or exclude) potential root causes. Therefore it is important to take time to plan the investigation steps in a logical sequence, preventing the disruption of important traces that could be useful for identifying the root cause.

The identification of the root cause is always a very challenging task. It can be relatively easy in the case of massive contamination of the filled units, an event not so infrequent mainly during the

initial assessment of new aseptic manufacturing facilities/filling lines. In these cases the problem is often linked to an equipment malfunctioning (example: a valve not working properly during a CIP/SIP cycle) and thus the resulting contamination may reoccur in multiple consecutive process simulations until the problem is identified and solved. When it is possible to clearly identify the root cause, it is generally easy to evaluate the impact of the problem on the previously filled product(s) (if any) and to implement the required corrective actions to correct it. The repetition of multiple (at least three) successful process simulation runs will serve to confirm that the actual root cause has been identified and the related source of contamination eliminated.

The investigation is much more difficult and often inconclusive in the case of the sporadic presence of contaminated units (example: 3 – 4 units per run). If repeated on multiple runs, it could indicate that this profile is representative of the "background noise" of the process, thus requiring significant changes to procedures (for example: change of garments/dressing code, disinfectants, air flow patterns, etc.) and related qualification/validation activities before performing a new set of process simulation runs.

The final outcome of a media fill failure investigation should be obtained in a reasonable time and approved by production and quality.
The final report should include:

- A summary of the event
- A list of the systems investigated (including those which have been found not related to the contamination event)
- Description of the contaminating micro-organism(s) and an assessment of the possible origin
- An assessment of the root cause (if identified) including the rationale and the supporting documentation
- An assessment of the impact on the batches previously manufactured by the same process
- Description of the CAPAs taken (or planned), including the results of additional media fill runs, if performed

14.27 Conclusion

The relevant improvements introduced in the aseptic processing technology during the last two decades make the aseptic process simulation/media fill test less effective in assessing the performance of aseptic processes as it was in the past. However it remains the most powerful tool currently available for this purpose and it is also a well consolidated and standardised regulatory requirement.

14.28 References

[1] U.S. Food and Drug Administration, Guidance for Industry on *Sterile Drug Products Produced by Aseptic Processing*, 1987.

[2] EU Guidelines to Good Manufacturing Practice, Medicinal Products for Human and Veterinary Use, 1992

[3] U.S. Food and Drug Administration, Guidance for Industry on *Sterile Drug Products Produced by Aseptic Processing*: *Current Good Manufacturing Practice,* 2004.

[4] EU Guidelines to Good Manufacturing Practice, Medicinal Products for Human and Veterinary Use, Annex 1, 2008.

[5] Agalloco, J "Process Selection for Sterile Products", *Microbiology and Sterility Assurance in Pharmaceutical and Medical Devices",* Business Horizons, pp 603 – 614, 2011.

[6] Recommendations of PQRI Aseptic Processing Working Group, Product Quality Research Institute; March, 2003.

[7] Parenteral Drug Association, Technical Report No. 22, *Process Simulation for Aseptically Filled Products*, 2011.

[8] Akers, J. and Agalloco, J.,"The Akers-Agalloco Method", *Pharmaceutical Technology*, Nov 2005.

[9] Akers, J. and Agalloco, J., "The Simplified Akers-Agalloco Method for Aseptic Processing Risk Analysis", *Pharmaceutical Technology*, July 2006.

[10] Tidswell, E, "Quantitative Risk Modeling in Aseptic Manufacture," *PDA Journal of Science and Technology* 2006; **60**(5): 267-83.

[11] ICH Q9, *Quality Risk Management*, 2005.

[12] Parenteral Drug Association, Technical Report No. 44, *Quality Risk Management for Aseptic Processing*, 2008.

Microbial risk management during aseptic manufacture

Tim Eaton, Sterile Manufacturing Specialist, AstraZeneca, UK

15.1 Introduction

A knowledge and understanding of the areas of greatest risk associated with any manufacturing process enables resource to be focused upon managing these areas, optimising the security and productivity of the process. For the manufacture or processing of sterile pharmaceutical products, the requirements for managing microbial contamination are prescribed in the regulatory documents published by both the European Commission[1] and the US FDA[2]. These documents include microbial concentrations that should not be exceeded, and methods to minimise the contamination of products. To achieve the requirements, risk management techniques are recommended by the regulatory authorities[3]. Aseptically prepared products require highly controlled and monitored environments for the manufacturing activities and typically a cleanroom is utilised. The cleanroom is likely to incorporate critical zones or workstations, such as safety cabinets, isolators or restrictive access barrier systems (RABS) where product may be exposed. The risk management system outlined in this chapter considers aseptic manufacturing within such a cleanroom and the principles discussed can be readily applied to any type of critical zone or system that has been incorporated into it.

15.2 Risk management system stages

An effective system to manage the risk from the various sources of microbial contamination during aseptic manufacturing has been published[4] and utilises the following stages:

1. Identify the sources of contamination and routes of transfer to the product.
2. Assess the risk from the sources and routes of contamination and, where appropriate, introduce new control methods, or improve existing control methods, to reduce risk.
3. Establish a monitoring schedule using valid sampling methods to monitor the hazards, or their control methods, or both. Establish alert and action levels with procedures to be followed, when these levels are exceeded.
4. Verify, on a scheduled basis, that the contamination control system is appropriate by reviewing the product contamination rate, the environmental monitoring results, the risk assessment and control methods and the action levels, and where appropriate modify accordingly.
5. Establish and maintain appropriate documentation.
6. Train the personnel.

The risk assessment stage is a major component in the overall risk management process, and calculates the level of risk associated with a contamination source and is usually the most difficult stage to undertake. An assessment method based upon the fundamental method of contamination transfer will provide the most accurate assessment as it utilises the actual contamination process. This approach is therefore appropriate for aseptic manufacturing processes and is used in this chapter for the risk assessment stage.

15.3 Stage 1: Identification of contamination sources and routes of transfer

The correct identification of all the sources of contamination and the routes of transfer to the product is a fundamental first stage of any risk management system. All sources of contamination

need be correctly identified. Grouping of the sources and the subsequent use of a contamination risk diagram to simplify the routes of transfer to product is a useful approach to help with this.

15.3.1 Sources of contamination

Examples of sources of contamination in a typical aseptic manufacturing operation are:

Supply and cleanroom air The air supplied to a cleanroom and the critical workstation within the cleanroom where product may be exposed, is typically made up of re-circulated air from the cleanroom areas and also a proportion of fresh intake air, and are sources of contamination resulting from both these constituents. The air within the cleanroom and the critical workstation, contain contaminants dispersed from people, equipment and machinery.

Personnel Personnel within the cleanroom are a major source of contamination and normally the sole source of micro-organisms. People disperse vast quantities of contamination from the skin, hair and mouth which can be transferred to the product through the air, or by contact with hands or clothing. Cleanroom clothing, gloves and masks are used to control the contamination being dispersed but these items can however become contaminated by the personnel wearing them and from other cleanroom sources.

Machinery and ancillary equipment Machines are a source of particles, as they can generate contamination by the movement of their constituent parts, or a secondary source from contamination deposited on them from the air or by contact with personnel. Some machine parts may not have direct contact with product, but other parts such as pipework or ancillary items such as forceps and scissors may directly contact product.

Product materials, containers, packaging Product materials, containers and primary packaging that are transferred into the cleanroom may be contaminated. The product itself, often as a liquid formulation, may also be a source of contamination.

Surfaces The floor, walls, ceilings, and other surfaces in the cleanroom, such as tables, tools, paper, etc, and so on, and ancillary equipment such as air samplers and calculators are examples of sources of surface contamination which is normally derived in a secondary way from personnel touching them, or from contamination deposited from the air. These surfaces can also be primary sources of contamination, if poor quality constructional components have been used that may break up and disperse contaminants, or if they have not been appropriately cleaned.

Adjacent areas Adjacent areas that interface with the cleanroom, such as change rooms, transfer hatches and external corridors, are likely to be more contaminated than the cleanroom itself due to the activities in these areas, and the reduced levels of controls utilised.

Compressed Gases Any compressed gases, such as air or nitrogen, may also be piped into the cleanroom and may make direct contact with the product, as an overlay gas during freeze drying for example.

15.3.2 Routes of transfer to product

The routes of transfer of contamination, by airborne and surface contact, must be identified so that the subsequent risk of contamination can also be reduced or prevented.

Airborne contamination is normally sourced from people and machines, dispersed into the air and then deposited onto the product. If the particles are small, such as human skin cells, they can be transported in the air before depositing. However, if they are large particles, like spittle or cuttings of plastic or glass, they will remain within a short distance from where they were generated, and fall directly into, or onto, the product. Contact contamination occurs when contaminated items such as machinery, ancillary equipment, cleanroom surfaces, containers, packaging, gloves and clothes come into contact with the product. Contact contamination can occur in many ways; one example is when personnel touch a contaminated surface with their gloves which then become contaminated. If product is then touched with that glove, contamination is transferred onto the product.

The sources and routes of transfer of contamination can be shown most appropriately using a contamination risk diagram.

15.3.3 Contamination risk diagrams

Construction of a contamination risk diagram is a good method of understanding the sources of contamination, their routes to the product as well as the methods to control either the route of transfer or the quantity of contamination. **Figure 1** is an example of a general type of contamination transfer diagram for a production cleanroom. The main sources of contamination have been grouped into seven categories, together with the main routes of contamination transfer and the typical control methods. The transfer of contamination around the cleanroom can be very complicated as, in theory, everything present in the cleanroom could be contaminated by everything else but practically it is only necessary to consider the major transfer routes. From this type of diagram, the importance of cleanroom and workstation air is clearly visible, and the role it plays in receiving and transporting many of the sources of contamination to the product.

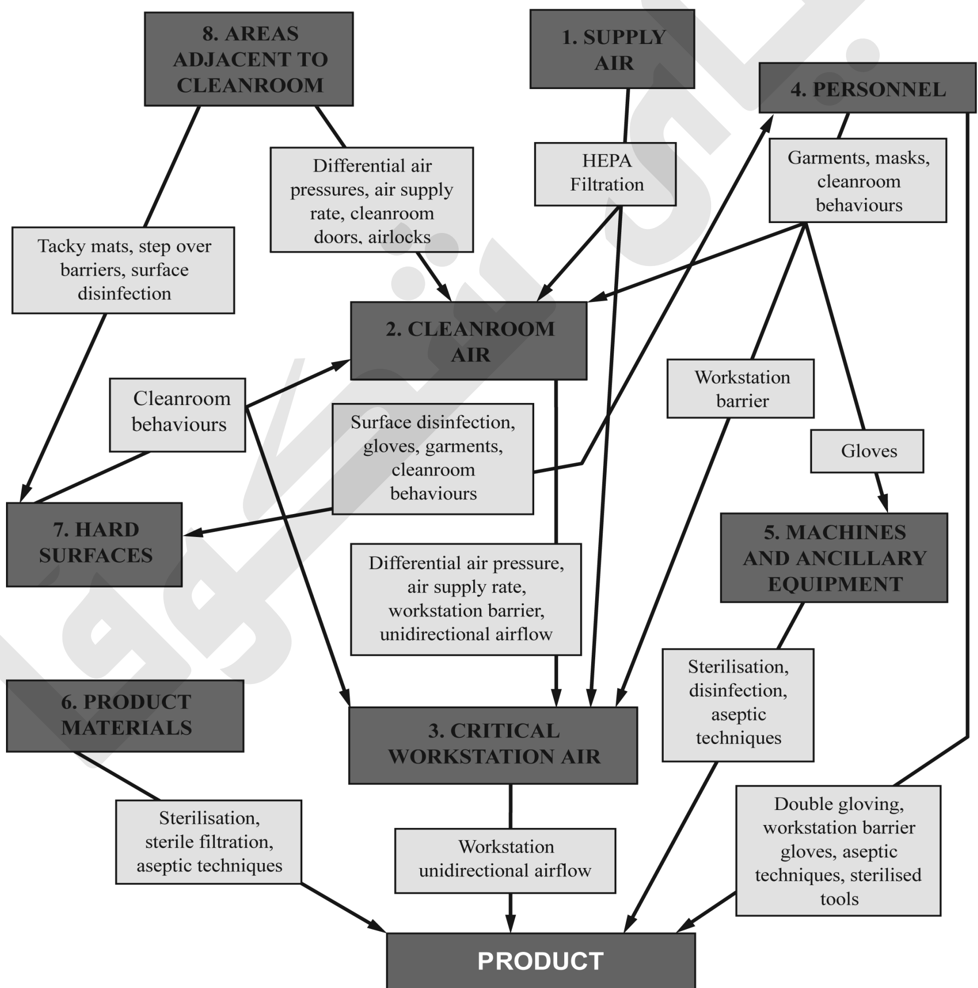


Figure 1: *Contamination risk diagram. Typical sources and routes of microbial contamination in a cleanroom and the associated preventative measures. The dark grey boxes indicate the contamination sources and the light grey boxes provide the associated means of control.*

15.4 Stage 2: Risk assessment method for microbial contamination

Risk assessments, for product microbial contamination, can only be undertaken accurately if the correct models of contamination are utilised. The fundamental mechanism of contamination transfer must therefore be understood and utilised in order to provide an effective assessment of the risk of microbial contamination to aseptically prepared products.

15.4.1 Fundamental contamination transfer model

The number of micro-organisms being transferred from a source of contamination to a product is dependent on the dispersal from the source, transmission to, and then deposition onto or into a product[5]. This process is shown in **Figure 2**.

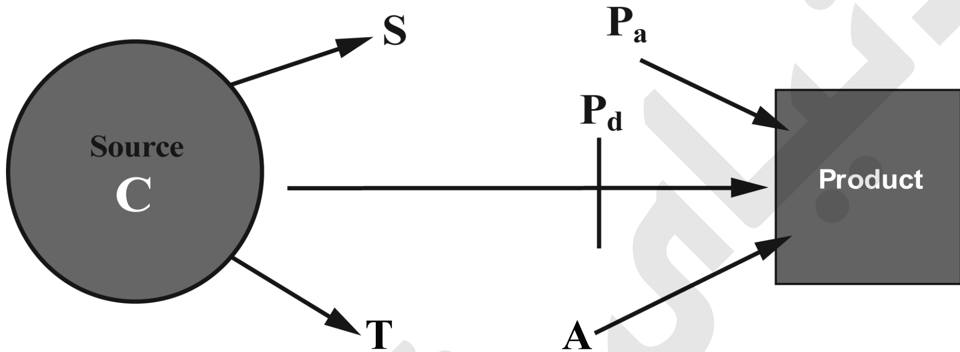


Figure 2: *Transfer of microbes from a contamination source to product.*

Utilising this model, the number of microbes deposited onto a given area of product in a given time, can be represented by **Equation 1**. This equation is universally applicable to all sources of contamination and all routes of transfer.

Equation 1:

$$\text{Number of microbes transferred to a product} = C \times S \times P_d \times P_a \times A \times T$$

where:

C = concentration of contamination on, or in, a source (number/cm^2 for a surface, or number/cm^3 for air);

S = the quantity of surface material, or air, that is dispersed or released from a source in a given time (cm^2/s for surfaces, and cm^3/s for air dispersion);

P_d = proportion of contamination dispersed from a source that is transferred to the area adjacent to the product;

P_a = proportion of contamination in the adjacent area that is deposited per unit area of the product (/cm^2);

A = area of surface onto which the contamination is deposited (cm^2);

T = time that transfer occurs, or number of contacts over time available

A practical example of the mechanisms described in **Equation 1** would be the airborne transfer of skin microbes from personnel to a product in a cleanroom. For this case, the number of microbes that would deposit on a product would depend on: (1) the concentration of microbes per area of a skin surface, (2) the surface area of skin that is dispersed in a given time, (3) the proportion of microbes dispersed and transmitted through the cleanroom clothing and the cleanroom air to the area adjacent to the product, (4) the proportion of microbes adjacent to product that will be deposited onto a given area of exposed product, and (5) the time over which this deposition occurs.

15.4.2 Simplified risk assessment model

The meaning of risk is well established, and defined in ISO 14644-6[6] as 'the combination of the probability of occurrence of harm and the severity of harm'. Risk can be defined mathematically by **Equation 2**:

Equation 2:

Risk = Severity of the occurrence x probability of occurrence

For aseptic manufacturing, 'severity' is the degree of undesirability of an event, and can be considered to be the amount of contamination that is transferred from a source of contamination to a product. The 'probability of occurrence' during aseptic manufacture is either the time a product is exposed to airborne contamination, or the number of times a product is touched by a contaminated surface. By comparing Equations 1 and 2, it can be seen that 'severity' is therefore the multiplication of the variables C, which is the concentration of microbes on a source, and S, Pd, Pa and A, which is the proportion of contamination that is dispersed from a source, transmitted towards the product, and then deposited onto a given area of the product. The 'probability of occurrence' is the variable T, the time over which transfer can occur, or the number of contacts that occur during the transfer time. Using this approach, the following simplified risk model, is shown in **Equation 3**.

Equation 3:

Risk from microbial contamination (risk rating) = A $\times$ B $\times$ C

where,
A = microbial concentration on, or in, a source of contamination
B = proportion of contamination transferred from a source to a product
C = time, or frequency, of the transfer of contamination.

It should be noted that the only difference from the fundamental Equation 1 is the missing variable of area (A). However, a unit area can be assumed and, should the area of the product be considered important or variable, it can be introduced at the end of the risk assessment. Simply, Equation 3 shows that the amount of microbial contamination is dependent on (a) the concentration on a contaminating surface, or within air, (b) the proportion of this contamination that is transferred to the product, and (c) the time or frequency over which this transfer occurs.

15.4.3 Risk scoring method

The risk model is used to assess the level of risk to product from sources of contamination during aseptic manufacture. To complete an assessment, risk factor scores are assigned to the three variables in **Equation 3**. The three variables in the model are known as risk factors and the score values assigned are multiplied together to obtain an overall risk rating for each source of contamination.

The simplest approach is to utilise descriptors as quantitative estimates of the variables, and a risk score consistent with each descriptor is assigned to the variable under consideration. If the actual numerical values are available, then a fully quantitative approach is possible and can be used to calculate the amount of contamination (the number of microbes) transferred from a given source of contamination to a product, and hence the level of risk associated with the source. However, it is likely that some of the actual numerical information will be unavailable, especially for risk factor B, the transfer of contamination. The approach with this assessment is to use actual numerical information for risk factors A and C, and descriptors with associated score values for risk factor B. **Table 1** shows risk scores that can be allocated to risk factors B, as well as the approach to be used with the actual numerical values for risk factors A and C.

The descriptors and associated risk scores used for risk factor B are distributed in a way such that a score of 1 is used for the 'Total' descriptor, where all the contamination is transferred, and the

other scores ranged in a linear way down to the ‘Low’ descriptor. This is the case where the transfer is controlled by a single method, and the proportion of microbes transferred can be high and often a simple proportion of 1. The scores for the descriptors ‘Low’ to ‘Extremely low’ are then distributed from 0.1 to 0.0001 in a logarithmic manner. This is consistent with the transfer of contamination from sources controlled by several methods, such as doors and ventilation, where a proportion of the remaining contamination is further removed by each control method. These controls result in step-wise reduction of the amount of the contamination transferred, represented by the logarithmic scoring system.

For each identified source of contamination, risk factor scores are assigned to each of the three risk factors (A, B and C) and multiplied together to obtain a risk rating.

Table 1: Risk scores

<table>
<tr><td colspan="7">Risk factor A – Microbial concentration on, or in, a source
Surface counts are scored as counts per cm^2 and airborne counts are scored as counts per cm^3.</td></tr>
<tr><td>Risk factor B – Proportion of contamination transferred from a source to product</td><td>Extremely low
0.0001</td><td>Very low
0.001</td><td>Low
0.01</td><td>Medium
0.1</td><td>High
0.5</td><td>Total
1</td></tr>
<tr><td colspan="7">Risk factor C – Time, or frequency, of the transfer of contamination.
For airborne transfer, the time available for microbial deposition into, or onto, a single product is scored.
For surface contact, the number of contacts with a single product is scored.</td></tr>
</table>

15.4.4 Allocation of risk factor scores

15.4.4.1 Risk factor A – Concentration of microbial contamination on, or in, a source

The risk factor score assigned to risk factor A is the concentration of microbes on, or in, the source of contamination. The units used for surface contamination are microbes per cm^2. Therefore, counts obtained from (5.5cm diameter) RODAC plates, which have a sampling area of 24cm^2, are to be divided by 24, and other results from swabbing and glove sampling should be similarly considered and converted to provide counts per cm^2.

The concentration of airborne microbes should be reported as counts per cm^3, and therefore the normal units reported for air sampling (counts per m^3), should be divided by 10^6. This will allow air and contact sources to be directly compared on an equal footing and the actual contamination rate of the product from each source to be calculated. Actual numerical data are likely to be available from monitoring results and can be utilised accordingly. If there are no available data, the maximum concentration that will meet the requirements of the EU GGMP[1] can be utilised, although this is likely to be significantly higher than the actual recorded levels and would therefore overestimate the calculated risk rating.

15.4.4.2 Risk factor B – Proportion of contamination transferred to product

Risk factor B assesses the proportion of microbes transferred from a source of microbial contamination to a product. Transfer is considered to be the dispersion of microbes from a source, the transmission to an area adjacent to the product, and then the deposition into, or onto, a product. The allocation of risk factor scores to air and contact transfers are approached differently.

15.4.4.2.1 Airborne transfer

The prime source of microbe-carrying particles in the air of a cleanroom is from the personnel working in the room. Microbes are carried on skin particles, and hundreds of microbe-carrying particles can be dispersed into the cleanroom air every minute[7]. However, in this risk assessment it is assumed that the microbes have been dispersed from personnel, and the source of microbes

is the air within various parts of the cleanroom or the critical workstation. For risk factor B, a descriptor that best describes the transfer from the source to the product is selected from **Table 1** and the risk score associated with it is allocated accordingly. This may be sufficiently accurate for this purpose, but if an increased accuracy is required, an estimate of the actual proportion of microbes transferred can be achieved by experiment, or by researching any appropriate and available scientific literature.

15.4.4.2.2 Contact transfer

Sources of surface contamination in a cleanroom include personnel's gloves and garments, machinery, the fabric of the room (ceiling, walls, doors, floors) and a variety of items such as trolleys, tables, weighing balances, alcohol dispensers containers, etc. When one of these contaminated surfaces comes into contact with another surface, microbes may be transferred, and the proportion of microbes on the contaminated surface that are transferred to the receiving surface, such as the product, is the transfer risk factor score. A descriptor that best describes the transfer from the source to the product is selected from Table 1 and the risk factor score associated with it is allocated accordingly. To help with this, and consistent with the experiences of the author, the proportion of microbes transferred between a glove and a surface is taken as 0.1 (10% of the microbes on the contaminated surface are transferred to the receiving surface) and for hard surface to hard surface contact, such as occurs between equipment or machine surfaces, the proportion transferred is 0.01 (1%). Primary transfer occurs when a contaminated surface touches the product as for example, a contaminated glove. Secondary transfer occurs when microbes on a contaminated surface are transferred to another surface, and subsequently transferred to the product.

15.4.4.3 Risk factor C – Time, or frequency, of contamination transfer

15.4.4.3.1 Airborne contamination

The actual time that microbe-carrying particles have to deposit into, or onto, a product is utilised and the unit of time is seconds (s). This may be, for example, the time a container is open during filling.

15.4.4.3.2 Surface contact

The frequency of contact between two surfaces should be determined and taken as the number of contacts. If 5,000 products were manufactured per batch and a single contact was made with one of the products, the frequency would be 1 in 5,000 i.e. 2×10^{-4}.

15.5 Stage 2: Practical risk assessments for aseptic manufacturing operation

15.5.1 Description of aseptic manufacturing operation

The relative importance of microbial sources of contamination in aseptic processing can be assessed by considering the pharmaceutical cleanroom shown in **Figure 3** where aqueous product is aseptically filled into containers which are then immediately sealed with stoppers. The filling zone is within a unidirectional airflow workstation (EU GGMP Grade A[1]), situated in a non-unidirectional airflow cleanroom (EU GGMP Grade B[1]). Access from the cleanroom into the workstation is through a full glass barrier door, used to complete the aseptic set up activities. During operation, the barrier door remains closed and the operator controls the filling operation from the cleanroom but enters into the workstation approximately 20 times, throughout the batch, to address any filling issues and to also replenish the supply of container stoppers into the vibrabowl that feeds the stoppers to the product filling zone. Two people work around the cleanroom as well as attending to the filling operation within the unidirectional workstation.

The aqueous solution of the product is prepared in an adjacent preparation cleanroom in a dedicated product vessel. The solution is transferred, under pressure in an enclosed system, from the preparation vessel through a sterilised, sterilising-grade filter, to the product filling manifold. An

aseptic connection is made in the workstation between the outlet of the transfer pipe from the filter and the pipe that connects to the filling manifold. The containers are sterilised in a tunnel that exits into the workstation, automatically fed to the filling zone, filled with product and then sealed using sterilised stoppers.

The product is manufactured in batches of 40,000, and each batch takes about 10 hours to complete and the time the container is open to airborne contamination (between exiting from the sterilising tunnel and the sealing of the filled container with a stopper), is about 10 minutes (600s). The concentrations of airborne and surface microbes, in the cleanroom and workstation areas, are routinely measured during the filling operation under full operational conditions, using standard microbiological monitoring methods.

The following contamination control measures within the cleanroom are utilised:

1. All air to the cleanroom and workstation is supplied through terminal ceiling HEPA filters.
2. Personnel enter the cleanroom from dedicated change areas and don sterilised, one-piece polyester coverall, hood, overboots and mask. Sterilised, latex, double gloves are worn over disinfected hands. Gloves are frequently disinfected with sterile 70% isopropyl alcohol in Water for Injection (70% IPA).
3. All surfaces, such as the product pipework and filling needles, which contact the product solution, are sterilised. The containers and stoppers are also sterilised. Other cleanroom and workstation surfaces are disinfected.

15.5.2 Identification of contamination sources

The first step in assessing the level of risk from the various sources of microbial contamination in the cleanroom is to identify the sources. Shown in **Figure 1** are the main sources of microbial contamination, grouped into seven categories. The contamination sources, associated with each of these categories, for the aseptic manufacturing operation shown in **Figure 3**, are detailed in

Figure 3: *Cleanroom and critical workstation used for aseptic preparations.*

Table 2. The sources in the change and personnel change area, and the preparation room, are not included because the degree of risk is much lower than those found in the cleanroom and critical workstation. However, if required, the methods described can be used to determine the importance of any other additional contamination sources of interest.

Table 2: Contamination sources for aseptic manufacturing operation

Contamination category	Source of contamination
1. Supply air	1.1 Air supplied to workstation 1.2 Air supplied to cleanroom
2. Cleanroom air	2.1 Air within cleanroom
3. Critical workstation air	3.1 Air within critical workstation
4. Personnel	4.1 Transfer to product from hands 4.2 Transfer to product via gloves with secondary contamination 4.3 Airborne contamination from personnel in workstation 4.4 Surface contamination from clothing from personnel in workstation
5. Machines and ancillary equipment	5.1 Machine surfaces not in contact with product 5.2 Surfaces in direct contact with product 5.3 Ancillaries in contact with product
6. Product materials	6.1 Liquid product from clean process area 6.2 Containers
7. Hard surfaces	7.1 Ceilings, walls, floors, doors in cleanroom 7.2 Trolleys, buckets, chairs, tables, calculator, waste bins, paperwork, pens, bin bags, labels, press buttons, etc. in cleanroom 7.3 Walls, floors and ancillaries (70% IPA container, microbial samplers, etc., in workstation)

15.5.3 Examples of the overall risk assessment method

The risk ratings for each source of contamination listed in **Table 2** can be calculated utilising the method described in section 4. These are to be calculated in the controlled state, when the established contamination control methods are utilised. To facilitate the assessments, some simple examples of the risk ratings associated with various sources of contamination are shown below. The microbial concentrations in the air and on surfaces have been determined from the operational environmental monitoring and these data are used in the calculations. Additionally, to understand and quantify the importance of some of these control methods, the example assessments also consider where control methods have been removed or reduced.

15.5.3.1 Risk from supply air

The air that is supplied to the cleanroom and workstation areas is a source of microbial contamination. This air is re-circulated from the cleanroom areas and the contamination is controlled by HEPA filters installed in terminal positions at the end of the supply ducts, found in the ceiling of the cleanroom, and the workstation. It is considered that the air has a microbial concentration very similar to the cleanroom, which, according to the EU GMP[1], should not exceed $10/m^3$ i.e. $1 \times 10^{-5}/cm^3$. This is taken as the concentration of the microbial challenge to the HEPA filter. The HEPA filters are *in-situ* integrity tested and against this test, have an efficiency of 99.99% (a penetration of 0.01%) against an inert particle that has a size that most easily penetrates through the filter (about 0.3mm). However, microbe-carrying particles in the cleanroom that are dispersed from humans have an average equivalent particle diameter of approximately 12µm[7,8]. These particles are at least 10,000 times less likely to penetrate HEPA filters than the most penetrating particle size. The cleanliness of the air supplied to the workstation and cleanroom areas therefore has a microbial content of $1 \times 10^{-5} \times (0.01/100) \times 10^{-4} = 1 \times 10^{-13}/cm^3$ and this is taken as the score for risk factor A.

For risk factor B, in the case of the workstation, air flows in a unidirectional way to the containers, and the proportion of the microbes transferred to the containers is considered to be high and a score of 0.5 is therefore allocated. For the air similarly supplied to the cleanroom, the rigid door barrier of the workstation, and then the effects of the unidirectional airflow within the workstation control the transfer of the contamination reaching the product containers. The proportion of the microbes transferred to the containers is considered to be very low and a score of 0.001 is therefore allocated.

The open containers are conveyed through the workstation, where they are filled and each container is open to airborne contamination for 10 minutes (600s) and risk factor C is therefore scored as 600s.

The risk rating for air supplied to the unidirectional airflow workstation is $1 \times 10^{-13} \times 0.5 \times 600 = 3 \times 10^{-11}$. For air supplied to the cleanroom, the risk rating is $1 \times 10^{-13} \times 0.001 \times 600 = 6 \times 10^{-14}$.

It can be seen that HEPA filters are the only contamination control method for the air supplied to the filling workstation. If it was decided that HEPA filters would not be required, risk factor A would increase to 1×10^{-5} within the workstation, and the risk from microbial contamination would increase to 3×10^{-3}, which for a batch size of 40,000 would be 120 contaminated units. This would be clearly unacceptable.

15.5.3.2 Risk from cleanroom surfaces

The walls within the cleanroom are a source of microbial contamination that may be transferred to product. The amount of contamination on the walls is controlled by scheduled disinfection and by ensuring the cleanroom personnel practice good cleanroom disciplines and do not lean on or make contact with them. The microbial concentration present on the walls should be below that set by the EU GMP[1] for a Grade B area, of 5 per ($24cm^2$) contact plate. However, with these controls in place, an actual concentration of $1.75 \times 10^{-1}/24cm^2$ has been recorded from the environmental monitoring and a value of $7.3 \times 10^{-3}/cm^2$ is taken as the score for risk factor A.

For risk factor B, the contamination will be adhered to the walls and will not be dispersed by the air in the cleanroom. A proportion may be transferred when a cleanroom operator touches the wall and then enters the workstation to manipulate the product or container. These types of manipulations are performed using tools and so the proportion of microbes transferred from the wall to the hands of the operator, then from the operator's hands to the tool, and then to product is considered to be extremely low and a score of 0.0001 is allocated for risk factor B.

The cleanroom operator is expected to have a reasonable understanding of cleanroom disciplines and will not touch the walls and product and it is assumed that the frequency of contact, as a worst case, is no more than once for each batch of 40,000 and so 2.5×10^{-5} is the score allocated for risk factor C.

The risk rating for cleanroom wall contamination is $7.3 \times 10^{-3} \times 0.0001 \times 2.5 \times 10^{-5} = 1.8 \times 10^{-11}$.
It can be seen that disinfection and cleanroom disciplines are the control measures used to minimise the contamination on the surfaces of the walls. If these control measures are discontinued, risk factor A may increase 100 fold to 7.3×10^{-1} and the risk rating would increase proportionally to 1.8×10^{-9}. However, even with this increase in the risk rating, the cleanroom walls are not considered to present an important product contamination risk.

15.5.3.3 Risk from operator's hands

The microbial concentration on the hands is variable and depends on the individual and the effectiveness of any disinfection procedure. Hands that have been washed with an antimicrobial

cleansing agent, and sampled by contact plate, have a microbial concentration of about 20 per $24cm^2$, and a concentration of $0.8/cm^2$ is taken as the score for risk factor A.

Risk factor B is the proportion of microbes transferred from the skin of hands and deposited onto the product. This transfer is controlled with double gloving, using sterilised gloves. The likelihood that both of the gloves are punctured and allow skin microbes to pass through to an area on the outer surface where transfer to the product can occur, is very low[9]. Additionally, operating personnel will not contact product directly but will utilise tools. Therefore, risk factor B is considered to be extremely low and a score of 0.0001 is allocated.

Risk factor C is the frequency that the operator manipulates product. The operator will enter approximately 20 times during each batch and it is assumed that on each of these occasions, there is some manipulation of the product, containers or stoppers. For a batch size of 40,000 this is a frequency of 5×10^{-4} and this is the score allocated for risk factor C.

The risk rating for contamination from washed hands with double gloves is $0.8 \times 0.0001 \times 5 \times 10^{-4} = 4 \times 10^{-8}$. The use of double gloves is considered to be a key contamination control method to prevent the transfer of microbes from the hands. If no gloves are worn, and tools are not utilised, then product may be directly contacted by hands and the proportion of microbes transferred may increase to certain. The score for risk factor B would be 1 and the risk rating would increase to 4×10^{-4}, which for a batch size of 40,000 would be 1 contaminated unit.

15.5.4 Calculation of risk ratings

Shown in **Table 3** are the risk factor scores and risk ratings from each microbial source found for the aseptic manufacturing operation (shown in **Figure 3**) that are listed in **Table 2**. The assessment has been completed for the manufacturing operation in the fully controlled state, utilising the contamination control measures included in the table shown in parenthesis. The in-operation environmental monitoring results have been utilised to provide data relating to the microbial counts. If any of the listed control measures are not utilised, by considering the principles described in the previous sections, the relevant risk factor scores can be readily re-assessed to determine the risk ratings applicable for the level of contamination control measures actually used.

15.6 Stage 2 (continued): Risk assessments for critical areas

The assessments shown in **Table 3** can be considered to be a first level estimate of the number of microbes that are transferred from a source to each container, from all general sources of contamination throughout the cleanroom suite. Due to potential inaccuracies, mainly associated with risk factor B (the proportion of contamination transferred), and the omission of a variable that includes the area of contamination associated with the product, the risk rating may be subject to a degree of error. However, it is obvious that contamination can only occur in the critical areas where the product is open to potential contamination. By applying a more detailed second level risk assessment, using separate derived equations[5] for contamination by airborne deposition or by contact contamination, further improvements can be made to the accuracy of the assessments within the critical areas. This may also be useful to investigate a particular contamination issue that may have occurred during the routine aseptic manufacture of a particular batch of product.

15.6.1 Airborne deposition

The fundamental **Equation 1** can be modified by combining the first four variables into a single variable that gives the number of micro-organisms that will deposit onto a given area of product in a given time, i.e. the deposition rate. The number of microbe carrying particles that will deposit onto a known exposed area in a given time can be determined by **Equation 4**.

Table 3: Sources of contamination risk and calculated risk ratings for aseptic manufacturing operation

Source	Risk factor A Microbial counts (surface- counts/cm^2 air- counts/cm^3)	Risk factor B Proportion of contamination transferred (dispersion, transmission and deposition)	Risk factor C Time, or frequency, of transfer of contamination	Risk rating
1. Supply air to unidirectional airflow workstation and non-unidirectional airflow cleanroom				
1.1 Air supplied to unidirectional airflow workstation	1×10^{-13} (Terminal HEPA air filtration)	0.5	600	3.0×10^{-11}
An explanation of the estimation of this risk rating is recorded in section 5.3.1				
1.2 Air supplied to non-unidirectional airflow cleanroom	1×10^{-13} (Terminal HEPA filtration)	0.001 (Workstation barrier, unidirectional air flow)	600	6.0×10^{-14}
An explanation of the estimation of this risk rating is recorded in section 5.3.1				
2. Air within non-unidirectional airflow cleanroom				
2.1 Air within non-unidirectional airflow cleanroom	1×10^{-7} (Rate of air supply, cleanroom garments, cleanroom disciplines)	0.001 (Workstation barrier, unidirectional air flow)	600	6.00×10^{-8}
The airborne cleanliness levels are routinely monitored and were found to have a microbial content of $0.1/m^3$ i.e. $1 \times 10^{-7}/cm^3$ The air would be well dispersed but the proportion transferred to product is considered to be very low (0.001) because of the workstation barrier and by the action of the unidirectional airflow in the workstation. The time the container is open to airborne contamination is 10 minutes.				
3. Air within non-unidirectional airflow cleanroom				
3.1 Air within unidirectional airflow workstation	3.5×10^{-9} (Cleanroom garments, cleanroom disciplines, unidirectional airflow)	0.1 (Unidirectional air flow)	600	2.1×10^{-7}
The airborne cleanliness levels are routinely monitored and were found to have a microbial content of $3.5 \times 10^{-3}/m^3$ and a score of 3.5×10^{-9} cm^3 is allocated to risk factor A. The unidirectional airflow will control the transfer from personnel to the product, although the movement of arms and body may disrupt and mix the airflow and the proportion transferred is considered to be medium (0.1). The time the product container is open to airborne contamination is 10 minutes.				
4. Personnel				
4.1 Transfer to product from	0.8 (Hand washing)	0.0001 (Double gloving, sterilised	5×10^{-4}	4×10^{-8}
An explanation of the estimation of this risk rating is recorded in section 5.3.3				
4.2 Transfer to product via gloves with secondary contamination	2.75×10^{-4} (Glove disinfection, cleanroom disciplines)	0.001 (Sterilised tools, aseptic techniques)	5×10^{-4}	1.4×10^{-10}

Table 3 continued on next page

Table 3: Sources of contamination risk and calculated risk ratings for aseptic manufacturing operation (continued)

Source	Risk factor A Microbial counts (surface- counts/cm^2 air- counts/cm^3)	Risk factor B Proportion of contamination transferred (dispersion, transmission and deposition)	Risk factor C Time, or frequency, of transfer of contamination	Risk rating
Microbial monitoring of finger dabs is undertaken. The average microbial concentration is 2.4 x 10^{-3} per plate and a plate sample includes 5 digits, each digit having an area of approximately 1.75 cm^2. The microbial concentration is therefore 2.75 x 10^{-4} per cm^2 and this is the score for risk factor A. Contamination may be transferred from the operator's hands to the tool used to manipulate product, and then transferred to product. The proportion transferred is considered to be low (0.001). The operator will enter approximately 20 times during each batch of 40,000 and so 5 x 10^{-4} is the score allocated for risk factor C.				
4.3 Airborne contamination from personnel in workstation	3.5 x 10^{-9} (Cleanroom garments, cleanroom disciplines, unidirectional airflow)	0.1 (Unidirectional air flow)	600	2.1 x 10^{-7}
This risk is considered to be the same as previously assessed as the air within the unidirectional airflow workstation, 3.1.				
4.4 Surface contamination from clothing from personnel in workstation	1.4 x 10^{-3} (Garment sterilisation, cleanroom disciplines)	0.01 (Aseptic techniques)	5 x 10^{-4}	7 x 10^{-9}
The microbial concentrations on the garments were determined from personnel exit monitoring, following completion of the manufacturing activities and are therefore worst case. The concentrations were found to be 3.4 x 10^{-2} per 24cm^2 and so a score of 1.4 x 10^{-3} cm^2 is allocated for risk factor A. The proportion transferred to product is considered to be low (0.01) and will occur when the operator enters the workstation approximately 20 times during each batch of 40,000 and so 5 x 10^{-4} is the score allocated for risk factor C.				
5. Machines and ancillaries in unidirectional airflow workstation				
5.1 Machine surfaces not in contact with product	5.2 x 10^{-5} (Disinfection)	0.0001 (Cleanroom disciplines)	5 x 10^{-4}	2.6 x 10^{-12}
Microbe-carrying particles strongly adhere to hard surfaces and will not be detached by air currents. A proportion may be transferred when a cleanroom operator touches the surface and then manipulates the product or container. These types of manipulations are performed using tools and so the proportion of microbes that are transferred from the surfaces to product is considered to be extremely low (0.0001). The operator will enter approximately 20 times during each batch of 40,000 and 5 x 10^{-4} is the score allocated for risk factor C.				
5.2 Surfaces in direct contact with product (pipework) hands	2.0 x 10^{-6} (Sterilisation, aseptic techniques)	1	2.5 x 10^{-5}	5 x 10^{-11}
The microbial concentrations on sterilised surfaces have been determined by sampling following completion of the manufacturing operation and will therefore represent worst case conditions. Microbial contamination in sterile pipework that is in direct contact with the product solution is likely to be readily transferred (washed) into the product solution and so the proportion transferred is considered to be total (1). The frequency of transfer is considered to be constant but is assumed to be distributed throughout the batch of 40,000 and 2.5 x 10^{-5} is therefore utilised for risk factor C.				

Table 3 continued on next page

Table 3: Sources of contamination risk and calculated risk ratings for aseptic manufacturing operation (continued)

Source	Risk factor A Microbial counts (surface- counts/cm^2 air- counts/cm^3)	Risk factor B Proportion of contamination transferred (dispersion, transmission and deposition)	Risk factor C Time, or frequency, of transfer of contamination	Risk rating
5.3 Ancillaries in contact with product (scissors, forceps, etc.)	2.0 x 10^{-6} (Sterilisation, aseptic techniques)	0.01 (Aseptic techniques)	5 x 10^{-4}	1 x 10^{-11}
Ancillaries such as forceps are likely to be utilised to manipulate stoppers or containers, instead of directly contacting liquid product. For these types of materials, the microbial contamination transferred is likely to be low (0.01) The operator will enter approximately 20 times during each batch of 40,000 and 5 x 10^{-4} and is the score allocated for risk factor C.				
6. Material – primary and packaging				
6.1 Liquid product from clean process area	1 x10^{-9} (Sterile filtration, sterilisation, aseptic techniques)	1	2.5 x 10^{-5}	2.5 x 10^{-14}
A sterilising grade filter must deliver a sterile effluent when challenged with 10^{-7} microbes per cm^2 of filter. For the size of filter actually utilised, a total challenge of 1 x10^{-11} was utilised and delivered a sterile effluent. The product solution has a volume of 80 litres and has a maximum allowable microbial concentration of 1 x 10^2/ml solution and after filtration a resultant microbial concentration of 1 x10^{-9}/ml which is the score used for risk factor A. Any contamination present in the sterile-filtered effluent would be completely dispersed and transmitted throughout the product solution and the proportion is assessed as total (1). The frequency of transfer through the filter is continuous to each container throughout the batch of 40,000 and 2.5 x 10^{-5} is therefore utilised for risk factor C.				
6.2 Product container	2.0 x 10^{-6} (Sterilisation, aseptic techniques)	1	2.5 x 10^{-5}	5 x 10^{-11}
The microbial concentrations on sterilised surfaces have been determined by sampling following completion of the manufacturing operation and will therefore represent worst case conditions. Microbial contamination in the container that is in direct contact with the product solution is likely to be readily transferred (washed) into the product solution and so the proportion transferred is considered to be total (1). The frequency of transfer is considered to be constant but is assumed to be distributed throughout the batch of 40,000 and 2.5 x 10^{-5} is therefore utilised for risk factor C.				
7. Hard Surfaces				
7.1 Ceilings, walls, floors, doors in cleanroom	7.3 x 10^{-3} (Disinfection, garments, cleanroom disciplines)	0.0001 (Cleanroom disciplines)	2.5 x 10^{-5}	1.8 x 10^{-10}
An explanation of the estimation of this risk rating is recorded in section 5.3.2				
7.2 Trolleys, buckets, chairs, tables, calculator, waste bins, paper-work, pens, press buttons, IPA can, etc. in cleanroom	1 x 10^{-2} (Sterilisation, disinfection)	0.0001 (Cleanroom disciplines)	5 x 10^{-4}	5 x 10^{-10}

Table 3 continued on next page

Table 3: Sources of contamination risk and calculated risk ratings for aseptic manufacturing operation (continued)

Source	Risk factor A Microbial counts (surface- counts/cm^2 air- counts/cm^3)	Risk factor B Proportion of contamination transferred (dispersion, transmission and deposition)	Risk factor C Time, or frequency, of transfer of contamination	Risk rating
The contamination will be adhered to the surfaces and will not be dispersed by the air in the cleanroom. A proportion of the contamination may be transferred when a cleanroom operator touches these items and then enters the workstation to manipulate the product or container. These types of manipulations are performed using tools and so the proportion of microbes that are transferred from the item to the hands of the operator and then from the operator's hands to the tool, and then to product is considered to be extremely low (0.0001). The operator will enter approximately 20 times during each batch of 40,000 and as a worst case, 5×10^{-4} and is the score allocated for risk factor C.				
7.3 walls and ancillaries, e.g. IPA can and microbial samplers in workstation	5.2×10^{-4} (Disinfection)	0.0001 (Cleanroom disciplines)	5×10^{-4}	2.6×10^{-11}
See 7.2 The operator will enter approximately 20 times during each batch of 40,000 contacting the IPA can each time, and so 5×10^{-4} and is the score allocated for risk factor C.				

Equation 4:

Number of airborne microbes deposited in a given time (no.) = deposition rate (no./cm^2.s) x area of product exposed (cm^2) x time of exposure (s)

Microbiological settle plates can provide information for micro-organisms regarding the deposition rate which is the number of microbes deposited per unit area of surface per unit of time. The area of the surface exposed to contamination, and the time it is exposed will be known, and the number of airborne micro-organisms that deposit onto a surface may then be calculated. If settle plate data are not available, the deposition rate can be found from knowledge of the airborne particle concentrations and the deposition velocity of the microbe carrying particles as shown in **Equation 5**.

Equation 5:

Number of airborne microbes deposited in a given time (no.) = number of microbes/cm^3 x deposition velocity (cm/s) x area of product exposed (cm^2) x time of exposure (s)

The deposition velocity is the velocity that microbe-carrying particles fall through the air. Within cleanroom areas, the average equivalent particle diameter of microbe carrying particles has been shown to be $12 \mu m^3$ and can deposit, mainly by gravity into, or onto, the product with a velocity calculated to be 0.46cm/s.

15.6.2 Surface contact

The equation used to calculate the number of micro-organisms deposited by surface contact is derived from the fundamental **Equation 1** by considering surface contamination to occur as discrete events, and by combining the dispersion, transfer and deposition variables into one overall term known as the deposition coefficient. The number of microbes deposited on a given area over a known time can be determined by **Equation 6**.

Equation 6:

Number of microbes deposited by surface contact (no.) = microbes on source surface (no./cm^2) x deposition coefficient x area of product contacted (cm^2) x number of contacts

Where, the deposition coefficient is the proportion of microbes transferred from a contaminated surface to a receiving surface.

15.6.3 Examples of risk assessments for critical areas

By applying the derived equations for contamination by airborne deposition or by contact contamination within the critical areas, accurate assessments of the number of microbes contaminating product can be determined. Two examples, one for the assessment of airborne contamination and one for contact contamination, are shown below.

15.6.3.1 Calculation of contamination rate from airborne deposition

Consider the aseptic manufacturing operation described in section 4. During the manufacture of a particular batch, the airborne microbial sampling within the workstation recorded a count of 1cfu per m^3, for each sample, throughout the entire filling operation. **Equation 3** can be used to determine the risk of product contamination from the air within the workstation.

The number of airborne microbes within the filling workstation was found to be $1/m^3$ ($1 \times 10^{-6}/cm^3$) and this is assumed to be the concentration of microbes around the product containers. The deposition velocity of a particle with an average equivalent particle diameter of 12μm is about 0.46cm/s, and the inner neck area of the container is known to be $1cm^2$. The open containers are conveyed through the unidirectional airflow workstation, and each container is open to airborne contamination for 10 minutes.

Therefore, number of airborne microbes deposited = $1 \times 10^{-6} \times 0.46 \times 1 \times 600 = 2.76 \times 10^{-4}$. This is the contamination per container and for a batch size of 40,000 this is a contamination rate of 11 containers.

15.6.3.2 Calculation of contamination rate from surface contact

A final aseptic connection is completed by the operator between the outlet pipe from the sterilising grade filter (which is connected to the product preparation vessel) and the filling head in the workstation. The pipework that subsequently transfers the liquid product from the sterilising filter outlet to the filling head, including the filling needles, are all pre-assembled and sterilised prior to use. This ensures that there will therefore be a minimum of opportunities for microbial contamination to occur during assembly. Therefore, during the aseptic connection, microbial contamination from the gloved hands into the aqueous product solution could occur, and this may then be dispensed into a container during filling. During the aseptic connection associated with a particular batch, the operator was observed to complete this activity using poor aseptic technique and the product solution contacting surfaces were contacted by the operator's fingers. **Equation 4** can be used to determine the risk of product contamination. Results of sampling of the microbial concentration on the gloved hands of the operator completing the aseptic connection recorded 2cfu. For 5 digit sampling, with each digit having a surface area of approximately $0.875cm^2$, this represents a surface microbial concentration of 4.57×10^{-1} per cm^2 which is assumed to be concentration of microbes on the source surface.

The deposition coefficient, from the glove to a point during the aseptic connection, where contamination of the product solution can occur, is 0.1. The area of contact of the glove during a faulty aseptic pipe connection was that associated with two digits, an area of $1.75cm^2$. This was a single contact and microbes transferred from the glove are assumed to subsequently make contact with the product solution and the microbes introduced will be transferred down the pipe and into the containers being filled. It is reasonable worst case assumption that each container will receive no more than a single microbe, although the contamination is likely to be concentrated in the containers at the start of filling rather than throughout the entire batch.

Therefore, number of microbes deposited by contact = $4.57 \times 10^{-1} \times 0.1 \times 1.75 \times 1 = 7.99 \times 10^{-2}$. For a batch size of 40,000, this is a contamination rate of 1.99×10^{-6}

15.7 Stage 3: Establishment of an effective monitoring programme

An effective programme is required to monitor the hazards, or their control measures, or both. In order to provide effective monitoring, valid sampling methods at identified locations with defined monitoring frequencies and appropriate action levels must be employed.

15.7.1 Valid sampling methods

Sampling methods must be able to confirm that the collection efficiencies of the sampling instruments or procedures are reasonably high and precise, and that the media and incubation conditions are correct for the type of microbes sought in the cleanroom to grow. The sampling instruments must also be regularly calibrated. There are well-established methods for measuring the concentration of micro-organisms on surfaces and in the air within cleanrooms. However, some of these have low collection efficiencies and the results obtained can be very variable. It is therefore necessary to have confidence that the collection efficiencies are high and precise. It is also necessary to determine that there is no loss of growth because of disinfection inhibition due to residues left on the surfaces, or dehydration of the microbial growth medium during air sampling. Methods to determine the instrument collection efficiencies and to validate the microbial growth conditions are available[10].

15.7.2 Sampling locations and monitoring frequency

Locations in the cleanroom that are to be monitored, and the associated frequency, should be related to the risk of contamination. Sources of contamination that have been shown by risk assessment to have higher risk ratings must be sampled and at a high frequency, whereas inconsequential hazards may be ignored. For example, the air where product or product containers are open to contamination must be comprehensively sampled at a number of locations continuously, whereas the ceiling surfaces may be sampled at a single location on a weekly schedule. Surfaces in the critical area should be sampled more thoroughly than the surfaces of the background cleanroom areas, which in turn should be sampled more thoroughly than surfaces in the supporting cleanroom areas.

The number of locations to be sampled in a given area should also be related to the risk of contamination. Thus, sampling locations and the associated frequency should be concentrated in the critical areas, and as the risk of contamination becomes less in the background and support areas there should be fewer sampling locations at reduced frequencies. Cleanroom interface areas, such as transfer hatches and personnel change rooms, may be an exception and may require proportionally more sampling because of the higher risk associated with the materials and personnel that are transferring to the cleanroom areas.

It will also be necessary to consider the type of product manufactured in the cleanroom. Terminally sterilised, or non sterile products, with a low risk to patients, will not require less sampling at less frequencies than products manufactured aseptically. Also, an aseptic product assessed to have a low risk to patients would need fewer sampling locations and reduced frequencies than one with a high risk.

There is little available information regarding this type of monitoring, as this is dependent on the type of product, the layout of the manufacturing cleanrooms, and the manufacturing processes. Some guidance however is provided in PDA Technical Report No.13.[11] It should also be considered that too frequent sampling could potentially introduce further risk. The intrusion into critical areas for the purpose of sampling can introduce contamination and the need for appropriate sampling should be balanced against the potential introduction of contamination. Initially, the identified sampling locations and frequencies may not be the most appropriate. When the contamination control system is verified, the opportunity should be taken to reduce, increase or change the locations and frequencies, depending on the data obtained.

15.7.3 Setting the monitoring limits

The EU GMP[1] and the FDA[2] regulatory documents include limits for microbial concentrations that should rarely be exceeded. However, where better environmental conditions are achieved, it is appropriate that more stringent standards should be maintained. Consequently, action levels may typically be set at the limits recommended by the regulatory authorities, with the alert levels being set at a lower value.

The alert level indicates that the contamination concentrations are higher than might normally be expected and can give an early warning of a potential drift from design conditions. It indicates that the conditions are still under control and no corrective actions will normally need to be initiated. However, if several alerts are obtained in a relatively short time, or another unusual pattern of results are recorded, then this may suggest that action is required.

An action level is a value which, when exceeded, requires immediate investigation to identify the cause and, if necessary, corrective action. The investigation should assess whether the excursion outside the naturally expected variation is due to sampling issues, or is a genuine excursion that exceeds the acceptable contamination concentration. For those considered to be genuine, the investigation should be completed according to an agreed method to assess whether or not the result is acceptable and, if unacceptable, what action is required to regain control. Useful information on how these types of results should be managed is given in ISO 14698-2[12] and methods that can be used to regain control are given in the PDA Technical Report[11].

Results from sampling can give a large variation in results due to the variation in the number of personnel present, their activity and changes to room air movements. If the alert and action limits for the samples are set too low, the limits will regularly be exceeded. These limits are best set after a reasonable amount of sampling has been completed under full operational conditions. A periodic review of the monitoring system should also be completed, and the limits may need to be modified as data are accumulated, although the action levels should never be set at higher levels than outlined in the regulatory standards. This can be a complicated subject, and is outside the scope of this Chapter. PDA Technical Report No.13[11] and PS Technical Monograph No. 2[13] are useful sources of information regarding the analysis of the monitoring results and the setting of alert and action levels.

15.7.4 Recording of the control and monitoring methods

Tabulation of the contamination control and monitoring methods, the location and frequency of sampling, and the associated alert and action levels, is a useful method of ensuring that the risk management process is satisfactorily completed. Shown in **Table 4** is such a record for the air supplied to the unidirectional airflow workstation, identified in section 3 as a source of contamination. All of the identified sources of contamination need to be recorded in a similar manner to provide a comprehensive record of the contamination control measures and the associated monitoring methods. The completed record, for all the identified sources of contamination, must be updated if the control measures or the sampling procedures are changed or amended.

15.8 Stage 4: Verification of the risk control system

The risk management system should be regularly verified to ensure that it is effectively controlling contamination.

15.8.1 Introduction

It is necessary to periodically verify that the control system is working well. This should be carried out by assessing results of product sterility tests, process simulation trials (PSTs, sometimes known as media fills) and environmental monitoring. A review of the risk assessment method and

Table 4: Tabulation of control and monitoring methods

Source to be controlled	Contamination Control Methods	Monitoring Methods	Location and Frequency of Sampling	Alert and Action Levels
2. Supply Air				
Air supplied to unidirectional airflow workstation	1. HVAC air filtration (HEPA bags)	1. *In-situ* filter integrity testing (DOP)	1. In supply air duct – 24 months	1. HEPA filter penetration of no more than 0.01%
	2. Terminal HEPA air filtration (H14 rated filters)	2.1 *In-situ* filter integrity testing (DOP)	2.1 At cleanroom filter face – every 6 months	2.1 HEPA filter penetration of no more than 0.01%
		2.2 Airborne particle concentrations	2.2 Within unidirectional airflow workstation – locations specified in SOP – Continuously	2.2 Alert: 10/ft^3 ≥ 0.5μm 1/ft^3 ≥ 5μm Action: 100/ft^3 ≥ 0.5μm 2/ft^3 ≥ 5μm
		2.3. Settle plates	2.3 Within unidirectional airflow workstation – locations specified in SOP – Each manufacturing batch	2.3 Action: 1cfu/settle plate
		2. 4 Active sampling of airborne microbes	2.4 Within unidirectional airflow workstation – locations specified in SOP – Each manufacturing batch	2.4 Action: 1cfu/m^3
		2.5 Microbial surface counts	2.5 Within unidirectional airflow workstation – locations specified in SOP – Each manufacturing batch	2.5 Action: 1cfu/contact plate

associated risk models and risk ratings, should then be completed. This will indicate if any of the control methods need to be changed (improved or relaxed) and if there are also any changes required to the environmental monitoring programme, and the established action or alert limits.

A risk management approach requires the contamination control standards to be set at a level appropriate to the manufacturing process under consideration. This is a bit different from the established approach in pharmaceutical manufacturing where if any contamination control standards can be improved, they generally are, regardless the degree of risk. The verification of the control system should also take account of the microbial risk to patients from the product, as different products have different degrees of risks to the patient. Different manufacturing processes may therefore have different levels of environmental control.

15.8.2 Assessment and reassessment of the overall effectiveness of the control system

The overall effectiveness of a risk management system is ideally established by demonstrating that every product is free from contamination. This is not possible in aseptic manufacture, as any associated sterility test destroys each product and so only a small proportion of the manufactured product is tested in this way. The first step in the verification is to confirm that the products tested

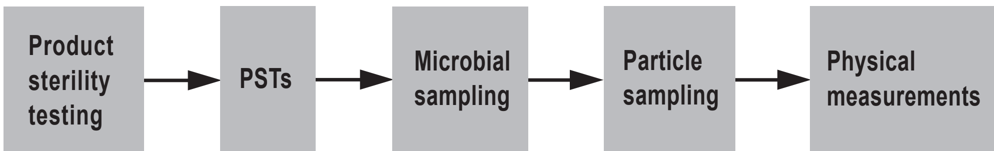


***Figure 5:** Criticality of testing to demonstrate aseptic assurance.*

by the sterility test were satisfactory, recognising however, that there is only a small chance that a non-sterile product will be detected using this test. Therefore, verification of the contamination control system also has to be completed by other less direct means. Simulation of the manufacturing process with microbiological media is a good method of assessing the likely sterility of the product.

However, in a similar manner to the sterility test, sufficient process simulation trial (PST) results are unlikely to be collected at a sufficiently frequency to provide the confidence that all manufactured units are contamination free. Further verification of the likely sterility of the product is required from the results obtained from microbial sampling of the sources and routes of contamination. It is necessary to show that the environmental sampling results have a low enough concentration to ensure that contamination of the product is at an acceptable minimum. This approach is not as scientifically rigorous as using the results obtained from the sterility and process simulation trials as there is no information on what concentrations of contamination are required to achieve practically-sterile products and what other undetected events occurred during the manufacturing process.

Shown in **Figure 5** are the tests used to confirm environmental control during aseptic manufacture. The tests are arranged so that the methods on the left provide a greater likelihood of the chance of a product being contaminated. Generally, the further the test method is away from measuring the prime parameter of product sterility, the easier it is to collect results. Physical measurement of cleanroom parameters, such as air pressure differential, air supply volumes or air velocities can be measured continuously. These tests give confidence that the cleanroom is working well within its design parameters but does not however demonstrate that the product is not contaminated by airborne deposition or by surface contact. Particle counting of air gives some indication of the likelihood of airborne contamination but does not directly measure airborne microbial deposition, and clearly does not measure surface contact. Air and surface microbial sampling provide more information about the likelihood of air and surface contamination but these tests take more time to gather, and may present some additional contamination risk because of their intrusive nature.

15.8.3 Review of the risk assessment method

It is necessary to reconsider the risk models and risk scoring methods used in the risk assessment for the aseptic manufacturing operation. Any improvements that can be made to the risk model or scoring method should be considered and any additional information regarding new control methods or additional test results, should be included into the reassessment. If an increase in the risk rating is thought to be significant, a review of the methods used to control risk as well as the environmental monitoring programme should be initiated. Conversely, if the assessment is shown to be acceptable but the risk assessment shows there a lower degree of risk of contamination from identified sources than previously indicated, consideration of whether the control method and, or, associated environmental sampling programme, could be modified and even made less stringent.

15.8.4 Modification of environmental sampling and alert or action levels

A review of the both the overall effectiveness of the control system, and the risk assessment method, may provide new information on the degree of risk of the sources of contamination. This information should be used to review the environmental sampling programme with regard to a

decrease or increase in the number of sampling points, or the frequency of sampling, as well as the alert and action limits. This can be done as follows:

1. If the overall effectiveness of the control system is shown to be acceptable, and the risk assessment results are unchanged, then there is unlikely to be a need for change in the environmental sampling programme and the alert or action levels.
2. If the overall effectiveness of the control system is acceptable, but the risk assessment results show that a particular risk source (or sources) has a reduced risk, then the amount of environmental sampling of the sources and action and alert levels could be reduced.
3. If the overall effectiveness of the control system shows that further control is required, or the risk assessment results show that a particular risk source of contamination has an increased risk, then the environmental sampling programme could be increased and, or, action and alert levels made more stringent.

15.9 Stage 5: Establishment of appropriate documentation

The control system expects that a standard type of documentation system is utilised. The methods and the monitoring procedures described in this chapter should be documented and regularly updated to incorporate any implemented changes. Reports should be produced regularly to detail an analysis of the monitoring results and any deviations from the expected results. When action levels are exceeded these should be reported, as well as the actions taken to correct the issue, or the explanations as to why no action was necessary. Alert levels can also be reported, particularly those associated with a multiple or unusual occurrence.

15.10 Stage 6: Training of personnel

Some risk management systems do not specify that it is expected that personnel must be trained. All efforts to control contamination through risk management will fail if the personnel working in the manufacturing areas are not trained to conduct themselves correctly. Personnel should be trained to understand how the cleanroom functions, and how to behave to minimise contamination. This training should be completed prior to entry into a cleanroom, and subsequently at defined intervals.

15.11 *References*

[1] Annex 1, Manufacture of Sterile Medicinal Products, European Union GMPs, European Commission, 2007.

[2] Guidance for industry sterile drug products produced by aseptic processing – Current good manufacturing practice. US Food and Drug Administration, 2004.

[3] Pharmaceutical cGMPs for the 21st Century – a Risk-Based Approach. US Food and Drug Administration, 2004.

[4] Risk management of contamination (RMC) during manufacturing operations in cleanrooms. The Parenteral Society and Scottish Society for Contamination Control, Technical Monograph No.14, 2005.

[5] Whyte W and Eaton T. Microbiological contamination models for use in risk assessment during pharmaceutical production. *European Journal of Parenteral and Pharmaceutical Sciences* 2004; **9**(1): 11-15.

[6] ISO 14664-6. Cleanrooms and associated controlled environments : Part 6: Vocabulary. Geneva, Switzerland, International Organization for Standardization, 2007.

[7] Noble WC, Lidwell OM, Kingston D. The size distribution of airborne particles carrying micro-organisms. *Journal of Hygiene* 1963; **61**: 385.

[8] Whyte W. Sterility assurance and models for assessing airborne bacterial contamination. *Journal of Parenteral Science and Technology* 1986; **40**: 188-197.

[9] Eaton T. A safe pair of hands – how secure are your gloves used for aseptically prepared pharmaceutical products? *European Journal of Parenteral and Pharmaceutical Sciences* 2005; **10**(3): 83-86.

[10] Whyte W. Collection efficiency of microbial methods used to monitor cleanrooms *Journal of Parenteral Science and Technology* 2005; **10**(2):3-7.

[11] Fundamentals of an environmental monitoring program. Parenteral Drug Association, Technical Report No.13. 2001.

[12] ISO 14698. Cleanrooms and associated controlled environments – biocontamination control. Part 2: Evaluation and interpretation of biocontamination data. Geneva, Switzerland, International Organization for Standardization, 2004.

[13] Environmental contamination control practice. Parenteral Society, Technical Monograph No 2, 2002.

Airflow studies and airflow mapping

Tim Sandle, Head of Microbiology, Bio Products Laboratory, UK
Marco Budini, Consultant, Pharmaceutical Technology & Compliance, Italy
Rajesh Thempadiyi, Manager, Technical Transfer, Dr Reddy's Laboratories, India

16.1 Introduction

Air is of fundamental importance to cleanrooms, either as a contamination source (micro-organisms carried in the air-stream) or as a control measure to minimise contamination (through the supply of clean air). Therefore, controlling a cleanroom requires careful attention to air filtration and airflow. The movement of the air in a cleanroom is an important contamination control step[1]. In a cleanroom, this air movement is normally in turbulent flow (that is air moving with a non-uniform velocity). Here, air is driven in through grills and ducts at ceiling height and removed through low level ducts. The air in the room with its initial supply velocity is sufficient to keep it in constant turbulence, which prevents particles and micro-organisms from settling (this is an ideal because dead air can occur, for example, beneath tables). With clean air devices, the air is designed to be unidirectional whereby the air direction and air velocity are designed to remove any contamination in the air-stream away from the critical area.

Understanding how air moves in a cleanroom or clean air device is critical in order to understand how the clean air functions in relation to any contamination that might be present in the air. This is undertaken through airflow visualisation studies (or what are sometimes called smoke studies). The purpose of flow visualisation is to confirm the smoothness, flow patterns and other spatial and temporal characteristics of airflow in an installation. For this, the airflow is examined through airflow visualisation mapping whereby smoke is generated and the behaviour of the smoke studied and then captured by a video camera.

Airflow studies can demonstrate a significant amount of information. This can relate to contamination control in assessing whether airflows are drawing potentially contaminated air towards a critical zone of whether certain objects in the air-stream cause contamination by forcing the air to change direction. Careful consideration must be made of equipment and other obstacles in the room. Where air strikes an object any contamination in the air can be deposited or where air becomes trapped then dead air spots can develop leading to localised turbulence. It is important that the manager of the cleanroom understands where these 'dead spots' can occur and then either attempts to engineer these out or undertakes a suitable risk assessment or targets environmental monitoring samples (viable and particulate monitoring) towards the areas of concern[2].

However, it is important to note that there are no objective criteria with which to assess airflow visualisation studies: the difference between a good and bad airflow study cannot be expressed parametrically. Thus any interpretation of an airflow study rests, to a degree, on subjectivity and when reporting airflow studies care should be taken to avoid commenting on things that cannot be clearly seen by the smoke distribution.

16.2 Regulatory requirements

Airflow visualisation studies are a requirement for the certification of certain types of clean air devices, as with the ISO 14644 standard, and a regulatory requirement for cleanrooms used for aseptic processing. With the ISO standard, air-flow studies are referenced in part 2 (described as 'airflow visualisation') and part 3 (described as 'flow visualisation')[3]. In terms of regulatory

requirements, the FDA Guide to Aseptic Processing[4] (2004), requires airflow studies for unidirectional airflow (UDAF) devices, within which product vials are exposed. The Guide requires that such studies are conducted under dynamic (in-use) conditions. The requirement for airflow studies is also referenced in the World Health Organisation (WHO) Good Manufacturing Practices for Pharmaceutical Products[5]. With the WHO guidance, specific reference is made to the need to perform airflow studies in Grade A environments in order to assess the uniformity and effectiveness of the unidirectional flow. In contrast, EU GMP, in Annex 1 for sterile products, does not necessarily limit the application of airflow studies to unidirectional airflow devices in describing the object of airflow studies as intended to demonstrate that air does not present a contamination risk to processing[6]. The Pharmaceutical Inspection Convention scheme[7], to which the EMA and FDA are members, is least specific of all in simply noting that 'smoke studies' need to be performed.

Notwithstanding the subtle differences between the regulatory requirements, there is commonality in that airflow visualization studies need to be performed within unidirectional airflow devices (ISO class 5/EU GMP Grade A environments) that are used for aseptic processing and where product or components are exposed. Within the context of aseptic filling this means, as a minimum, the aseptic filling zone and where aseptic connections (if any) are performed. Arguably a meaningful assessment cannot be made without accounting for the impact of the surrounding environment. Therefore, many airflow visualization studies include an assessment of the cleanroom within which the unidirectional airflow device is located (ISO class 7 in operation/EU GMP Grade B).

There is a difference in terminology between laminar airflow and unidirectional airflow. It is important to understand and to define this difference prior to starting an airflow study. The terms are defined as:

- Laminar flow (also known as streamline flow: An airflow without cross-currents perpendicular to the direction of flow and no eddies or swirls. The airflow movement is in single direction and in parallel layers at constant velocity from the beginning to the end of a straight line vector.[8]
- Unidirectional flow: An airflow moving in a single direction, in a robust and uniform manner, and at sufficient speed to reproducibly sweep particles away from the critical processing or testing area. Being pragmatic, the usage of terminology of unidirectional airflow suits the industry as it can be achieved at lower velocity than the specified guidance value as well.

In addition to published regulatory standards, important information can be gleaned from a review of inspectorate trends. FDA 483 notifications concerning airflow studies have highlighted the following concerns[9,10]:

- Adequate exhaust systems or other systems to control contaminants are lacking in areas where air contamination occurs during production. Review of the smoke studies revealed:
- No studies were performed in the sterile filtration room located
- Personnel were not properly gowned
- Only one of the filling technicians was clearly visible
- Pooling was visible between the technician and filling equipment and supplies
- Equipment and supplies normally present in the filling suite were not present during the study
- Air returns were not visible
- Filling activities were not consistent with those observed during a filling operation
- There was no documentation that the studies were reviewed and evaluated
- The technician participating in the smoke study was observed with exposed skin
- The technician's activities were not consistent with those observed during actual sterile operations
- Pooling and eddying was visible in and around the sterile filling assembly

- There was no documentary evidence of *in-situ* air pattern analysis (e.g. smoke studies) conducted at critical areas to demonstrate unidirectional airflow and sweeping action over and away from the product under dynamic conditions. Please note that proper design and control prevents turbulence and stagnant air in the critical area. It is crucial that airflow patterns be evaluated for turbulence that can act as a channel for air contamination. The studies should be well documented with written conclusions, and evaluation impact of aseptic manipulations (e.g. interventions) and equipment design

16.3 Operational state

Airflow visualisation studies should be conducted with cleanrooms and devices in different states of operation. These can be divided into the 'at rest' (or static state) and the in operation (or dynamic state). The static state is with equipment on, but not running, and without personnel present. The dynamic state is with all machinery normally operating running and with the number of personnel normally present. Undertaking airflow studies for the at-rest and in-operation states allows the at-rest state to be compared with the dynamic state study. This can indicate if a problematic airflow in operation is due to the activity being undertaken or due to the room design. Such comparative studies also provide important information relating to modifications to the room or HVAC system. Furthermore, for newly built cleanrooms, the difference between the static state airflow pattern and the dynamic state are similar if the cleanroom is performing as it was designed[11]. It is rare, however, that a static state study alone can be presented to a regulator in lieu of a dynamic state study.

Airflow studies should also be taken at different stages of operation. The activities undertaken should be representative of standard operations. Such activities may be simulated where, for example, the aseptic filling of product being examined is water or some other innocuous substance normally used due to the risk of product contamination.

After completion of an airflow study, the cleanroom user will need to decide the extent of cleaning. Although low particulate smoke can be purchased, many types of generated smoke leave a residue and thus pose a contamination risk. In many circumstances, the room or unidirectional device will require cleaning and disinfection (with appropriate environmental monitoring) at the cessation of the activity.

16.4 Smoke generating devices

In order to conduct an airflow visualisation study a smoke-generating device is required. This could be a smoke pencil for localised, smaller scale examinations, or an industrial smoke generator for examining a larger area of an aseptic processing room. Such devices are sometimes described as ‘smoke machines’ or ‘fog machines’.

The substance used to generate the smoke is an important consideration. If the substance used to produce smoke is heavier than air, then there is a risk that the substance could fall out of the air and may give an incorrect impression that the air is being removed from the room at a faster rate than is actually taking place in practice. This risk exists more with CO_2 based smoke systems than with glycol-based smoke. Ideally the smoke produced should be of a small particle size as the smaller the particle size the lower the settling velocity of the smoke (that is the rate at which a particle will fall, due to gravity). Research has shown, for example that a smoke particle of unit density, 0.2 micron in diameter, will fall at 8 mm /hr, compared to a 2.0 micron particle, which will fall at a rate of 468mm/hr. Therefore the smaller the particle of smoke produced, the greater is the chance that the smoke stays suspended in the air which eventually reduces the occurrence of deposition of the particles because there is a greater probability of the air being removed from the cleanroom (through room air changes) than there is of particle deposition.

The most common type of smoke generators uses a water-based fluid which mainly constitutes pharmaceutical grade glycol and water (either Water for Injections or deionised water). Here

'smoke' is formed as water vapour condenses into tiny liquid water droplets in the air. It is important that the smoke generated is as clean as possible so that the residual contamination is minimised. Other types of smoke machine include dry ice machines which create a low lying heavy fog that uses dry ice (solid carbon dioxide); liquid nitrogen machines (which similarly produce a low lying fog) and oil-based smoke machines, which leave the greatest amount of residue. Dry ice smoke machines present some health and safety concerns, especially when used in confined spaces due to the high volumes of carbon dioxide produced. Smoke pencils normally contain a porous filler chemical impregnated with fuming sulphuric acid. Therefore when smoke particles are emitted from the end of the tube they have acidic properties due to sulphuric acid aerosols. The smoke produced must conform to national health and safety requirements.

Table 1 displays the most commonly available types of devices for generating smoke for airflow studies together with the advantages and disadvantages of the methods.

Table 1: Smoke generating devices for conducting cleanroom airflow patterns

Type of smoke generator	Advantages	Disadvantages
Smoke pencil	Inexpensive Easy to use	Low volume of smoke Acidic smoke (leaves residues that can damage some materials)
Glycol/water	High volume of persistent smoke	Leaves residues (water soluble)
	No safety concerns	
Dry ice Liquid Nitrogen	High volume of smoke No residues	The smoke is heavy and can alter the test results Some safety concerns

As an alternative to hand-held smoke generation devices, smoke generators can be ducted. This allows for a steady flow of smoke to be produced (in a curtain like effect) **(Figure 1**). This can be useful for examining larger aseptic filling machines where the object is to note any ingress from the external air into the critical zone (sometimes referred to as the 'work zone'). To achieve the required distribution of smoke this can function either through the inherent velocity of smoke produced by the generator or by incorporating fan assistance where a rapid distribution of the smoke is required.

For ducting, this should be as short and straight as possible with as smooth a bore as practical, so that frictional losses within the duct are kept to a minimum. Ideally the smoke output should be controlled so that a low output can be used to show unidirectional flow and a higher output used where the impact of the surrounding turbulent air upon the unidirectional flow is required.

Figure 1: *Example of ducted smoke through a perforated pipe in order to obtain a curtain-like effect.*

Glycol/water smoke generation devices work by heating a chemical (such as propylene glycol or triethylene glycol, mixed with 20 percent water) above its boiling point within a heat exchanger. The

chemical is then vaporised and as the vapour passes out of the heat exchanger and starts to mix with the relatively colder atmospheric air, the rapid condensation of the vapour results in a visible smoke (or technically the more accurate term ‘fog’). The configuration of the heat exchanger is important in order to avoid smoke of too large a particulate size being produced and thus avoiding the production of smoke of variable quality. If the heat exchanger becomes too hot the machine will malfunction; if the heat exchanger becomes too cold it will produce liquid rather than smoke.

Smoke pencils are small, chemical-filled glass tubes. When both tips are snapped off and air is squeezed through the tube, a jet of white chemical 'smoke' is produced. Smoke pencils are best used for small, localised studies, such as for detecting air leaks in ductwork, cracks around doors, etc.

16.5 Video recording

Airflow studies should be captured by video recording. This not only captures all the required information but also provides a better historical record than can be provided from photographs or from hand drawings. The other advantage of video recordings is that they can be shown to auditors and enable the findings of the airflow study to be communicated explicitly and easier than is possible through diagrams or written descriptions. The use of video tapes or DVD recordings is generally a regulatory expectation.

Care must be taken when using video recording that a suitable video camera is used, with sufficient definition to allow for the smoke to be seen clearly on the finished recording. The use of a tripod is recommended so that any shaking of the camera by the camera operator is minimised. It is also important that the recording is secure and stored in a way which does not damage the digitally captured images for future playback. Making a second copy or transferring onto a computer hard drive or server is recommended.

16.6 Undertaking an airflow visualisation study

As stated earlier, before starting an airflow visualisation study the area to be examined must be prepared as normally in terms of equipment, personnel and simulated activities. The staff present should wear appropriate cleanroom clothing as if the event were an actual aseptic operation, even if the cleanroom is temporarily declassified, otherwise the completed recording will not indicate to a regulator the full extent of normal operations. Often regulators use the airflow visualisation studies for the evaluation of the aseptic technique, since many activities performed in the critical area (example: some equipment set-up operations) are not easily visible from the external windows, therefore a great attention to this aspect must be addressed during the video takes.

It is generally a good idea to have two people, separately from what is happening in relation to the process, to run the airflow study (although care must be taken to avoid these additional people from disrupting the study itself). One person is tasked with operating the smoke generator and for ‘directing’ the study, and the second person is tasked with operating the video camera.

It is important, before the study starts, that a script is written and consideration be given as to what will or will not be an acceptable outcome (this is largely a qualitative assessment). This will ensure that the people who will be recording the study and generating the smoke know what they should be looking for. This will include consideration of ‘what are the activities being performed?’, ‘how many times with any activity take place?’, ‘how long will the study last for?’ and so forth. The person who will be operating the video camera will need to understand when close-up shots are required (such as, examining the impact of the airflow around a filling needle or for an intervention involving the use of tools) and when a wider-angle shot is needed (to show the path that the smoke takes). Furthermore, it may be necessary to shoot the same activity using different angles so that all aspects of the activity and smoke distribution can be seen. Care should be taken when filming to avoid glare from the cleanroom lights reflecting from certain surfaces or glass, and situations when

personnel move or stand immediately in front of the camera. The use of a polarized filter on the camera lens can help in reducing reflections and glare. In some situations the smoke may not be clear against a white background and it may be necessary to have a darker material (such as a dark plastic material fixed to the wall) in order to provide sufficient contrast[12]. In some cases, additional light sources may be useful in increasing the overall contrast, mainly if the light beam is orthogonal to the direction of the video capture (the Tyndall effect).

When conducting the study, the person who operates the smoke generating device must be careful not to introduce large volumes of smoke into an air stream, otherwise the smoke will either be too heavy and it will be unclear as to what is happening with the airflow, or that the velocity of the smoke will be too great and the airflow disrupted. Instead the smoke should be introduced gently at a low velocity so that the smoke produced is steady and 'light', to the extent that the smoke should appear to float in the air and then dissipate leaving little residue. It is equally important that the smoke remains visible for the required time.

The use of a video monitor or a PC directly connected to the digital video camera during the video takes can help in evaluating immediately if the recording is meeting the expectations. This is not easy on the small screen of the video camera. Considering the efforts required in an airflow visualisation study, it could be disappointing to discover that a video take is not satisfactory at a later stage, when repeating the recoding could be problematic.

16.7 Key objective of airflow visualisation studies

There are a number of essential aspects of the airflow that need to be examined by conducting an airflow study. When examining aseptic processing, the primary aim is to indicate if there is adequate protection of exposed product, product contact components and surfaces. Other considerations include:

a) To understand the difference between static and dynamic states and the direction of air laminarity away from critical zone. Here it is important to establish if the airflow is smooth, free from disturbances (such as small, temporary vortices or eddies) and unimpeded. The direction of the air pattern should be noted. The question to frame analysis is: Is the air movement as expected? For example:
   - i) Is the air avoiding the floor?
   - ii) Is the air moving into a lower grade area?

At rest, airflow studies can also be used to make the following important observations:

- a) Demonstrating that airflow patterns do not present a contamination risk, such as considering if the air from background environment is getting mixed with the air stream from the UDAF source. It should be proven if the clean zone is positively pressurized in relation to the background environment. During this test the "induction" effect can also be evaluated.
- b) Evaluation of routine operational conditions can be examined, such as changes to the airflow during one or several door opening of the background clean room.
- c) To evaluate the unidirectional airflow from the airflow device. The Institute of Environmental Sciences and Technology (IEST) and National Environmental Balancing Bureau recommends maintaining not more than a 14° angle of deflection from vertical.
- d) To evaluate the installation and operation of equipment beneath the airflow and to evaluate impact of equipment on the unidirectional airflow.
- e) To evaluate the 'sweep away' action of air during the operation of the filling/ stoppering and sealing stations, and its effect on the product containers or closures.
- f) Evaluation of airflow between the stoppering and capping station should be performed in order to ensure that air flow is from stoppering station to capping (lesser clean/particle generating) station is satisfactory.

g) To evaluate the interfaces with the filling line. For example a depyrogenating tunnel (the exhaust fan of the depyrogenating tunnel shall be designed to exhaust air from the filling zone isolator as well, since airflow should be from filling station to cool zone of tunnel), freeze-dryer, mousehole (the exit path for vials from isolators is termed as a mousehole. The isolator may have other exit ports through which product is discharged, opening the isolator to the outside environment. Airflow at this station should be towards the outside, which should also be justified with sufficient overpressure in a continuous manner to ensure that isolation is maintained at this location), etc.

Prior to the actual test for airflow pattern, a trial run may be conducted by keeping to the above objectives with clearly defined acceptance criteria. This will help in optimizing the operational parameters of the equipment and surrounding environment. Operating parameters such as speed of either inlet or exhaust fans (Proportional Integral Derivative (PID) controlled fans), pressure differential between two stations in the clean zone, room pressure differential, etc.

b) Within cleanrooms, where a turbulent flow is expected (an air-pattern characterised by small and temporary fluctuations caused by instabilities) the impact of any air eddying must be examined to note any impact upon critical process steps. Within cleanrooms, the air streams should be quickly dispersed. Areas of poor air movement should be noted. A combination of single 'puffs', 'streams' or 'multiple streams' of smoke can be used to investigate possible problems.

With both ‘a’ and ‘b’ above, examples of problems or points of concern that may be found include:

- Heat rising from machinery and disrupting the airflow
- Obstructions preventing the supply of air getting to the critical area
- Obstructions, or machinery design, turning a unidirectional flow into a turbulent flow
- Entrainment: contaminant drawn into the clean air
- Stagnant or turbulent areas acting as conduits for entry of contamination from areas of lower contamination
- Air flowing from personnel towards the product
- Examination of HEPA filters to detect any adverse affect on air flow patterns, which will eventually have an affect on unidirectional airflow
- Evaluation of disruption of Active Air Sampling/particle counting in the clean zone

c) To demonstrate the impact of operator interventions and other personnel activity, including environmental monitoring (the placement and operation of active air-samplers can be particularly disruptive to the air pattern). Studies of interventions must take into account:

1) Prime objective of such studies is to demonstrate that air-flow patterns do not present a contamination risk during performing routine interventions e.g. care should be taken to ensure that air flows do not distribute particles from a particle generating person, operation or machine to a zone of higher product risk. Insertion of hand into all the gloves of a positive pressure isolator should also be considered.

2) Such studies must ensure that that, during performing of any intervention, there is no obstruction to the airflow over the container/closure/product is in open condition. All the interventions shall be evaluated against this criterion by performing and assessing the air flow pattern.

3) It is important that UDAF devices are operating as normal while performing interventions in the clean zone.

Airflow pattern studies that examine interventions can be conducted prior to media fill simulation runs to finalize the *modus operandi* of aseptic interventions. These studies are helpful in developing the interventions in a complaint manner. Video records of these studies can also be made in order to train the technicians involved in machine assembling, filling, and stoppering activities. The impact of other personnel operations should be assessed as appropriate;

d) To show the impact of equipment operating. One important observation which can be made is assessing the impact of a change in vial size (minimum or maximum can be considered) in order to evaluate the air flow along different stations in a filling line. This can be assessed at different states: at the start of batch (front line is empty and impact of automatic opening of tunnel door); during batch (vials completely filled in the line) and at end of the batch (rear side is getting empty).

e) To demonstrate the set-up of equipment

f) The area outside of the UDAF units (within the ISO Class 7/EU GMP Grade B cleanroom). Arguably, the biggest contamination risk in these areas is when potential airborne particles could accumulate in vortex regions. This can happen when an unidirectional air-flow strikes an object and creates a ‘wake region’. It is prudent to confirm the level of particles in such regions through the use of a discrete particle counter.

In general, when examining airflows the streamlines should be noted. A streamline is defined as the route the air is taking and a streamline will be the path that any contamination by ventilation could take (so-called convective transport). When a streamline meets an object and causes turbulence (or meets air which is turbulent), then contamination could potentially be dispersed (or so-called turbulent diffusion). Regions of stagnation should also be noted. These can often occur in front of machinery and by work surfaces that are perpendicular from the airflow. The air velocity in these areas can be unpredictable.

16.8 What to include in airflow studies

There is a range of aspects of an airflow that should be examined at the time the airflow is conducted and when playing back the video recording[13]. These items are listed and discussed below:

a) Examination of the unidirectional airflow.

The unidirectional airflow is a key parameter of a clean air device in terms of the air velocity (operating at 0.45 metres per second (±20%) and direction[14]. These devices contain HEPA filters, which control the air speed and direction. The object of the unidirectional airflow is to push outward any contamination which might be deposited into the air-stream and to avoid the potential for contamination dropping out of the air, either though gravity or by striking a object, and falling onto a critical surface. To measure the aerodynamic performance of the unidirectional airflow unit, smoke should be introduced at the filter face so that the distribution of the smoke downwards and away from the critical zone can be seen. Smoke should also be introduced at the working height, immediately above the area where product or product components are exposed.

The assessment should note the impact of the machine upon the airflow. Does the smoke, for example, entrail inwards when the air impacts upon the filling machine guarding? What is the effect of disturbances caused by the motion of machine operations? The biggest risk will be when potential airborne particles accumulate in vortex regions. When a unidirectional air flow strikes an object, the obstacle will create a 'wake region'. Such regions should be studied for vortices and potential particle accumulation.

Any regions of stagnation should be detected. When unidirectional airflow meets an object, wakes and vortex streets can be formed. This causes turbulence that can lead to pockets of stagnation in front of machinery and work surfaces that are perpendicular to the main direction of the airflow. Such pockets can be unpredictable in speed and direction and require mapping. Another potential risk from large surfaces is that wake regions can entrain ambient air into clean zones. A consideration of the impact of Grade B areas upon Grade A zones should be considered in such circumstances.

Ideally, the pattern should show that the air is characterised by a smooth flow, free of any disturbances (such as small and temporary vortices or eddies) and unimpeded.

b) Examination of the operation of the aseptic process.

Where a filling machine is examined, for example, the machine should be filmed running with bottles or vials moving along a conveyor belt. The filling operation should be simulated with filling needles moving upwards and downwards. Ideally a placebo should be used in place of product, such as filling vials with water.

For filling machines, steps such as machine set up, loading of vials, stoppers and caps; cleaning of spilled product; cleaning vials; removing jammed stoppers; gauntlet changes, etc. should be captured in the study.

c) Examination of in-process interventions.

Where interventions by personnel are commonly performed during aseptic processing, these should be simulated during the study. This may include activities like picking up fallen vials using forceps, adjusting stopper grippers, fixing filling needles, etc. The air patterns should be carefully assessed for turbulent regions where contamination may be dispersed in an unwanted direction or accumulate[15]. In ideal circumstances, the airflow should continue to flow out of ISO Class 5/Grade A and into ISO Class 7/Grade B. Where excessive turbulence is created and air flows back from ISO Class 7/Grade B into ISO Class 5/Grade A, this will indicate a potential problem as shown in **Figures 2** and **3**.

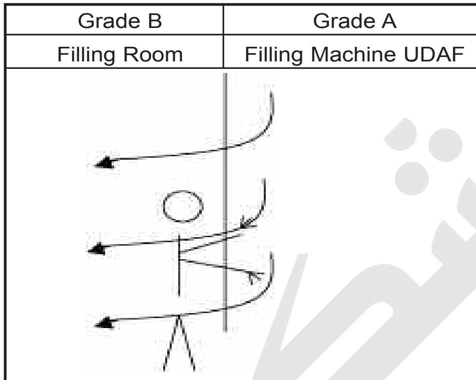


Figure 2: *Representation of an intervention by an operator into an ISO Class 5/Grade A zone. Individual performs intervention. Very little turbulence is generated and air stream is from Grade A to Grade B. This situation is satisfactory.*

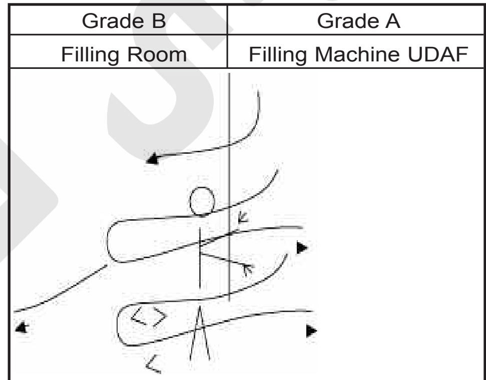
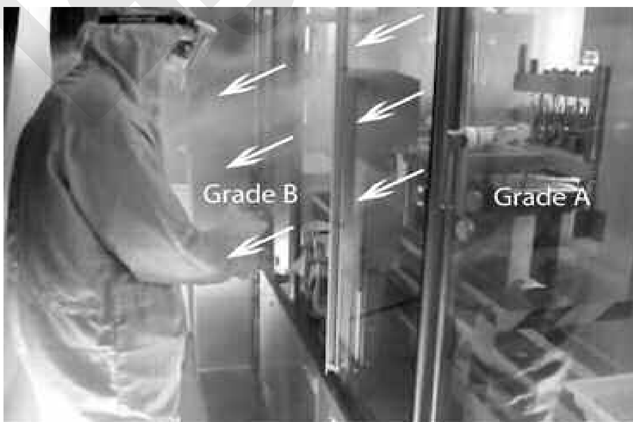


Figure 3: *Second representation of an intervention by an operator. Individual performs intervention. Turbulent airflows are formed resulting in some air being driven back into Grade A. This situation is not satisfactory.*



In addition the effect of the operator breeching the barrier between the critical zone and the cleanroom (such as between ISO class 5 and class 7) should be noted.

Figure 4: *Typical smoke pattern when a Grade A filling machine cabinet window is opened (note the additional light behind the operator to enhance the smoke visualization).*

d) Examination of the cleanroom.

The room in which the unidirectional airflow device is located must also be studied. This is important in order to determine if there is any impact of the room upon the unidirectional device (particularly any possibility of air ingress either in standard operation or where the filling machine doors are opened). It is also important to study the effect of personnel and equipment movement within the turbulent flow[16], in terms of general contamination control (this may indicate, for example, where there is dead air, particularly arising from local impediments[17] and thus any areas of potential contamination). It is important to see if the air in a cleanroom is supplied and mixed well to ensure that all contaminants are removed. For room studies, smoke should be delivered at the HEPA filter face and its distribution across the room noted.

Ideally, the air pattern will show that the airflow is characterised by small and temporary fluctuations and eddies caused by instabilities. The flow is not necessarily constant and will fluctuate around an average value. Such patterns will occur under UDAF when air meets an object. The impact of the temporary eddy should be assessed. Some surface materials can attract airborne particles based on the quality of the material (such as roughness, electrostatic properties). Where this occurs, the impact and potential concentration of contamination should be considered as appropriate. The contamination rate will depend on:

- Concentration of contaminants and motion of contaminants

  When cleanrooms are examined, the effect of the 'clean-up' time can also be measured. Here the room can be filled with smoke and then the time taken for the smoke to clear (as it is extracted and the room replaced with clean air from the HVAC system) captured. This part of the study will also indicate if there are any areas of the cleanroom that clear more slowly than others. Such information can be useful for future reference should a problem with particle counts occur in the future as the time taken for the room to clear is known.

d) Dynamic characteristics of the door opening and closing operation[18].

Doors of filling rooms and filling machines should be opened in order to simulate what might happen during processing. With filling rooms, if equipment is required to be transferred during an operation, this activity should be simulated. When examining the impact of door opening, the extent of backflow velocity, and thus the potential for less clean air ingression into a cleanroom, should be noted. This can vary depending upon the type of cleanroom door.

Information relating to the air distribution in the cleanroom can also provide supporting information about the function of pressure differentials. Most importantly, there should be a cascading positive air pressure differential system, where the "cleanest" space is maintained at the highest pressure and the lesser cleaner spaces decrease in pressure. For this the impact of the pressure gradient upon the airflow can be examined. The function of increased pressure is to force clean air outwards, thus preventing the ingress of contaminated outside air entering the clean zone. A pressure differential also prevents the entry of outside air when the door is open.

16.9 Reviewing airflow studies

Once complete, airflow studies should be reviewed against the pre-set acceptance criteria for the clean air zone, room or activity. The airflow pattern study report should contain details about the operating parameters and set points for the operating equipment involved in the study. The set/operating parameters could be the inlet fan speed, the exhaust fan speed, position of automated air control valves, pressure differentials, pressure differential between tunnel and isolators, etc. Problems should be noted and preventative actions considered. The most effective actions are those that lead to a change in practice or redesign, so as to eliminate the risk. Only where risks cannot be eliminated should monitoring be targeted[19]. Where significant changes take place, the airflow study should be repeated so that the effectiveness of the action can be measured.

Examples of problems often detected during airflow studies are reported in the table below with possible corrective actions:

Table 2: Common concerns from airflow visualisation studies and suggestions for corrective actions

Problem	Possible corrective action
Low air velocity inside a Grade A/ISO class 5 cabinet at the working height (vertical flow). Stagnant smoke pockets at the bottom side of the cabinet.	This problem is often caused by insufficient air exhaust at the bottom level of the cabinet windows. The windows should have openings at the bottom side big enough for allowing an easy exhaust of the air coming from the HEPA filters.
The air pattern inside the Grade A/ISO Class 5 is not unidirectional. There are turbulences immediately downstream the surface of the HEPA filters.	This can be caused by obstacles hidden behind the HEPA filters grid (lights, grid frame). Remove the obstacle; use lights specifically designed for Grade A/ISO class 5 cabinets. Another root cause for this kind of problems could be a not uniform velocity of the air coming from every single HEPA filter units installed in the Grade A/ISO class 5 cabinet. It is necessary to check the air velocity of each filter with a calibrated anemometer and replace any partially clogged filter.
There are turbulences at the interface between the Grade A/ISO class 5 cabinet (filling machine) and the adjacent pieces of equipment (example: depyrogenating tunnel, crimping machine)	Turbulences in these areas are almost unavoidable. It is important that the airflow study confirms that the air is always flowing from the inside of the most critical area (the filling machine) towards the adjacent areas. If the turbulence is too evident it can be minimized by differential pressure adjustments.

16.10 Documenting airflow studies

As for other qualification/validation activities, it is advisable that each airflow visualization study is executed following a pre-approved protocol. The protocol is also useful for planning accurately all the activities necessary for an effective execution of the study. Executing an airflow study is a relatively complex and time consuming activity that can require several days of work and consequent shutdown of the involved production area. Therefore it is very important that all the activities are appropriately planned and that all the required materials are available when needed. For example, it would be wise to have available a back-up for critical instruments (smoke generator, video camera) and plenty of consumables (dry ice, liquid nitrogen, or glycol solution), since it is not easily predictable how many times a specific production step could need to be repeated (mainly if it is the first study for a specific department/line).

An example of content of an air flow study protocol could be:

1. Title and approval page
2. Scope
3. Description of the area/equipment to be tested and selected conditions (use of a risk assessment could be advisable for selecting the appropriate conditions, such as: vial size, line speed, number of operators present, etc.)
4. Responsibilities
5. Instruments/materials
6. Activities to be covered (for example):

    a. Filling line set-up
    b. Filling line in static conditions after set-up
    c. Filling line in dynamic conditions
    d. Simulated interventions
    e. Environmental Monitoring
7. Acceptance criteria
8. Cleaning of the line and environment post execution

For each activity listed at point 6., a more detailed description of each single step to be covered should be included in the protocol, the execution of the activity must be similar to what happens during the aseptic filling simulation studies (Media Fill).

An example of a form to be used for describing the activities to be executed for simulating critical interventions and then for documenting their execution is attached (see **Appendix I**).

16.11 Reporting airflow studies

Once completed, the airflow visualisation study should be copied to permanent media (such as disc or computer hard-drive). The study should be given a unique reference number (or the protocol number) and the date and time of the study should accompany the recording. It is not advisable to edit the master study (original recording), as this could create suspicion to a regulator that the study has been adulterated. If a scene has been shot incorrectly, this should be noted in the final report. However, editing the study (example on a DVD divided in chapters) can be helpful for presenting it to the inspectors provided that the “master recording” remains unaltered in the event that a regulatory inspector wishes to view it. Furthermore, narration can be added to the recording if it is considered helpful, although in most circumstances this will be unnecessary if the accompanying report is written sufficiently well. Adding music to the recording is an unnecessary frivolity and should be avoided.

The study should then be reviewed by a competent person and a report generated. The amount of detail which goes into the report will depend upon the user (to the extent that the report describes each scene). Nonetheless, notable events should be described with reference to the time or frame number of the recording.

In compiling the report, the results of airflow studies should be carefully examined to see if the design of the room is appropriate and to determine if the activities conducted in the area are suitable for minimising airflow disruption (and where disruption occurs this will lead to decisions being required whether the design needs to be changed or the activities modified).

In addition, airflow studies should be considered in relation to the environmental monitoring undertaken within the cleanroom or unidirectional devices under normal operations. Where there is dead air or risk of turbulence which cannot be easily eliminated, then it is important that environmental monitoring takes place. This will involve consideration of the use of settle plates or active air-samplers (noting the concern that active air-samplers can themselves disrupt the airflow). The selection of sites for environmental monitoring should be documented[20].

16.12 Frequency of airflow studies

Airflow studies are normally undertaken for newly built cleanrooms and after significant modifications. The airflow studies presented to regulatory inspector should reflect normal operations.

One consideration is the frequency of repeating airflow studies so as to demonstrate a continuous state of control. The user will need to decide if airflow studies are undertaken only after significant modifications have taken place or whether they are carried out at regular intervals (as with the requalification of other critical cleanroom parameters). The frequency of requalification would need to be determined by some type of risk assessment.

16.13 Alternatives to airflow studies

Computational design systems are sometimes used as alternatives to airflow studies. Such approaches are more common within the microelectronics and semiconductor industries than with pharmaceutical cleanrooms (nevertheless such approaches can be used during the design phase for a new cleanroom). Embarking on alternative and novel approaches is more difficult in pharmaceutical cleanrooms because of the pharmaceutical stringent regulatory requirements for an airflow study.

Alternative approaches include numerical flow and particle transport modelling[21]. Here comparisons can be made between experimental data from an operating clean room and where theoretical predictions indicate that the model is sufficiently accurate to predict the main features of the flow and particle transport for various configurations and operating conditions[22].

16.14 Conclusion

This chapter has examined the importance of airflow studies as a means to visually understand the airflow within a cleanroom or clean air device, and has indicated that understanding the air pattern is important in order to know the level of contamination risk and to identify any potential weak areas within cleanroom design and operations. This chapter has also provided pragmatic tips in conducting a meaningful airflow study that will satisfy the regulatory considerations.

Although airflow visualisation studies are a necessary part of cleanroom assessments and can reveal valuable information they do not provide the complete picture of contamination concern or allow a complete assessment of contamination risks to be made. As Ljungqvist and Reinmüller have pointed out, for example, airflow studies must be combined with other observations and environmental monitoring data in order to entirely understand the cleanroom risks[23].

16.15 References

1. Jinno N. Study of airflow pattern in the clean room for manufacturing semi-conductors. *Proceedings of the Institute of Environmental Sciences* 1986:556-564.
2. Sandle T. Environmental Monitoring. In: Saghee MR, Sandle T and Tidswell EC. eds. Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices; New Delhi, India. Business Horizons, 2011, pp293-326.
3. International Organisation of Standardisation. Cleanrooms and Associated Controlled Environments. I. Classification of air cleanliness; II. Specifications for testing and monitoring to probe continued compliance with ISO 14644-1; III. Metrology and test methods. BS EN ISO 14644 Parts 1,2 and 3. 1999 (Part 3: revised 2005).
4. Food and Drug Administration. Guideline on Sterile Drug Products Produced by Aseptic Processing FDA, Rockville, MD, USA, 2004.
5. World Health Organisation. WHO GMP for Sterile Pharmaceutical Products, Working document QAS/09.295 Rev.1, Geneva: World Health Organisation, 2009.
6. European Commission. EU GMP Guide to Good Manufacturing Practice. Revised Annex 1: Manufacture of sterile medicinal products. Brussels, Belgium. European Commission, 2008.
7. Pharmaceutical Inspection Convention (PIC/S). GMP Annex 1 Revision 2008 Interpretation of the Most Important Changes for the Manufacture of Sterile Medicinal Products – Recommendation, January 2010.
8. Batchelor GK. Introduction to Fluid Mechanics. Cambridge: Cambridge University Press, 2000.

[9] GMP Trends Inc. GMP Trends Issue 20714. GMP Trends, Colorado, USA.

[10] GMP Trends Inc. GMP Trends Issue 20716. GMP Trends, Colorado, USA.

[11] Kuehn T, Marple VA, Han H, Liu D, Shanmugavelu I,Yourself SW. Comparison of measured and predicted airflow patterns in a clean room. *Proceedings of the Institute of the Environmental Sciences* 2008: 331-336.

[12] Klinberg S. Smoke studies: Clearing the mystery of air flow visualisation. *Journal of Validation Technology* Summer 2010:11-17.

[13] Ross S and Sandle T. Air pattern analysis of a filtration transfer. *Pharmaceutical Microbiology Interest Group News* 2007,**26**:3-4.

[14] Gail L. Facts and fiction in cleanroom metrology. *Journal of IEST 2010;***53**(1).

[15] Ljungqvist B and Reinmüller B. Risk assessment with the LR method. *Eur J Parenteral Sci* 2002; **7**(4):105–109.

[16] Boyle DR and Golay MW. Measurement of a recirculating, two-dimensional, turbulent flow and comparison to turbulence model predictions: steady state case, *ASME J Fluids Engineering* 1983; **105**:439-454.

[17] Settles GS, Huitema BC, McIntyre SS and Via GG. Visualization of clean room flows for contamination control in microelectronics manufacturing. In: *Flow visualization IV*: *Proceedings of the Fourth International Symposium*, Paris, France, Aug. 26-29, 1986 (A87-52301 23-34). Washington, DC, Hemisphere Publishing Corp., 1987, pp 833-838.

[18] Shigeo H, Kazutomo I, Yoshiaki K, Kaoru M. Dynamic characteristics of the door opening and closing operation and transfer of airborne particles in a cleanroom at solid tablet manufacturing factories. *Transactions of the Society of Heating, Air-Conditioning and Sanitary Engineers of Japan* 2004;**95**:63-71.

[19] Katayama H, Higo T, Tokunaga Y, Hiyama Y, Morikawa K. Establishment of critical contamination risk locations ("hot spots") in environmental monitoring by means of three-dimensional airflow analysis and particulate evaluation. *PDA J Pharm Sci Technol* 2005; **59**(1):49-63.

[20] Sandle T. Environmental Monitoring Risk Assessment. *Journal of GXP Compliance* 2006; **10(2)**:54-73.

[21] Murakami S, Kato S and Suyama Y. Three-dimensional numerical simulation of turbulent airflow in a ventilated room by means of a two-equation model. *ASHRAE Trans* 1987; **93**(2):621-641.

[22] Kuehna T H. Clean room aerosols: Predicting air flow patterns and particle contamination in clean rooms. *Journal of Aerosol Science*,1988; **19**(7):1405-1408.

[23] Ljungqvist B, Reinmüller B. The LR method in critical areas: airflow patterns and the design of aseptic interventions.

Appendix I

Example of a form for documenting an airflow study covering critical interventions on a filling line to be included in the study protocol. Similar forms may be used for the other steps (e.g. equipment set-up, environmental monitoring, etc.).

It is based on the use of a Digital Video camera (DV tape recording). The indication of the time code (that identifies every single frame) allows for easy retrieval of each simulation step. It is important that the date and time of the camera internal clock are synchronised with the local time in order to document on the video recording the exact time of the video takes.

Filling Line _______________
(Critical interventions to be executed)

Operation to be executed/simulated	Video recording data			Done by
	Tape #	Time code start	Time code end	
Vial removal from the accumulation table at the exit of the tunnel using the sterile forceps.				
Stopper removal from the guide using the sterile tool.				
Dosing pump and needle replacement (opening window A).				
Vial removal from the filling line with sterile forceps (opening window B).				
Simulation of line cleaning and sanitisation after vial breakage.				

COMMENTS

Executed by ______________________________ Date__________________

Reviewed by ______________________________ Date__________________

Cleanroom contamination sources and control measures

Eric Strauss, Senior Director, Global Head of Quality, Sterile Technology
Teva Pharmaceuticals, Israel

17.1 Introduction

In the pharmaceutical industry risk of microbial contamination is of the utmost concern. In order to keep the risk low, we must protect the product throughout the manufacturing process. Cleanrooms are used in order to control the environment where products most susceptible to microbial contamination are manufactured. While the highest cleanroom standards are naturally needed where the highest risk operations are performed, many of the potential sources of contamination are common to all classifications of cleanrooms. In this chapter, the cleanrooms discussed will typically be that used for high risk manufacture such as for aseptic processing.

Cleanrooms are classified to different grades based on the amount of particulate matter of a given size being present in a set volume (e.g. cubic foot or cubic meter) in the cleanroom air. There are different classification nomenclatures in use (e.g. ISO, EU Grade, FDA) but they all share a common basic premise that the lower the numbers of particles that are present, the higher the cleanroom standard. The table below will summarise the room classifications when measured in a dynamic operational mode.

The aseptic filling process is performed under ISO 5 (EU Grade A, FDA Class 100[a]) conditions.

This chapter will review various sources of potential microbial contamination that may affect the cleanroom and by association create risk to the product. While we will regularly refer to

Table 1: Cleanroom Classifications – Operational

ISO	EU Grade			FDA Class	
		*Number of particles =/> 0.5 µm per m³	*Number of particles =/> 5.0 µm per m³		Number of particles =/> 0.5 µm per ft³
5	Grade A	3,520	20	Class 100	100
6	ND	35,200	293	Class 1000	1000
7	Grade B	352,000	2,930	Class 10,000	10,000
8	Grade C	3,520,000	29,300	Class 100,000	100,000

ND – Not Defined
*These values are taken from ISO 14644-1 (3) and are approximate to those in the EU GMP guide

contamination of the cleanroom, it should be clear that the ultimate risk is the product itself. Common control measures used for prevention of the potential sources of contamination will be reviewed.

The main sources of microbial contamination of a clean room cleanroom and control measures to be taken can be divided into the familiar 4M categories of Materials, Machines, Method and Men. While we will approach each area separately, it should be noted that all four areas interact closely with each other and a failure in one area may affect another.

4M Sources of Cleanroom Contamination and Control Measures

17.2 Materials

Materials used in a cleanroom can be generated from an internal critical system on an ongoing basis or be a distinct object sourced and transferred as needed.

Materials entering a cleanroom can be further subdivided into:

17.2.1 Utilities

HVAC

Air supplied into a cleanroom has the potential to be a major source of contamination as it is the most copious material found in the room. Cleanroom air is typically supplied by an HVAC system (Heating, Ventilation, and Air Conditioning) that allows control of temperature, relative humidity as well as particulate matter concentration in the incoming air. Particulate matter is a source of physical contamination to the exposed product as well as a potential source of microbial contamination, as microbes may ingress into the room associated with the particulate matter.

In order to control the air quality and thus control these potential sources of contamination, multiple control measures related to the air handling systems are used. These include the purity of the air supplied, the air flow pattern within the room, the volume of air used and the relative pressure provided to the rooms.

The quality of the air relates directly to the particulate matter load. The particulate matter concentration of the air is controlled by using increasingly more restrictive filtration of the incoming and re-circulated air within the air handling system. In practice, the highest level of filtration usually required is via a High-Efficiency Particulate Air (HEPA) filter with the capability of filtering out 99.97% particles of 0.3µm or larger[1]. The temperature and humidity of the incoming air is also controlled, as these parameters can influence operator comfort[2]. The potential contribution of contamination by the operator will be discussed below.

The air flow pattern should be designed to provide sufficient air to constantly cover all critical areas where risk is high (e.g. where product is exposed). The physical bathing of the critical areas with the filtered air acts as a shield to help prevent contaminates from reaching the exposed products. While room designs can vary based on the process, a typical configuration will have ceiling mounted HEPA filters supplying air with sufficient velocity and volume to unidirectional sweep over the critical areas. Low positioned return vents are used to aid in sustaining the vertical air flow pattern from top to bottom.

It is typical to provide sufficient air volume to allow multiple complete air changes in the cleanroom. This in essence flushes the room with filtered air and is intended to continually remove potential contaminants that may enter or accumulate in the room during normal use. The amount of air changes required for cleanrooms where product is exposed is often sited to a minimum standard *not less than* 20 air changes per hour[3], but in practice it is common to see cleanrooms designed to provide a much higher number of air changes.

The amount of air supplied and exhausted in a cleanroom compared to that supplied to the adjacent rooms is also used to create a pressure differential cascade between adjacent rooms. This is an essential tool to control contamination entering from room to room together with materials and personnel. The pressure cascade should be designed to have a higher pressure where the more critical operations are being performed. Guidance values of 10-15 Pascal between rooms of different grades are suggested[4].

It is clear that the HVAC system with its multiple control measures is a fundamental element of creating cleanroom conditions. In order to maintain the control measures, and judge the status of the cleanroom, the air handling system must be monitored closely and maintained regularly. This includes routine monitoring of particulate matter, viable (microbial) environmental monitoring, differential pressures, temperature and relative humidity. Annual testing the HEPA filters (e.g. integrity-leak tests, air velocity) and air flow patterns via smoke tests are also important parts of regular maintenance of the air handling system.

Compressed Gases
Compressed gases (e.g. Air, Nitrogen) are often used in cleanrooms for operation of machinery or for direct product contacts such as when using Nitrogen gas overlay. While compressed gases themselves are not known to be a hospitable environment for microbes, they are not typically sourced from suppliers who document the microbial quality of the gas provided. In addition, as the storage and initial sections of the distribution systems are often situated in an uncontrolled environment, there is a possibility that the system can become contaminated.

This potential source of contamination is controlled by the use of 0.2µm sterilising grade gas filters within the distribution system, particularly near the point of use. The filters must be integrity tested and replaced at a pre-determined frequency. Gas filters are hydrophobic and common integrity tests used include Forward flow (diffusion) test, the Bubble point test and the Water Intrusion test[5]. The basic test principles for the Bubble point and Forward flow are similar with the filter being thoroughly wetted with a test solution such as Isopropyl Alcohol (IPA) and pressured air being applied to the upstream side of the filter. The integrity of the filter is determined versus pre-set criteria such as minimal pressure required to force the wetting solution from the filter membrane pours (inducing bubbles) in the Bubble point test, or maximal flow rate of air diffusing through the wetting solution at a given pressure in the diffusion test[6]. The Water Intrusion test has the advantage of not using IPA (that could be considered a downstream contaminant if tested in situ)[7].

In order to ensure the microbial quality of the compressed gas, a microbial bioburden sample is taken. As the overall risk for this source of contamination is low, the frequency of testing can be quarterly or less often depending on the history of the system (the cleanroom user should assess this by way of risk assessment).

17.2.2 Product materials

The topics in this chapter relate to potential contamination sources of a cleanroom that may then result in passing contamination to products. Starting material's quality impact on the final product is self-evident, but they will be evaluated here as a potential source of contamination of the cleanroom and subsequent manufacturing in the room that may be affected.

Starting Materials
Starting Materials for manufacturing products are usually not a major source of contamination of the cleanroom. This is mainly due to the practice of using materials of high microbial quality (i.e. low bioburden) and inclusion of bioburden reduction steps (e.g. sterilising procedures) in the manufacturing process[3,4].

Control measures on starting material microbial quality include sourcing from approved suppliers whose quality systems have been verified and receipt of materials with Certificates of Analysis which includes microbial testing. Alternatively in house testing for microbial attributes may be part of the material release procedure[8]. Further control measures include proper storage and handling of the materials in controlled warehouses and detailed instructions for sampling and dispensing materials within suitable protected areas to prevent adventitious contamination[9]. Pre-sterilised starting materials require greater attention to storage conditions and handling and will be addressed later together with pre-sterilised packaging components.

Bulk Product

Many cleanrooms are used for manufacturing products intended to be sterile. Some products can be terminally sterilised in their final container while others cannot and need to rely on pre-sterilisation of the components and bulk product before being aseptically assembled within the cleanroom. In the former case where a terminal sterilisation process is to be performed, the bulk product filled in the cleanroom can be in a non-sterile state and thus itself be a potential source of contamination of the cleanroom. This source of contamination of the room is controlled in two main ways. Firstly, control is gained by the use of high microbial quality starting materials as mentioned above. Using low microbial quality starting materials for products destined to be terminally sterilised is not acceptable as the initial bioburden of the bulk material must be controlled in order to ensure that the validated terminal sterilisation method capability is not surpassed. Secondly, it is common practice to include additional bioburden reduction steps, such as filtration through 0.2 μm rated membranes, were possible, regardless of the use of terminal sterilisation. This adds an additional level of sterility assurance as well as protecting the cleanroom.

Primary Packaging components

As with product starting materials, primary packaging components (such as vials and stoppers) are not considered a major source of cleanroom contamination. Primary packaging components are either pre-sterilised directly before entrance into the cleanroom, or during terminal sterilisation of the product. Control measures in place include use of validated sterilisation methods (e.g. steam sterilisation of stoppers, depyrogenation/sterilisation of glass vials), sourcing components from approved suppliers and compiling data on incoming bioburden of the components. In addition, we protect the sterilised components (e.g. stoppers) by controlled storage and handling. This can be accomplished by only exposing them to HEPA filtered air in controlled conditions and for limited times within the cleanroom.

Pre-sterilised primary packaging components (as well as pre-sterilised starting materials) received from a supplier are naturally a negligible source of potential contamination due to their sterility, but do require added attention. Correct storage and handling of these components may be a challenge when using a general warehouse in order not to compromise the sterile integrity. As opposed to non-sterile starting materials and components, the pre-sterilised items will not undergo a further sterilisation process during manufacture and thus maintenance of the sterile state is critical. Passage into the cleanroom of pre-sterilised components is also more complex. Controlling the sterility integrity during storage and handling is often accomplished by use of multiple protection coverings (e.g. double bags). The passage of the components into the cleanroom is performed in stages where subsequent layers of protection are removed together with the sanitisation of the surfaces. This allows protection of the sterilised components as well as reducing risk of introducing contamination into the cleanroom.

17.2.3 Supplies

Environmental monitoring media and supplies

Environmental monitoring is a control measure used to assess the status of the cleanroom. To this end, culture media (e.g. settle plates, contact plates) and supplies (e.g. swabs) need to be passed into the

cleanroom. It is also common practice for culture media used in aseptic filling areas to be supplied irradiated. The transfer of the media and supplies is accomplished by use of multiple protection coverings (e.g. double bags). Auxiliary equipment needed for environmental monitoring (e.g. active air sampling devices) are disinfected before entrance into the cleanroom. It is common practice to dedicate pieces of equipment for cleanroom use and avoid removing them from the cleanroom.

Cleaning Materials

Cleanrooms need to be cleaned and disinfected at a high frequency. While the materials (e.g. disinfectants) are by nature antimicrobial, they themselves may become a source of potential contamination if not prepared and handled properly. This is due to the fact that not all disinfectants are effective against all types of microbes. Control measures to avoid this type of contamination include using disinfectants shown to be effective against the routine flora isolated in the cleanroom while being compatible with materials of construction found in the room. An additional control measure is to reduce potential bioburden of the cleaning and disinfecting materials via filtration (e.g. 0.2µm rated membrane) before entering them into the cleanroom. This filtration is especially directed to prevent spores, which are microbial forms very resistant to destruction and are not typically killed by routine (e.g. non-sporicidal) disinfectant materials. The use of specialised sporicidal disinfectant materials are required if spore contamination has been found in the cleanroom. Documentation of their preparation, including filtration step and setting of an end of use date, are measures typically taken to control the quality of the materials.

Periodic rotation (i.e. use of multiple types) of disinfectants has been a topic for a long time with the potential for selecting out resistant strains to the disinfectant type being debated. Although there is an argument that the naturally low bioburden present in a controlled cleanroom does not lend itself to this selection process, use of multiple disinfectant types is still encouraged by some authorities[4]. One strategy that is often seen is to rotate the use of routine disinfectant with a sporicide, even in cases where spores are only rarely detected.

Cleaning aids used in the cleanroom are likewise controlled by using sterilised items (e.g. wipes) made of material compatible to a cleanroom environment (e.g. non-shedding). Auxiliary equipment such as mop handles and buckets need to be made of materials that can withstand repeated contact with the cleaning materials and are themselves readily cleanable and disinfected. It is common practice to dedicate cleaning equipment for use in the cleanrooms to avoid their use in rooms of lesser classification.

17.3 Machine

Machines can be further subdivided into:

17.3.1 Component Preparation equipment

Vial/Ampoule washers

Vials and ampoules are common primary packaging components used in the preparation of sterile dosage forms. They are commonly sourced directly from the supplier and require washing and sterilisation before entrance into the cleanroom. The equipment used to wash the components needs to be of suitable design and employ utilities of sufficient quality in order to prevent the components from being contaminated. The washing cycle includes internal rinsing, external rinsing and drying stages to remove debris remaining from the manufacturing and handling of the components. The water and air quantities and pressure of the stages are controlled. The quality of the water used is at least that of pharmacopoeia grade Purified Water for initial rinses with Water for Injection (WFI) quality for the final rinse although it is common practice to use WFI for all rinses if available. WFI is tested to a rigorous standard with microbial specifications of less than 10 colony forming units (CFU) per 100 millilitres and not more than 0.25 Endotoxin Units per millilitre[10, 11].

If water is re circulated in the washing machine, it should be filtered through a bioburden reduction membrane. Compressed air is used for drying and it is also filtered through a bioburden reduction membrane. Once the components are washed, they need to be handled in a manner to prevent contamination until the next step of their preparation for use (e.g. sterilisation/depyrogenation). The most common way to ensure integrity is to configure the washing machine to directly feed a depyrogenation tunnel. This avoids the need to load the components into trays and transfer them to the depyrogenation oven. If the later tray method is used, care should be taken to control the cleanliness of the trays and to protect the components exposure to the environment.

17.3.2 Component Sterilisation equipment

Autoclaves

Autoclaves are designed to sterilise equipment, supplies, components and finished products. They use pressurised steam or super heated water to transfer high heat to the material being treated. This heat effectively sterilises the material by killing any contaminating micro-organisms via coagulating or denaturing microbial proteins.

While an autoclave's potential for adding microbial contamination to the cleanroom is minimal, improper design or use of an autoclave may play a factor in the cleanroom status. Autoclaves used for steam sterilisation of equipment to be used in the cleanroom, are preferably of the double door design. In this manner, the sterilised goods can be unloaded directly into a cleanroom. This allows the separation of sterilised from non-sterilised goods as well as avoidance of potential contamination of sterilised goods if unloaded into areas where non- sterile items are prepped and wrapped for autoclaving. The cleanroom used for unloading the autoclave is typically not the cleanroom where aseptic filling occurs but rather an adjacent area. This prevents excess steam or condensation that may be present in the autoclave from entering the aseptic core when the autoclave door is open. Cycles developed with sufficient drying phases and localised exhausts near the door are measures to reduce this phenomenon. Proper use of a double door autoclave includes not using it to pass through materials that have not undergone a sterilisation cycle.

Depyrogenation ovens/tunnels

Depyrogenation units are intended to both sterilise and depyrogenate (i.e. Destroy endotoxin). These units use dry heat conducted to the contaminating micro-organisms to coagulate and denature the microbial proteins. Although these units require higher temperatures and longer exposure times than autoclaves to accomplish sterilisation (due to the absence of moisture which aids the transfer of the heat), the higher temperature allows the destruction of endotoxin which is a heat stabile component of the wall of many Gram-negative bacteria[12].

Depyrogenation ovens should be designed and treated as described for autoclaves. As mentioned above it is preferable to use a depyrogenation tunnel rather than an oven as this lowers the risk of contamination of the components while being transferred into the cleanroom. In any case, the depyrogenation equipment does have an additional potential source of contamination than an autoclave due to the large quantity of air they use to cool the components after depyrogenation. The air is typically drawn from the room surrounding the tunnel or oven and must be HEPA filtered in order not to contaminate the components before they enter the cleanroom. The HEPA filters associated with the depyrogenation equipment are qualified at a routine frequency (e.g. annually).

17.3.3 Pass-through devices

Pass-through devices allow the transfer of materials between rooms. The most common type of pass-through device is the double door pass-through box and we shall use this as our example. Where the pass-through connects rooms of different classifications, there is a chance of the contamination. Control measures taken to protect the cleanroom start with the design of the pass-

through device. An active pass-through box should contain a source of HEPA filtered air to create a local pressure differential between the pass-through and the adjoining rooms. The pressure cascade should follow the principles outlined before with the flow going from the higher classification to the lower classification. Passive pass-through boxes lack the air source and rely on other measures. These measures include interlocking doors that prevent both doors from being opened simultaneously and time delay locks that prevent opening of the second door for a pre-set resting time once the first door was closed.

The pass-through boxes must be cleaned and sanitised regularly. It is common practice to sanitise them with each passage of materials into the cleanroom. Another control measure to prevent contamination through the pass-through box is the use of germicidal lights (e.g. ultraviolet light) inside the box. These lights should not be used while materials are present in the box that may be adversely affected by the emitted radiation. The use of these lights is not as wide spread as they may have been in the past and their effectiveness is often not known. Proper procedures for use of the pass-through box while transferring materials are important. Sanitisation of materials entering the box along with sufficient contact time for the sanitising solution to be effective allow reduction of bioburden that may be on the outer surfaces of the materials being transferred. Use of double bag protection covers mentioned above find their use when materials are passed into the cleanroom via the pass-through boxes. It is sometimes possible to extract the innermost bagged material during the transfer while leaving the outer bag in the pass-through box to be removed out via the room of lesser classification.

Other pass-through devices may have surface sanitisation processes integrally associated with them such as Continuous Exterior Surface Decontamination (e.g. E-beam) tunnels and Vaporised Hydrogen Peroxide (VHP) transfer units and afford a high level of protection to the filling machine during the material transfer. Generally these types of transfer devices are coupled with Isolator barrier systems (reviewed below). As Isolators are usually situated in rooms of a lower cleanliness class than traditional lines, the extra protection granted by the surface sanitation during the material transfer may compensate for any risk of contamination to the material prior to the transfer.

17.3.4 Filling machines

The machinery with the highest potential risk of contaminating the product is the filling machines. The filling machine needs to both provide continuous protection of the critical areas where product is exposed, as well as act as a barrier to prevent the major source of cleanroom contamination (e.g. human operators) from approaching the critical areas during routine operations. Due to critical manufacturing activities occurring within the filling machines (e.g. aseptic filling and stoppering), the direct contamination risks to the product will be discussed in this section rather than potential contamination of the cleanroom in general.

As mentioned above, the filling machine needs to be designed to provide sufficient air to constantly cover all critical areas where risk is high (e.g. where product is exposed). The machine can itself include integral air handling units supplying HEPA filtered air or be configured to channel air supplied by ceiling mounted HEPA units. In either case the control measures relating to air quality, air flow patterns, differential pressures and preventative maintenance of the air handling system are essential.

Just as important as supplying a source of high quality air to the filling machine is preventing unwanted lesser quality air from entering. The design and use of barrier systems helps control the ingress of air as well as providing a physical barrier protecting against operator proximity and other potentially contaminating activities occurring in the cleanroom. There are many variations available today and we will only review the basic types. All materials used for the barriers need to be easily cleanable and withstand repeated treatment with disinfecting agents

The simplest type of barrier around the filling machine is a strip curtain. These are traditionally made of overlapping slates of plastic material (e.g. Lexan®). While these barriers are easily configurable to the machine layout, the ease at which they can be crossed is in itself a potential weakness. Although they may be fitting for local protection of large items that require ease of accessibility (e.g. large mobile tanks), they are not the barrier of choice for protecting critical areas within the filling machine were access needs to be restricted. In addition, cleaning of the strips can be difficult.

Other barrier systems use a variety of rigid walls to protect the critical aseptic core of the filling machine. As visibility of the filling machine is important, the materials of construction for the rigid wall are made of glass or transparent plastic material.

The walls of the barrier can be designed as movable doors or rigid plates with access to the internal surfaces of the filling machine via glove ports. When gloves ports are used, the gloves require suitable maintenance. The frequency of sterilising/sanitising and change out of the gloves, as well as routine integrity testing for leaks must be determined. The environmental monitoring program should include sampling of the gloves used post use.

The manner of use of the barrier system is of utmost importance as a control measure to limit potential contamination of the critical areas of the filling machine. Access to the interior of the machine should be via the glove ports whenever possible. Use of the doors to gain access to the interior of the machine should be limited to set up activities that cannot be accomplished via the glove ports and should be followed by sanitisation of the affected areas. Door panels are often supplied with external over-arching HEPA filtered Unidirectional Airflow (UDAF) units, also referred to as Laminar Flow units[13], to provide flow air coverage to help protect the door openings if utilised. If access via a door panel is required during routine operation, this is considered a critical intervention. A procedure should be in place to clear the line of any exposed product during the intervention and include re-sanitisation of the line before production can be restarted. A risk assessment of the intervention types, including challenge via media simulation is standard practice. Please note that these types of interventions are to be avoided whenever possible and successful challenge in a media simulation is not in itself sufficient to qualify the performance of a poor practice. Interventions should be recorded in the batch documentation and reviewed as part of the product batch record review.

Transfer ports need to be designed into the barrier system to allow regular introduction of the materials for routine use (e.g. stoppers). The type of ports used can range from simple flap openings above a hopper to rapid transfer ports (RTP) that provide a high level of protection. Use of the transfer ports likewise needs thought in order to reduce their use to a minimum. For instance, considerations regarding environmental monitoring should include the amount of materials required during the manufacturing session, as well as their placement within the filling machine in order to minimise the number of passages through the transfer ports.

The use of rigid barriers is a widespread industry practice and has been evolving. Restricted Access Barrier Systems (RABS) are common types of rigid barrier protections that includes multiple concepts discussed above together to create a high-level protection system. RABS include the use of glove ports (or equivalent), unidirectional airflow, material transfer systems and mandated procedural activities when interventions are performed. The RABS concept incorporates the used of Sterilisation-in-place (SIP) for product contact pathways whenever possible. RABS can also be designed to allow hermetically sealing of the barrier (i.e. Closed RABS) and use of a sporicidal surface sanitisation such as VHP to control bioburden after the filling machine has been set up and before manufacturing has commenced. The use of this type of surface sanitisation is an added control measure to limit risk of contamination of the filling machine, but does not replace the

need for sterilising product contact equipment. Large product contract parts not connected to a SIP system (e.g. stopper hopper bowls) are sterilised outside the filling machine and set-up using aseptic technique even when VHP of the surfaces is used.

The highest level of barrier protection to a filling machine is provided by Isolators. These barrier systems are similar to closed RABS but include no option to perform an open door intervention during manufacturing. Primary packaging components such as vials will typically enter only via depyrogenation tunnels and RTPs are used exclusively to introduce or remove materials from the filling machine. SIP is used to sterilise product contact surfaces and surface disinfectant is used such as VHP. As no breach of the Isolator is allowed during production, the classification of the cleanroom surrounding the Isolator can be of a lower class than for other barrier systems[3].

17.4 Method

Poorly considered or executed methods could themselves contribute to potential contamination of the cleanroom.

Methods can be further subdivided into:

17.4.1 Work Instructions

Cleaning

Suitable cleaning and disinfecting of the cleanroom is the principal control measures used in maintaining the room as “clean”. Work instructions are used to describe methods for cleaning and disinfecting cleanrooms. These instructions need to be clear and comprehensive. Disinfectant agents and equipment to be used need to detailed, as well as the frequency and level of cleaning required. As with all materials entering the cleanroom, strict control of cleaning and disinfectant agent quality is needed to ensure they are fitting for their intended purpose, as discussed above. Work instructions should include the techniques to be used in performing the actual cleaning and disinfection of each surface of the cleanroom. In addition, any required contact times for the disinfectants to achieve their desired effect should be documented. Documentation of all materials used and actions performed is an important part of providing evidence that the cleanroom was prepared and maintained as required.

Manufacturing

Manufacturing instructions have an important role in controlling potential contamination of the product[14]. Specifically, instructions for handling sterilised materials must be included in the manufacturing instructions to reduce the risk of contamination. Use of sanitising agents (e.g. 70% alcohol) for cleaning work surfaces and frequently sanitising gloves should be incorporated in the manufacturing instructions. Types of tools to be used (e.g. sterile forceps) should be part of the manufacturing instructions or clearly defined in associated SOP’s for working in a cleanroom. Directions for operating machinery in the cleanroom must likewise be unambiguous. The manufacturing instructions may include a step to confirm the status of critical utilities (e.g. HVAC) by recording differential pressures from local gauges when on-line building management systems are not available. Simple steps in a manufacturing record to verify and document that a local UDAF unit is in the working position before initiation of manufacturing can prevent potential compromise of the cleanroom environment. To help reduce potential contamination related to recording activities, specially treated paper is available that is non-shedding and sterilisable. Use of paperless production records (electronic systems) can further reduce these risks.

17.4.2 Environmental Monitoring

Environmental monitoring is used to follow the performance of the cleanroom. The monitoring program must be designed to cover both viable and non-viable sources of contamination. Sampling sites, methods of sampling and monitoring frequency are all important elements of a robust environmental monitoring program. Airborne, surface and personnel viable monitoring, as well as

particulate matter (non-viable) monitoring are typically included in the program with the critical areas requiring more frequent sampling. The section below will only describe basic principles of an environmental monitoring program and is not exhaustive of the subject.

For particulate (non-viable) monitoring, it is common practice to install remote continuous monitoring systems for the critical areas (such as filling lines and surrounding rooms) thereby removing the need for placing and removing mobile probes. Sampling points in critical areas should be chosen adjacent to where product is exposed (e.g. filling point, stoppering point). Sampling probes should be directing into the unidirectional airflow and be close (30 cm) to the working surface. Acceptance criteria for action limits are set in standard regulations[3]. In this author's experience alert limits are often being set at 70% of the action limit. In order to utilise the particulate matter system as an on-line monitoring tool, alarms (e.g. visual and audible) should be available to the cleanroom operators with a clear action plan to follow when alarms occur. The alarms should be carefully recorded in order to facilitate evaluation of the particulate matter data during product batch record review to determine the status of the cleanroom. This evaluation may allow correlation of particulate matter excursions to activities or interventions in the manufacturing procedure. This knowledge may lead to identification of risky activities and their correction.

Viable monitoring involves the use of microbial culture media capable to detecting typical cleanroom microflora, such as tryptone soya agar. Care must be taken that the media is prepared and handled in a manner to be effective (e.g. sustain its growth promoting ability) and maintain sterility so as not to potentially contaminate the cleanrooms. Methods used for viable monitoring include passive air sampling (e.g. settle plates) and active air sampling where devices are used to draw a set volume of sampled air across the surface of a culture media unit. Placement of the media should represent the critical working areas and sampling times should represent the critical activities performed. Active air sampling devices should not themselves disturb the air flow within the critical area. Repeated interventions to place/replace media units within the critical area may in itself create some risk. Validating a four hour exposure time for settle plates is one way to reduce the number of interventions required. Use of remote sampling heads for active air systems is another way to reduce interventions by eliminating the need to introduce and remove the active air sampling device.

Surface sampling is another important part of viable environmental monitoring. Methods commonly used include direct media contact plating (e.g. contact plates) for smooth surfaces and swab sampling for hard to reach and non smooth surfaces. As these methods involve direct contact to sterile surfaces, the risk of contamination of the surfaces by the act of sampling is high. Therefore surface sampling is only performed after manufacturing activities have concluded.

Personnel monitoring involves sampling operator's garments and gloved hands. Garments are typically monitored at multiple sites (e.g. arm, chest, and facemask). Frequency of sampling can range from once a week to once a day. Operator's gloved hands are of a larger concern and are monitored at a high frequency. Finger dabs for both hands are typically performed upon an exit from the cleanroom and may be required at each exit or at a minimum of once per shift. Where the manufacturing process requires a large number of filling machine interventions, finger dabs may be required after each intervention. If such sampling is performed, the operator should thoroughly disinfect his gloves after sampling or exit to the appropriate area to replace his gloves. While it is common practice for operators to frequently disinfect their gloved hands while in the cleanroom, this should not be done directly before finger dab sampling otherwise an inaccurate assessment of personnel hygiene will be obtained. EU based recommended limits for finger dab monitoring is less than 1cfu/glove (5 finger dab) for operators in the ISO 5 area and 5cfu/glove for operators in ISO 7 areas (4). The USP suggests in a non-binding informational chapter limits of 3cfu/glove and 10cfu/glove for ISO 5 & ISO 7 areas respectively[15]. Although no explicit limits are

given in the FDA Aseptic Guide, it is this author's opinion that the FDA would expect limits set as per the EU and not per the USP.

Out of limits results from environmental monitoring programmes should be investigated as described in a clearly defined in a SOP. Corrective and preventative actions should be taken, as appropriate. In addition, the results of environmental monitoring should be periodically assessed for trends and actions taken if adverse drift or upward trends develop.

17.5 Men (Personnel)

It is universally accepted that the operators are the single largest potential source of contamination in the cleanroom[16]. We naturally harbour micro-organisms and can act as vectors transporting these micro-organisms into the manufacturing environment upon our clothes, skin and hair. Humans shed millions of skin cells every hour and although not every skin cell may harbour microbes, the potential contribution to the cleanroom bioburden from skin borne micro-organisms is huge[13, 17]. Control measures used to combat *people* as a main source of cleanroom contamination can be divided into three main categories. The first category deals with distancing the operator from the critical areas if at all possible. These principles were covered above when discussing the barrier systems built around the filling machines. Barriers that discourage proximity of operators from critical areas reduce the risk of contamination. Isolator barrier technology is at the forefront of removing the operator from the critical areas of the filling machine. In addition, filling lines using more automation and less human intervention in principle lessen the risk of contamination from the human operator.

This next section will deal with two other categories of control measures that help reduce potential contamination from *people* in cleanrooms where human operators are still required. These are gowning which deals with containment of the contamination, and training which helps its reduction of occurrence.

Personnel can be further subdivided into:

17.5.1 Gowning

Garments

In order to contain potential contaminating skin cells or other sources of contamination emanating for our bodies (e.g. spittle) we use special gowning. For gowning to be an effective control measure, the gowning concept used by the facility must be clearly defined in working instructions and include the basic *where* (different garments are required), *when (*are the garments worn), *what* (garments are needed) and *how* (the garments are to be used) of gowning.

Firstly, it is common practice in all pharmaceutical environments to reduce the level of potential contaminants from the outdoors by having all personnel who may enter the manufacturing areas perform a basic garment change into plant garments upon entrance into the factory. Depending on where the operator may be required to enter, the garment change may be as little as donning an overcoat and shoe covers, to full removal of personal outer clothing and changing into plant scrubs. For personnel that will enter cleanrooms, additional garmenting steps will be taken. The changing regime is in incremental stages with successively more comprehensive gowning used as the classification of rooms required to enter is higher. For instance, personnel required to enter the lower level classified area (e.g. Grade D or C) may change their plant garments into a clean coverall or fresh overcoat. New shoe covers and use of hair/beard covers are commonly used. Please note that while the exact type of garment used should be commensurate with the type of activity to be performed, it is a general principle that the garments themselves are to be clean and be constructed of material that is non-shedding of particles and fibres. Dedicated changing rooms are used for entry into classified areas.

Subsequent entry into the higher classified cleanrooms (e.g. Grade B and Grade A), require an additional change. Grade D/C clothing is replaced with Grade A cleanroom gowns. This clothing is

constructed of material that is non-shedding of particles and fibres. As the garments themselves should not be a source of potential microbial contamination they are sterilised or sanitised before use. In order to maintain their status, they are individually wrapped and sterilised (e.g. steam sterilisation, irradiation). One time use disposable or multiple use re-usable garments may be used. Where re-usable garments are used, care must be taken when they are laundered and handled so as not to add contamination (e.g. particulate). Procedures should be in place for controlling the quality of the garment that includes knowledge of the maximum number of laundering/sterilisation cycles the garment can withstand as well as examination of the integrity of the garment.

The gowning system typically consists of a full body coverall with additional full head (hood) and foot (boot) coverings. Facemasks and goggles are worn to cover all exposed skin surfaces of the face. Non-powdered gloves are worn and garment openings such as arm and leg cuffs are often closed via elastic banding or snaps. Tucking hood into neck, sleeves into gloves and pant legs into foot covering provides an additional overlap to help contain particles from escaping the gown.

The gowning components need to be of suitable size to fit the operators. Items that are too tight may tear and thus loose integrity or cause irritation to the operator which may lead to enhanced shedding of particles. Likewise too loose garments may not provide sufficient closure or obstruct operator movement.

The changing rooms and gowning procedures are designed to allow operator to protect the gowning integrity as the gowning proceeds. Step over benches are often used to facilitate a separation within the changing room that demarks the area where the gowning change is initiated, from where the fully gowned operator is to end up. This later area of the changing is generally required to be at the same classification as to the room it leads into.

17.5.2 Training

As even the most comprehensive gowning system cannot totally prevent contamination from personnel in the cleanroom, training is needed to help reduce the risk of such contamination occurring.

Basic Microbiology and Hygiene

Training starts with familiarising personnel with basic concepts of microbiology and hygiene. This type of training is often part of general GMP training and is not restricted to those personnel who will be entering a cleanroom. Understanding that microbes cannot be seen with the naked eye and are ubiquitous to our environment, may impress upon an operator the scale of the challenge we face in preventing contamination. Explaining how they can multiple quickly with minimal growth requirements and their potential adverse medical implications to patients will emphasise even more so the need to be vigilant in sustaining control measures that have been put in place. Highlighting the importance of personal hygiene including the need to report any medical conditions that may increase risk of contamination should be part of this basic GMP training. Medical examinations initially and periodically thereafter are also fundamental control measures to prevent operator sourced contamination of the cleanroom.

Gowning procedure and qualification

Gowning training is performed for all personnel entering a cleanroom. The garments can be unwieldy to put on as they are often one piece jumpsuits. As we wish to keep them in as clean a state as possible, agility is often needed to keep the garment from touching unclean surfaces such as floors or exposed parts of our skin. Instruction is given on the need to remove all make-up and jewellery. The sequence of donning the garments (e.g. top down staring with head cover, mask, body suit, shoe covers and finally gloves) along with in which change room area each of the actions is performed is a part of the training. Visual aids in the changing room, such as detailed

pictures of each gowning step, can help with operator compliance to the gowning procedure. Before leaving the changing room to enter the cleanroom, the operator checks his appearance in a mirror to confirm the gowning is correctly in place. Gloved hands are sanitised with non-water based sanitiser as water sources and sinks are not allowed in close proximity to higher classified cleanrooms.

Qualification of the gowning procedure is performed by sampling with culture media (e.g. contact plates) at several points of the gown to judge the effectiveness of the gowning procedure training. A visual observation by a competent member of staff is also normally performed. Typically three successful gowning with minimal or no contamination is required for the operator to be qualified in the gowning procedure. Once personnel are qualified in the gowning procedure, they can enter the cleanroom.

Cleanroom Behaviour

Training on cleanroom behaviour should start before the first entrance into the cleanroom. In order to reduce the chance of generating and release of particulate matter, guidelines to cleanroom behaviour include using only slow movements, no touching of the garment especially the facemask, no hand contact with the floor, minimal talking and keeping distance from critical areas whenever possible. In addition, loose garments or torn gloves should only be remedied outside the cleanroom. Overcrowding of the cleanroom should be avoided with the area being staffed with only the number of personnel required for the activities being performed.

17.5.3 Aseptic Technique

Aseptic techniques are procedures employed to reduce the chance of contamination being transferred to the product during manufacturing activities. Some of the principles include using only sterilised and disinfected tools to perform operations, not working over open exposed components, keeping distance from the critical areas and limiting the time an activity is performed to a minimum. Actual aseptic handling techniques can be taught in a laboratory setting with culture media manipulations as an initial training. Successful participation in a media fill simulation is a required part of the cleanroom operators' overall qualification and attests to the mastering of aseptic technique.

17.6 Summary

The sole purpose of a cleanroom in pharmaceutical manufacturing is to provide a controlled environment wherein medicines can be processed with low risk of contamination. This chapter was intended to give a basic overview of the potential sources of cleanroom contamination and review control measures typically used to combat them and thereby reduce risk. All materials entering the cleanroom are potential sources of contamination and as such need to be controlled as to their initial microbiological quality as well as preserving this quality as they are transferred into the room. Proper design and usage of machinery used within, and communicating to, the cleanroom are essential to prevent ingress of contamination as well. Working procedures appropriate to the cleanroom must be in place in order not to compromise the cleanroom environment during operation. Lastly, the greatest potential source of cleanroom contamination is the human operator. Control measures designed to negate this risk revolve significantly around how the operators are gowned and trained to perform their jobs. For control measures to continually sustain the cleanroom in the desired state, they require vigilance in their execution and maintenance.

17.7 References

[1] International Society for Pharmaceutical Engineering (2007). *ISPE Active Pharmaceutical Ingredients Baseline® Guide Second Edition.* s.l.: The Society for Pharmaceutical and Medical Device Professionals, Vol. 3., Tampa, Florida, USA

[2] Code of Federal Regulations. Part 211.42 Design and Construction features. *The Code of Federal Regulations Title 21- Food and Drugs.* Subpart C – Buildings and Facilities, Parenteral Drug Association, Bethesda, MD USA.

[3] Food and Drug Administration. (2004). "Guideline on Sterile Drug Products Produced by Aseptic Processing", FDA, Rockville, MD, USA.

[4] Euradlex. (2009). "The Rules Governing Medicinal Products in the European Community", Annex 1, published by the European Commission, Brussels.

[5] Jarmey-Swan, C. (2008). "Filtration". In Hodges, N. and Hanlon, G. [Eds.]. *Industrial Pharmaceutical Microbiology Standards & Controls.* Haslemere: Basingstoke: Euromed Communications, S7.

[6] Parenteral Drug Association. (2008 rev.)."Sterilising Filtraion of Liquids", *PDA Technical Report.* Technical Report No. 26, March 1998 (revised 2008), Vol. 62, S5.

[7] Dosmar, M, *et al*. "The Water Pressure Integrity Test – A new Integrity Test for Hydrophobic Membrane Filters", 4, July- August 1992, *Journal of Parenteral Science & Technology*, Vol. 46, pp. 102-106.

[8] Code of Federal Regulations. Part 211.84 (d)(6) "Testing and approval or rejection of components, drug product containers, and closures". *The Code of Federal Regulations Title 21- Food and Drugs.* Subpart E- Control of Components and Drug Product Containers and Closures. Parenteral Drug Association, Bethesda, MD USA.

[9] Code of Federal Regulations. Part 211.80 General requirements. *The Code of Federal Regulations Title 21- Food and Drugs.* Subpart E- Control of Components and Drug Product Containers and Closures. Parenteral Drug Association, Bethesda, MD USA.

[10] European Pharmacopoeia. (2011). Water For Injections. Vol. 7.0, 0169. European Directorate for the Quality of Medicines and HealthCare, Brussels.

[11] United States Pharmacopeia and National Formulary (2011). Water for Injection. *United States Pharmacopeial Convention, Inc.*,Vol. 34, Rockville, MD, USA.,

[12] Guy, D. (2003): "Endotoxins and depyrogenation". [In Hodges, N. and Hanlon, G. [Eds.]. *Industrial Pharmaceutical Microbiology Standards & Controls.* Haslemere: Euromed Communications, s.l.: Basingstoke: Euromed Communications, p12.

[13] Whyte, W. (1991): "An Introduction to the Design of Clean and Containment Areas". In Whyte, W. (Ed.) *Cleanroom Design.* Chichester : John Wiley & Sons,.

[14] Code of Federal Regulations. Part 211.113 Control of microbiological contamination. *The Code of Federal Regulations Title 21- Food and Drugs.* Subpart F- Production and Process Controls. Parenteral Drug Associaiton, Bethesda, MD USA.

[15] United States Pharmacopeia and National Formulary (2011). Microbiological Evaluation of Clean Rooms. Vol. 34, <1116>, *United States Pharmacopeial Convention, Inc.*, Rockville, MD, USA.,

[16] Sharp, J. *et al.* (2010): "Contamination of cleanrooms by people", *European Journal of Parenteral and Pharmaceutical Sciences*, Vol. 15, No.3, pp. pp73-81.

[17] Owers, K. L., James, E. and Bannister, G.C. (2004): "Source of bacterial shedding in laminar flow theaters", *J. Hosp. Infection*, Vol. 58, pp. 230-232.

Notes:

[a] FDA has officially adopted ISO nomenclature, but the *Class XXX* nomenclature is still commonly used within industry when referring to FDA cleanroom classification.

Particle counters and particle counting

Tony Harrison, Pharmagraph, Sandhurst, U.K.

18.1 Introduction

Air particle counters are used in many industries as a method to determine the number and size of airborne particles. In the GMP cleanroom environment, they are used to provide an indication that the manufacturing environment is safe to use for its intended purpose and are in particular used in areas for sterile drug manufacturing.

This chapter describes how particle counters work, the required calibration and performance requirements for air particle counters that are used to classify, qualify and monitor the GMP cleanroom environment, some practical guidance on using particle counters and some hints and tips on troubleshooting.

18.2 Air particle counters and the GMP cleanroom

Generally, GMP sterile cleanroom manufacturing areas are supplied with a HEPA-filtered air supply at a positive pressure relative to the surrounding areas to prevent contamination entering from these areas. This air supply is also used to flush away any contamination that may be generated inside the cleanroom by the cleanroom operators themselves or by their actions. As the typical, most penetrating particle size for HEPA filters used in cleanrooms is around 0.3μm, large numbers of particles bigger than this can be an indication of either a potential contamination event inside the cleanroom, or of a failure in the cleanroom containment system.

Most countries or regions of the world have their own GMP documents (EU GMP[1] in Europe) and all but the United States of America require the cleanroom monitoring system to measure and count particles that are >0.5μm and particles that are >5.0μm (the US cGMP[2] documents only require cleanroom monitoring of particles >0.5μm and do not mention particles >5.0μm).

Modern cleanroom clothing systems are designed to prevent cleanroom operators shedding large numbers of airborne particles into the cleanroom air, despite the fact that human beings shed skin particles at a high rate. Nevertheless, each cleanroom is capable of flushing away only a certain amount of particles generated inside the cleanroom. The cleanroom design must therefore take into account the expected number of cleanroom operational staff who will be present in the room at any one time to ensure that the room design can cope with the expected level of contamination challenge.

Studies have shown that about 75% of the microbe-carrying particles in an occupied room will have an equivalent diameter larger than 4μm[3]. EU GMP requires that particles >5μm are measured in addition to particles >0.5μm. The particular significance of particles >5μm is that they may well be skin cells, or parts of skin cells carrying viable organisms.

Although it could be argued that the real threat to a sterile product is the presence of a potentially contaminating airborne viable micro-organism-carrying particle, the technology is not yet available to detect and identify the species of airborne microbes in real time, so airborne particle counters are used as a surrogate method to give real-time indication of a possible airborne contamination event by detecting particles >5μm.

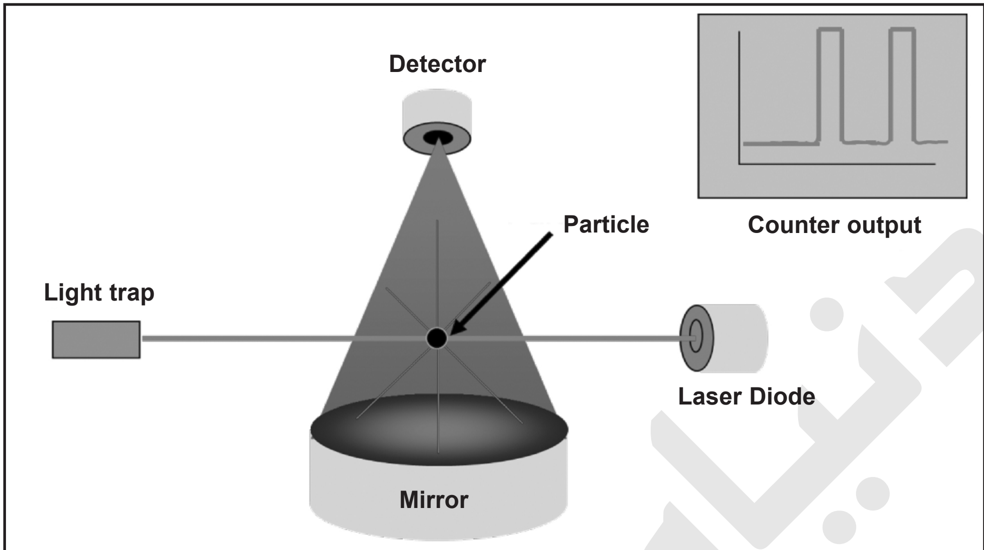


Figure 1: *How a particle counter works.*

Airborne particle counters can provide a real-time indication that the air handling system, the cleanroom containment system and the cleanroom operators themselves are all working correctly to deliver the desired level of controlled environment suitable for the cleanroom application.

18.3 How particle counters work

Modern particle counters utilise a laser beam to create a unidirectional light source. A sampling pump draws air through the sampling chamber of the particle counter and particles captured in this air sample pass through the laser beam. As the particles pass through the laser beam, the laser light is reflected off the particle and scattered. Mirrors collect this scattered light and focus it onto a photo-electric detector that turns the light into an electrical signal, the size of which is dependent on the amount of light reflected onto it by the focusing mirrors. The larger the particle size, the more light it scatters, the more light is focussed onto the photo-electric detector and the bigger the electrical signal given out. Sensitive electronic circuitry takes the electrical signals from the photo-electric sensor and interprets them into the number and size of the particles (**Figure 1**).

The particles of interest for GMP cleanrooms are generally >0.5μm (1μm = 10^{-6} of a metre) and the practical sampling and measurement limitation of the typical particle counter used in this application is around a maximum of 10μm. These particles are far smaller than the human eye can see, but are generally considered to be in the correct size range to detect any particles that could be either a clump of micro-organisms, or a particle large enough to carry micro-organisms, such as a human skin cell.

The amount of light scattered by these very small particles is tiny and the subsequent electrical signal generated is also very small, making accurate measurement of particle size and enumeration of the particles very difficult. Extremely sensitive electronic circuitry is required which, at the same time, has to be immune to interference to avoid incorrectly reporting particle counts or even generating false-positive counts.

18.4 Performance and calibration of particle counters

For many years, the reference design and calibration standard for air particle counters was the

Japanese standard, JIS 9921[4], which defined the calibration tests and performance requirements for this type of device. In 2007, ISO 21501-4[5] was published which captured the contents of JIS 9921. In addition, ISO 14644-3 (2005)[6] defines the required performance characteristics for airborne particle counters for cleanroom use and these match those laid down in both JIS 9921 and ISO 21501-4. The calibration procedures defined in ISO 21501-4 can be carried out on any air particle counter. However, the performance of the calibrated counter (**Figure 2**) must deliver count accuracy of at least +/-20% (termed "Count Efficiency") in order to be used in the cleanroom environment defined by ISO 14644-1[7].

Particle counters have to measure the size and number of particles that are smaller than the human eye can see. In addition, the particles observed in the cleanroom are not likely to be of uniform shape. So, in an attempt to ensure that particle counters all report similar results in the same cleanroom environment, uniform spherical spheres are used as the calibration reference standard. The particles are usually certified to have a nominal diameter at the required size and follow a normal distribution of size around this nominal diameter, with 50% of the particles being larger than the nominal size and 50% being smaller.

The certified spheres are nebulised into the airstream entering the particle counter and the electrical response set as being equivalent to that particular particle size. The counter now 'knows' to report any electrical signals of that size as being equivalent to a particle of equivalent size to the spheres used in calibration.

ISO 21501-4 then calls for the calibration of the particle counter to be verified against a 'gold standard' instrument (**Figure 3**) with a high accuracy performance and capable of accurately measuring particles 1.5 times smaller than 0.5µm. Typically, these 'gold standard' instruments are specialist, highly accuracy instruments and the tests are undertaken either by specialist contractors, or the calibration service teams from the particle counter manufacturers). This test is called counter "counting efficiency" and the counter under test must demonstrate that it is capable of counting particles to an accuracy of +/-20% when compared to the gold standard counter (**Figure 4**).

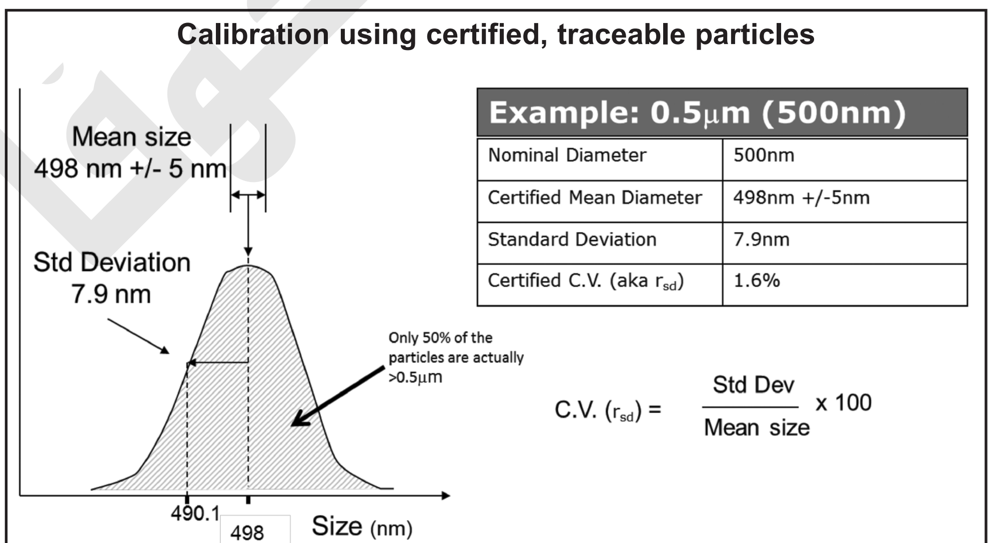


Figure 2: *Calibration of particles.*

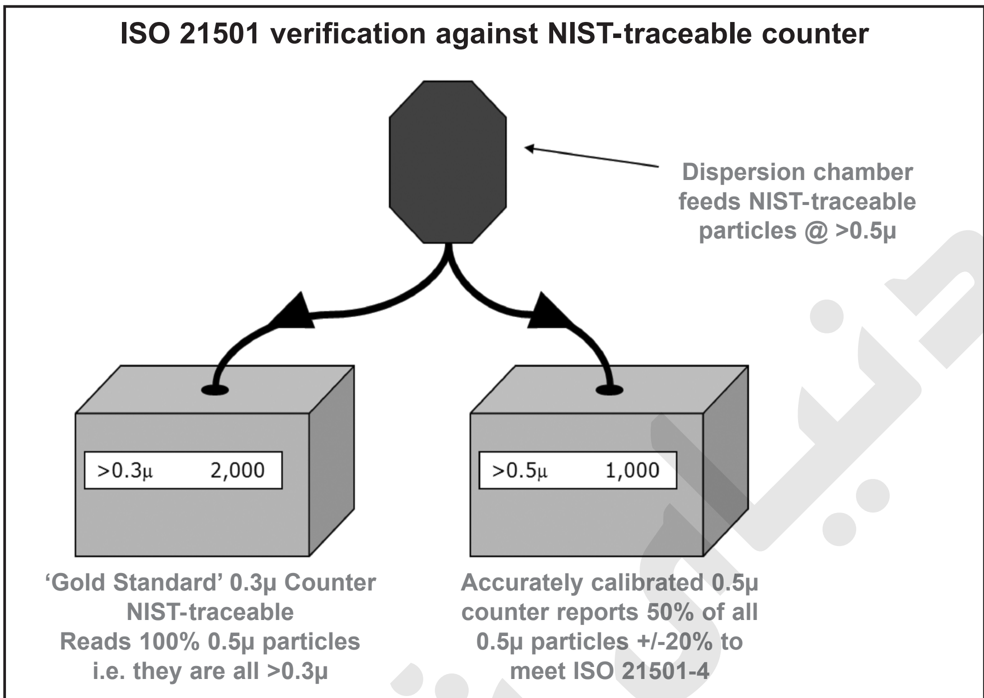


Figure 3: *Verification against a 'gold standard'.*

Each particle counter design will have a point at which the density of particles per cubic metre prevents the counter from identifying individual particles and starts to under-report. This is called the coincidence loss and is generally expressed as the concentration of particles per cubic metre at which a percentage chance of two particles being reported occurs, e.g. 5% at 14,126,000 particles/m^3.

In some particle counter designs, the sample flow-rate is too slow to capture larger particles and this is particularly noticeable in designs with a flow rate of 0.1 cubic feet/minute (CFM), where particles >1.0µm tend not to be captured and counted. In general, if particles >1.0µm are of interest, then a particle counter should have a minimum sample flow rate of at least 1CFM.

Other design parameters have a direct impact on the accuracy of the particle counter. One crucial design parameter is the intensity and coverage of the laser light beam in the sample flow. This is often termed the 'view volume' (**Figure 5**). If the intensity of the laser light varies across the view volume, then particles having the same size, but passing through different parts of the view volume will scatter different amounts of light and will therefore be reported as being of a different size. In a well-designed particle counter, the optical and electrical design will deliver an even performance for particles passing through any part of the view volume and this is expressed as having "100% view volume".

Other critical parameters include flow rate, sampling time and sampling volume, which are all critical if the counter is to accurately express the number of particles counted as a number per cubic metre of air.

In some poorly-designed particle counters, the electronics interpreting the electrical signals from

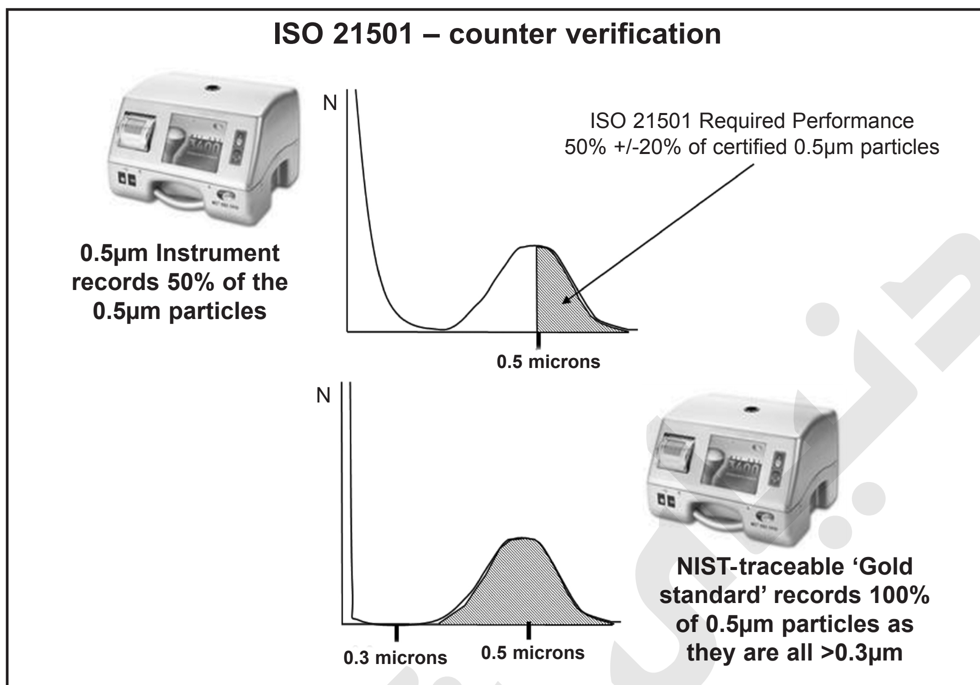


Figure 4: *Counting efficiency must be +/-20%.*

the photo-electric detector is unable to distinguish between the signals from the detector and background electrical 'noise' and this is termed 'poor signal-to-noise ratio' (**Figure 6**). Counters designed in this way may under- or over-report particle counts, or may indicate particles are present when in fact it is just a false count due to interference.

In summary, ISO 21501-4 and ISO 14644-3 direct us to the following performance requirement for particle counters used in the GMP environment:

- Sensitivity/resolution: Chosen between 0.1µm – 5µm with 10% size resolution
- Uncertainty of measurement: ±20% of concentration error at the size setting
- Calibration interval: 12 months maximum or specified performance verification
- Counting efficiency: (50 ± 20)% at minimum size threshold and (100 ± 10)% for particles greater than or equal to 1.5 times the minimum threshold size

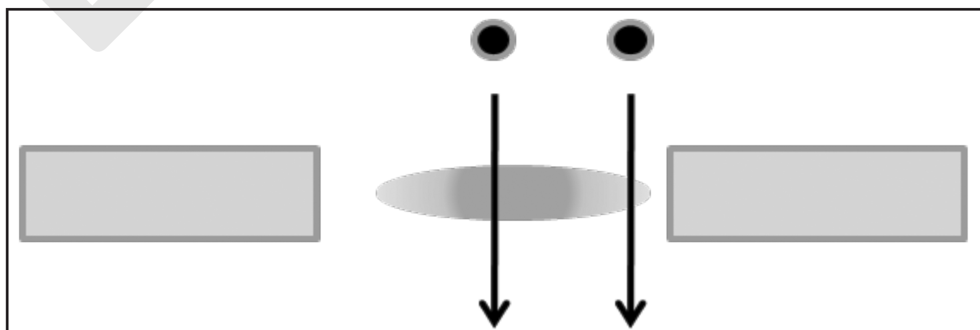


Figure 5: *Particle counter view volume.*

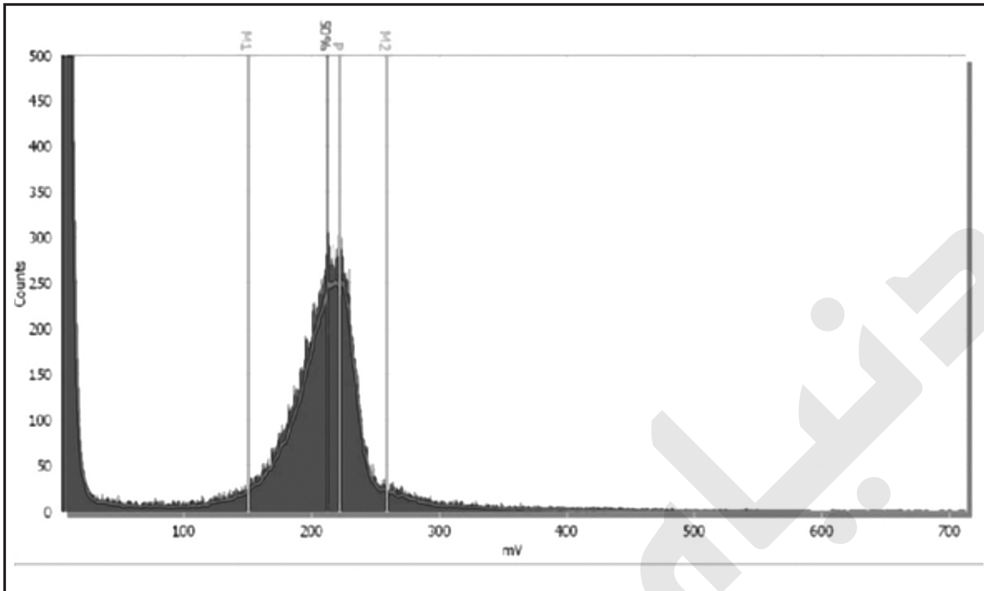


Figure 6: *Signal-to-noise ratio.*

– Lower concentration range: False count rate is insignificant in comparison to actually expected minimum counting rate. The low count rate should be zero particles for a certain time (e.g. no counts for 5 min)

– Upper concentration range: Two times greater than upper limit of the installation cleanliness class concentration at point of use, and no more than 75% of the manufacturer's maximum recommended concentration

18.5 Practical operations

18.5.1 Sample tubing

Sample tubing used for particle counters is typically of a specialist design and it is recommended that particle counter users only use the tubing supplied by the manufacturer of the particle counter. Manufacturers typically supply tubing that has an inner coating of an anti-static material that prevents the smaller airborne particles from being attracted to the tubing walls by a build-up of static electricity caused by the friction effect of air rubbing past the tubing walls as the air sample is drawn down the tube and into the particle counter.

One of the biggest challenges facing air particle counter users is correct sampling procedure. For example, it is common for air particle counters to be supplied by the manufacturer with a sampling tube of up to 3 metres in length. Tubing of this length, especially if coiled and not vertical, will cause a transport challenge to particles >1.0μm. Particles >1.0μm may have sufficient mass to either simply drop out of the air-stream as it passes through the sample tube, or, if the sample tubing is coiled, these larger particles may impact onto the side walls of the tubing and stick there as the tube bends.

Although most sample tubing is supplied with anti-static inner coating in an attempt to prevent the impacting particles from sticking, particle drop-out through these two effects is very common and is often displayed as an initial under-counting of the larger particles followed by a sudden ‘spike’, or increase in numbers of large particles as they dislodge from the tubing and pass into the counter, for example when the sample tubing is moved or knocked.

Whenever possible, if larger particles are of interest, the sample tubing should be vertically oriented, with the particle counter itself located with its sampling nozzle also facing vertical. In general, tubing lengths in these applications must be kept as short as possible. Although there are no rules in GMP concerning the length of the sample tubing, research undertaken by one particle counter manufacturer indicated that sample tubing as short as 1metre in length may have an impact on the efficient sampling of particles >1.0µm, especially those >5.0µm.

18.5.2 Sampling in unidirectional airflow

Unidirectional airflow is used in critical areas partly to avoid contamination events from spreading contamination across the entire work area, but also to ensure that any contamination is flushed away from the critical area. In these unidirectional flow air streams, care should be taken to ensure that the sample flow rate of the particle counter does not upset the unidirectional nature of the air flow. To achieve this, the particle counter manufacturer will supply a special sampling nozzle called an ‘isokinetic head’. These heads are funnel-shaped and will sample a sufficiently wide cross-section of the unidirectional airflow to match the sample rate of the counter. Note particularly that these isokinetic heads are of different sizes for different particle counter flow rates and it is important to use the correct size as supplied by the particle counter manufacturer.

18.5.3 Sampling process gases

When using air particle counters to sample process gases, the effect of pressure of the gas being sampled on the measurement should be taken into consideration. Most modern particle counters will adjust their sample pump speed to accommodate different sample pressures, but this compensation will only work for nominal variations and is generally not capable of coping with high pressure, process gas sample lines. In these instances, it is necessary to use a pressure reducer to allow a proportion of the gas being sampled to vent so that the proportion passing into the counter is actually within the operating sample pressure range of the counter. Pressure reducer designs that simply vent the excess process gas to the atmosphere can cause issues if the process gas pressure is too low because it may be possible for the air particle counter to draw air back in via the reducer’s vent, thus actually sampling a mixture of the process gas and the ambient air surrounding the reducer itself.

18.5.4 Interference from cleanroom activities

In many instances, the manual operations carried out in the GMP environment generate particles themselves, such as opening paper-backed packaging, or operating alcohol sprays for hand-or product/surface-sanitisation. These particles will be counted by the particle counter and, if there are sufficient particles present, the counter may indicate that the environment is no longer in compliance and sound an alarm. In addition, the alcohol sprays may occlude or damage the particle counter’s sensitive optics. Wherever possible, process should be changed and operators re-trained to avoid carrying out these particle-generating processes when the aseptic processing is being carried out, otherwise it will not be possible to guarantee that the environment was in compliance.

However, if it simply is not possible to eliminate these particles and where the particles can be demonstrated to not have a detrimental effect on the product quality, then it may be practical to ‘bracket’ or ‘book-end’ the production by monitoring the air quality before production and after production to demonstrate that the air quality was good before manufacturing commenced and to give an indication that it was good all the way through production by testing at the end of the production run. This may well be particularly suitable for powder fill applications where the product itself tends to float into the air and be counted as potentially contaminating particles. In this case the product itself is clearly not a contaminant and to cease production because the particle counters count the product powder as high particulate contamination level is clearly wrong.

18.5.5 Monitoring in closed barrier systems

In many cases, to achieve the required sterile processing environment, the cleanroom operators are separated from the production area via a closed Rapid Access Barrier System (RABS) or an isolator. The air particle counter must draw a sample from the inside of the closed RABS or isolator although this can present a problem if the isolator is to be leak/pressure tested or gassed with a sanitiser such as hydrogen peroxide gas. In order to support these processes, the sample vacuum pump for the particle counter and the sample pathway into the isolator must both be controlled by a signal from the isolator leak/pressure test and gassing controls to ensure that the sample pathway is closed during these operations.

Some particle counter manufacturers have indicated that their counters are tolerant to hydrogen peroxide gassing, but, as hydrogen peroxide gas works by depositing onto the surfaces to carry out sanitisation, it is not good practice to allow the particle counter to continue to sample during the sanitising gassing cycle. This is because the deposition from the gas will affect the accuracy of the counter optics over time and also because the particle counter will withdraw a proportion of the sanitising gas and may compromise the sanitisation cycle efficacy.

18.5.6 Continuous particle monitoring and alarms

Whilst ISO 14644-1 defines the sample volume required to carry out classification/re-qualification of cleanroom air quality, there is no sample volume defined for continuous air particle monitoring. In fact most air particle counters deployed in continuous monitoring system report air particle counts on a minute-by-minute basis, i.e. they sample for a minute then report the results.

Typically, the sample flow rate of a continuous particle counter is 1CFM, so in a one minute sample this will result in one cubic foot of air passing through the particle counter. Hence the particle counter will report particles/ft^3 and these results will need to be interpreted to provide counts/m^3. There are 35.2ft^3 in 1m^3, but it is not practical to simply multiply the particles/ft^3 by 35.2, especially when reporting particles >5.0µm in Grade A and B areas that are defined in GMP as having limits of 20 and 29 particles/m^3 respectively. So, if one particle at >5.0µm was detected in a 1ft^3 sample, then multiplying it by 35.2 would deliver 35.2 particles at >5.0µm per m^3, i.e. an out of compliance condition.

Most systems either simply count the particles in a discrete 36 x 1 minute sample set to estimate the total per cubic metre, or gather a ‘rolling window’ of samples, where each new 1 minute sample replaces the first 1 minute sample particle value in the calculation. Both techniques have their values, but it is certainly important to provide alarms based on each individual one minute sample period rather than wait for 36 minutes to pass before providing an alarm that something is going wrong.

18.6 Troubleshooting

Interpreting the results from air particle counters can easily go wrong, especially as the counters often have complex user interface screens and set-up programs for sampling and are often deployed by non-technical staff who are simply following a written SOP without in-depth understanding of how the particle counter works. For example, during routine environmental monitoring, it is quite common to take three, one-minute samples at each sampling location and average the results. Some particle counters can be programmed to calculate the results of this average and extrapolate them to display counts/m^3. However, if this set-up configuration is accidentally changed, or if the user is not familiar with the fact that most particle counters only sample 1ft^3 of air in a one minute sample, then the opportunity for incorrectly reporting the particle count levels is high. Wherever possible, if particle counters are to be configured to carry out automatic averaging and extrapolation of results to report in counts/m^3, the programming should be password protected to prevent accidental alterations by users.

This problem gets worse where particle counters from different manufacturers or with different flow rates are used within the same facility. A small number of higher flow particle counters may have been purchased to speed up cleanroom classification/re-qualification, but, if these are used for routine environmental monitoring programs, they will deliver very different results for a 1-minute sample than a slower sampling-rate counter for obvious reasons.

GMP calls for the particle counts to be reported in cumulative count mode, i.e. the particles reported as being >0.5µm should include all particles >0.5µm, including those >5.0µm. So, if there were 100 particles/m^3 in the size range >0.5µm but 10 of them were actually >5.0µm, then the counter should be set to report 100 counts at >0.5µm and 10 counts at >5.0µm. Particle counters can, however, be configured to report in differential mode, i.e. to report all particles >0.5µm, but smaller than 5.0µm. If the counter was configured this way, it would report these same particle results as 90 counts at >0.5µm and 10 counts at >5.0µm. Users should always check that the particle counter is set to report in cumulative mode for GMP applications.

Generally speaking, it is good practice to establish that the particle counter has not been contaminated and that the sample pathway is sealed by using a ‘zero-count’ filter to establish that the particle counter will report zero in the absence of any particles >0.5µm before it is used. The zero count filter is a HEPA filter designed to fit in the sample path and filter out all particles >0.3µm. When attached to the particle counter sample pathway, the particles entering the counter should all be <0.3µm and the particle counter should report zero counts at >0.5 micron and >5.0µm channels.

If the results from a particle counter measurement appear to be inconsistent with the normally observed results for that sampling location, then the root cause investigation should certainly include checking whether a different particle counter has been used previously, if the user is new, if the sample tubing has become damaged or contaminated, or if the counter programming has been altered to establish that the results are indeed different and that it is not just a measurement error. A simple check list is as follows:

- *Has a different particle counter been used this time which has differences in the measurement accuracy, or configuration to the usual test instruments used, e.g. has a high flow rate counter been used in the place of a lower flow rate counter, hence capturing more air and therefore more particles in the sample time?*
- *Are there any differences in the methods used by different operators?*
- *Has there been any significant change in the manufacturing environment?*
- *Were there any untrained workers in the cleanroom at that time, e.g. external contractors, inspectors from external companies, or staff not usually working in the cleanroom?*
- *Are there more people in the cleanroom than usual, e.g. external contractors called in to re-qualify the cleanroom?*
- *Has the particle counter been stored in such a way that the sample tubing has become contaminated with particles?*
- *Has the particle counter been operated in a more dirty area and become contaminated (a test with a zero-pass filter will help pick this up)?*
- *Has a new, potentially contaminated, sample tube been used with the particle counter?*
- *Have suppliers of raw materials indicated any potential issues, or has there been a change in supplier?*
- *Is the process affected by operator fatigue, e.g. have there been extended shifts?*
- *Have there been any changes in maintenance procedures?*
- *Have results always been reported truthfully/accurately in the past?*

18.7 Summary

This chapter has discussed the importance of airborne particle monitoring in the GMP cleanroom environment, the performance requirements of instruments used for this function and given some guidance on using these devices to achieve accurate results. It is important to understand how particle counters work, how to use them correctly and how to interpret the results in the context of potential failures in the containment system, the HEPA filters or poor cleanroom operator practice.

18.8 References

1. European Commission. EudraLex. The Rules Governing Medicinal Products in the European Union. Volume 4. EU Guidelines to Good Manufacturing Practice. Medicinal products for human and veterinary use, Annex 1: Manufacture of Sterile Medicinal Products, February 14, 2008. European Commission Enterprise and Industry Directorate-General, B-1049 Bruxelles/European Commission, B-1049 Brussel – Belgium.
2. Food and Drug Administration. Guidance for industry. Sterile drug products produced by aseptic processing – current good manufacturing practice, 2004. US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER) Office of Regulatory affairs (ORA) Division of Drug Information, HFD-240 Center for Drug Evaluation and Research Food and Drug Administration, Rockville, MD, USA.
3. Collection efficiency and design of microbial air samplers. W Whyte, Green G. Albisu A. *Journal of Aerosol Science* 2007; **38**:101-114.
4. JIS B 9921:1997 Light scattering automatic particle counter. Japanese Standards Association, 4-1-24, Akasaka, Minato-ku, 107-8440, Japan.
5. ISO 21501-4 Determination of particle size distribution – Single particle light interaction methods – Part 4: Light scattering airborne particle counter for clean spaces 2007. International Organization for Standardization Case Postale 56, CH-1211 Genève 20, Switzerland.
6. ISO 14644-3:2005 Cleanrooms and associated controlled environments – Part 3: Test Methods, December 20, 2005. International Organization for Standardization Case Postale 56, CH-1211 Genève 20, Switzerland.
7. ISO 14644-1:1999(E) Cleanrooms and associated controlled environments – Part 1: Classification of air cleanliness May 1, 1999. International Organization for Standardization Case Postale 56, CH-1211 Genève 20, Switzerland.

Environmental monitoring in cleanrooms

Tim Sandle, Head of Microbiology, Bio Products Laboratory, St Albans, UK
Madhu Raju Saghee, Assistant Manager – QA, Novartis India Ltd, Thane, India

19.1 Introduction

Controlled environments – cleanrooms – are required for the manufacture of many pharmaceutical products and are assigned a class or a grade through meeting a set standard for airborne particles. However once a grade is assigned, a series of other physical and microbial parameters must be met. These include HEPA filtration, pressure differentials, wearing appropriate garments, etc. In order to achieve microbial control, the use of defined cleaning techniques, together with the application of detergents and disinfectants, is important.

In order to assess the effectiveness of environmental control systems, an environmental monitoring programme is required and forms an essential part of any well planned pharmaceutical quality management system[1]. Though this chapter provides information on assessment of both non-viable particulates and viable micro-organisms, this chapter is mainly focused on microbiological monitoring.

Many aspects of pharmaceutical manufacturing are regulated through Good Manufacturing Practice (GMP). In many cases this is through detailed regulations or guidelines. With environmental monitoring, however, the expectations are less well defined. The FDA Guide to aseptic manufacturing[2] and the EU GMP Guide[3] provide frameworks for sterile production (although each site is required to define its own approach and to construct a rationale). With non-sterile production, although GMPs imply expectations for environmental monitoring, the approaches to be taken are less well defined, with little detail provided.

The applicable guidelines are:

- EU GMP Annex 1: in relation to sterile manufacturing, which provides guidance on the maximal values for viable counts and non-viable particulates, as well as detailing the accepted monitoring methods.
- EU GMP 1.4 ‘Quality Control’: in relation to monitoring environmental conditions for GMP purposes
- EU GMP Section 5: in relation to Production areas being protected from ‘microbial and other contamination’ (5.10), to avoiding cross-contamination (5.19), and the need to monitor process areas (5.20)
- EU GMP Annex 7: for avoidance of contamination when manufacturing herbal medicinal products
- EU GMP Annex 9: providing warning about the susceptibility of liquids, creams and ointments to microbial contamination
- CFR 211.113: ‘Control of microbiological contamination’, with an emphasis on the risk of objectionable micro-organisms
- FDA Guideline on Sterile Drug Products Produced by Aseptic Processing, September 2004
- USP 35-NF30 <1116>: Microbiological control and monitoring of aseptic processing environments
- ISO Standard 14644: Cleanrooms and associated controlled environments, Parts 1 to 9

- ISO 14698-1: Cleanrooms and associated controlled environments–Biocontamination Control': Part 1: General principles and methods. Geneva, Switzerland, International Organisation for Standardisation 2003

This variation in approach has led to a variety of different regimes being adopted[4]. The objective of this chapter is to outline the best practices for environmental monitoring and to help quality professionals define and develop an environmental monitoring programme for pharmaceutical grade cleanrooms.

In recent years the direction of environmental monitoring has been orientated towards risk assessment rather than simply gathering data for badly thought out purposes[5]. This chapter embraces some of the current thinking on risk assessment. Risk-based approaches involve identifying risks, assessing their impact by accounting for the severity of the risk and the likelihood of its occurrence, and then either accepting the risk or eliminating it. Where a risk cannot be eliminated or reduced to a satisfactory level, the risk should be monitored. It is around such detection systems that environmental monitoring is based. Therefore, the regulatory expectation is that pharmaceutical manufacturers adopt a risk-based approach to microbiological and environmental control[6]. The chapter also acknowledges the increased use of rapid methods.

In putting the chapter together, the emphasis has been on the practical aspects of environmental monitoring rather than the theoretical (although some elements of what is happening and why are included) aspects. This is because the objective of the chapter, and indeed this book, is to help users develop and review their own programmes. Thus this chapter provides a useful reference for both novices and experts alike.

Therefore the construction of an environmental monitoring programme is one of the most important considerations for microbiologist and Quality Assurance personnel. This requires a well thought out rationale, policy and procedures, with an emphasis upon monitoring, data analysis and trending, and follow-up actions for out of limits results.

This chapter considers environmental monitoring in its widest sense in relation to cleanrooms. This scope can be divided into three areas:

- Physical operation and certification of cleanrooms
- Control of non-viable particle counts
- Control of viable micro-organisms

There is, as indicated above, an important distinction between environmental monitoring and environmental control. The physical operation of the cleanrooms, along with managerial oversight and trained and qualified personnel, provides the control aspect. The important consideration here is within the design and the associated hazards which relate to certain parts of process operations. The viable and non-viable methods provide the monitoring aspect. Such data aids understanding of the ongoing performance of the facility.

In approaching environmental monitoring, it is important to note that monitoring only provides a 'snap shot' of conditions of a process of cleanroom at one point in time. Single events are rarely of significance in themselves; what is more important is the direction of the trend and the measure of confidence and speed the contamination issue can be addressed[7].

19.2 Personnel and responsibilities

19.2.1 Responsibilities

There is a range of responsibilities in relation to the environmental control and monitoring of

cleanrooms. Current GMPs require these roles to be defined. The role of Quality Assurance is to set standards, policy and to provide an oversight of operations. This includes overseeing change controls where they relate to modifications to cleanrooms or new builds, and where aspects of processing operations may affect the room environment (such as the use of milling which would generate a higher level of particulates); and into overseeing deviations into significant contamination control events.

It is the role of Engineering to aid in the design and operation of cleanrooms to the required standard. It is the role of Production staff to use the cleanrooms correctly and to assess aspects of the physical operation (such as checking pressure differentials between different areas). The role of Microbiology is to construct and to direct the particulate and viable environmental monitoring programme. This includes determining monitoring locations, setting alert and action levels, trending and interpreting data, delivering training, reviewing proposed new process equipment and investigating out of limits results.

19.2.2 Personnel

The major source of microbial contamination within cleanrooms is from personnel. This not only includes production staff, but also from other staff present in process areas such as cleaning staff and engineers. It is therefore important that all personnel entering cleanrooms have been suitably trained in the way to behave and with the gowning procedures. Additional training should be given for hygiene and in relation to a basic understanding of microbiology.

19.3 Cleanrooms

Cleanrooms and clean zones are areas especially designed to control the concentration of airborne particles. Considerable detail concerning cleanrooms is discussed in several chapters within this book and the operation of cleanrooms will only be mentioned briefly here in the context of environmental monitoring.

Clean areas are constructed in a way which is designed to minimise the introduction, generation and retention of particles. The cleanliness level ascribed to a cleanroom is determined by the use of the room and the number of particles in the air. The cleanliness of the air is controlled by an HVAC system (Heating, Ventilation and Air-Conditioning). The key aspect is that the level of cleanliness is controlled.

In addition, clean areas are constructed in a way where pressure, air supply, temperature and humidity are controlled. Therefore, the design, construction and operation of cleanrooms are important in order to achieve environmental control.

Cleanrooms are designed to minimise and to control contamination. There are many sources of contamination. The atmosphere contains dust, micro-organisms, condensates and gases. Manufacturing processes will also produce a range of contaminants. Wherever there is a process which grinds, corrodes, fumes, heats, sprays, turns, etc., particles and fumes are emitted and will contaminate the surroundings. Contamination can lead to expensive downtime and increased production costs. Once a cleanroom is built, it must be maintained and cleaned to the same high standards. It has been found that many of these contaminants are generated from five basic sources: the facilities, people, tools, fluids and the product being manufactured can all contribute to contamination.

People, in clean environments, are the greatest contributors to contamination, emitting body vapours, dead skin, micro-organisms, skin oils, etc. The average person sheds 1,000,000,000 skin cells per day, of which 10% have micro-organisms on them. Due to natural reasons, man releases a large number of particles. Most of these arise from the outer layer of our skin surface, but

particles can also come in contact with the surrounding air via our mouth and nose, i.e. when we breathe, cough, sneeze, laugh, sing and shout. The particles, released from people via the mouth and nose, are minute liquid droplets that are heavily contaminated with micro-organisms. This demonstrates the importance of wearing cleanroom clothing and wearing this clothing correctly[8].

Contaminants are of central importance within the field of contamination control and cleanroom technology. **Figure 1** shows a schematic representation of the source and dispersion as well as deposition of contaminants, from a general and holistic perspective. The figure shows the four general sources from which different contaminants may arise. Furthermore, the figure shows the various ways that contaminants can be distributed from the source and finally also where these contaminants can settle.

The four major sources shown in this figure, personnel (i.e. the human being) the surrounding air, all surfaces within the room and the product and (or) process itself, are all interconnected. All four sources are equally important from a general point of view. From a practical point, however, the question often stated is: "Which of these four sources has the highest significance with respect to the work being undertaken?" The answer to this question is that all four are important, but their individual impact on the outcome of the production process depends on the nature of the product, its sensitivity as well as how the product is to be used.

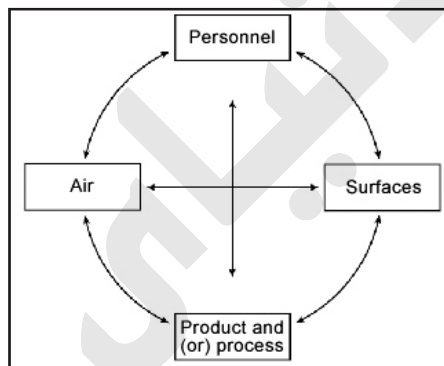


***Figure 1:** The relationship between the four major contamination sources, personnel, air, surfaces and the production process. The lines connecting the sources are the dispersion routes for contaminants, and the sources are also the places where contaminants can settle[9].*

As can be seen from **Figure 1**, personnel can contaminate the product or the production process through the air, through direct as well as indirect contact with different surfaces and also by direct contact with the product and (or) process equipment. The most important contamination by personnel is through the natural process of regular shedding of epidermal cells, as stated previously. Sitting totally still and keeping quiet, a person is said to release approximately 100,000 particles per minute. When moving, even just slightly, the number of particles will rise dramatically. Most cleanroom micro-organisms are in the air. If they settle on a dry surface they are unlikely to survive and ideally any contamination is removed from the room[10].

Within cleanrooms are various clean air devices. The terminology of ISO 14644-7, Cleanrooms and associate controlled environments – Part 7, uses the term 'Separative Devices' to collectively describe clean air hoods, glove boxes, isolators and mini-environments[11]. These devices include laminar airflows (more commonly described as Uni-Directional Airflow (UDAF) Devices in the context of pharmaceutical manufacturing given that 'true' laminarity cannot be easily demonstrated[a]), biosafety cabinets and isolators. Such devices normally operate at EU GMP Grade A/ISO Class 5[12]. The term 'cabinet' is used more widely within Europe and the term 'hood' used more widely in the USA.

19.3.1 Design of cleanrooms

From the above discussion on cleanrooms it can be seen that an important element of contamination control is with cleanroom design. Cleanrooms in pharmaceutical industries not usually comprise of a single room, but more likely of a suite of rooms adjacent to one another. A cleanroom suite includes a number of cleanrooms and interconnecting air locks traditionally placed

in a row, leading from the outer uncontrolled environment and in a step-wise manner into cleaner, and thus more, controlled environments. The basic function of a cleanroom is to protect the manufactured product from contamination[13].

The design objectives[14] to be considered for a suite of pharmaceutical cleanrooms can be summarised as below:

- Exclusion of the internal cleanroom suite from the external, uncontrolled environment
- Elimination of the contaminants developed from the manufacturing process and (or) the products handled
- Reduction of the contaminants developed from the personnel working within the cleanroom suites
- Isolation of hazards arising for the product or the process, both in regards to personnel and (or) product produced
- Elimination of product to product cross contamination
- Control of the material flow and personnel flow
- Security of entering by personnel as well as material
- Optimal comfort for the product, temperature as well as humidity
- Optimal comfort for the personnel, temperature, humidity as well as garment worn
- Storage areas for special tooling and cleaning equipment hardware
- Monitoring of cleanroom conditions, particles, micro-organisms, temperature, humidity and pressure

19.3.2 Cleanrooms and contamination control

There are four principles applying to the control of air-borne micro-organisms in cleanrooms.

- Filtration (through the use of HEPA filters)
- Dilution (to ensure that particles generated in clean rooms, in addition to those which pass the filters, are carried away by diluting the clean area with new “clean” air)
- Directional Air Flow (to ensure that air blows away from critical zones, as particles and micro-organisms cannot “swim upstream” against a directional air flow). This is achieved by employing a cascading air pressure differential system, where the “cleanest” class is maintained at the highest pressure and the lower classes decrease in pressure respectively.
- Air Movement (rapid air movement is important for as long as particles and micro-organisms stay suspended in the air they are not really a problem, for it is only when they settle out that they become an actual cause of contamination)

These important operational characteristics are built into the design of cleanrooms.

19.3.3 Classification of cleanrooms

Cleanrooms and zones are typically classified according to their use (the main activity within each room or zone) and confirmed by the cleanliness of the air by the measurement of particles. For pharmaceutical cleanrooms, air cleanliness is either based on EU GMP guidance for aseptically filled products and the EU GMP alphabetic notations are adopted; or by using the International Standard ISO14644, where numerical classes are adopted. Cleanroom classifications are confirmed by measuring particle counts per cubic metre in the dynamic state (this is based on the concentration (number of individual particles per unit volume of air) of airborne particles that fall within a cumulative distribution based on a lower limit size (such as 0.5mm). Both EU GMP and FDA require classification to be undertaken according to the ISO standard. ISO 14644-1 details three occupancy states for clean room classification: ‘as built’, ‘static’ and ‘dynamic’. The ISO standard provides limits for the static and dynamic states.

In summary, the principles of classification/re-classification are to show that:

a) Air supplied into the cleanroom is of a sufficient quantity to dilute or remove contamination generated in the room
b) Air within the cleanroom moves from a clean to a less clean area (i.e. from a higher grade to a lower grade).
c) Air supplied into the cleanroom is of a sufficient quality so it will not add to contamination within the room
d) Air movement within the cleanroom should ensure that there is no area within the room with high areas of contamination
e) With Grade A/ISO class 5 zones (unidirectional airflow devices in Grade B/ISO class 7 rooms) there is a requirement for controlled air velocity and unidirectional air flow (either horizontal or vertical).

Cleanroom classification grades and classes are discussed elsewhere in this book and are not discussed here in any more detail. It is important to note, however, that one of the key distinctions with cleanroom classification is the difference between the 'as built', 'static' and 'dynamic' states. The 'dynamic' (or 'in-use' or 'occupied') state is the optimal cleanroom state for when the environmental monitoring programme is executed. This is because it represents the worst case: a room, with equipment running and people present.

In addition to periodic classification, it is recommended that cleanrooms are also monitored in the dynamic state for process monitoring at periodic intervals (here particulate monitoring is performed for both 0.5μm and 5.0μm particle sizes – 0.5μm is a recommended by the FDA Guideline on Sterile Drug Products Produced by Aseptic Processing, but both sizes are required by EU GMP). Static monitoring may be performed in some areas, particularly for investigating particle count excursions during dynamic state monitoring.

19.3.4 Cleaning and sanitisation

Cleanrooms and clean areas must be regularly cleaned and disinfected. This is normally undertaken using a detergent step, followed by the application of a disinfectant. It may be necessary to remove the residue of the disinfectant with water.

When cleaning rooms, the equipment used (mops and buckets) should be of an appropriate design for the grade of cleanroom. When undertaking cleaning a strict cleaning regime should be followed. Cleaning and disinfection, using cloths and mop heads, is ideally performed by saturating the cleaning item and wiping the area using a series of parallel, overlapping strokes (with an approximate one quarter overlap) and never in circular motions. The direction of the cleaning should be towards the operator (from top to bottom, from back to front). Only one application of the disinfectant or detergent should be applied to avoid over concentration. Cleaning and disinfection should begin with the visually 'cleanest' area first and towards the 'dirtiest' area last.

Cleaning is normally undertaken in each process area before use. In general, the frequency of cleaning should be established through risk assessment. In order to assess the effectiveness of cleaning and sanitisation programmes, important information is provided from the environmental monitoring programme (especially from surface monitoring)[15].

19.4 Environmental monitoring programme: Non-viable particle counts

The monitoring of cleanrooms and clean area for non-viable particle counts is an important part of an environmental monitoring programme. Such monitoring is in addition to cleanroom classification.

'Particle' in the context of a cleanroom is a general term for sub-visible matter (a particle is defined as something that is either solid or liquid and with a size ranging from one thousandth of a micrometer (0.001μm) and up to one thousand micrometers (1000μm)[16]. In some cases there is also a need to define the word fibre. A fibre is generally defined as a particle, following the definition above, that has a relation between its length and its diameter that is equal to or larger than 10). Airborne Particles, refers to particles suspended in air. Air contains a variety of different particles of a range of different sizes. These are particles of dust, dirt, skin, micro-organisms, etc. As discussed above, the function of cleanrooms is to reduce the number of airborne particles (for example, an office building air contains from 500,000 to 1,000,000 particles (0.5 microns or larger) per cubic foot of air. In contrast, an ISO Class 5/EU GMP Grade A cleanroom is designed not to allow more than 100 particles (0.5 microns or larger) per cubic foot of air. A human hair is about 75-100 microns in diameter. A particle 200 times smaller (0.5 micron) than the human hair can cause major disaster in a cleanroom.

Particles are generated from a variety of sources. These can include:

- Facilities, such as: walls, floors and ceilings; paint and coatings; construction material; air conditioning debris; room air and vapours; spills and leaks
- People, including: skin flakes and oil; cosmetics and perfume; spittle; clothing debris (lint, fibres, etc.); hair
- Equipment, including: friction and wear particles, lubricants and emissions, vibrations
- Cleaning equipment, such as: brooms, mops and dusters; cleaning chemicals
- Fluids, arising from spillages
- Particulates floating in air, primarily: bacteria, fungi, organic material and moisture
- Compressed gasses
- Product generated

The unit of measurement for particles is the micrometer (or 'micron'). This is symbolised as μm. The micron is a unit of length equal to one millionth (10^{-6}) of a metre. With cleanrooms the regulatory standards focus on two sizes of particles which are selected due to the potential risk that they pose. These are:

- 0.5μm size particles, which are close in size to many micro-organisms;
- 5.0μm size particles, which are close in size to skin flakes, onto which many micro-organisms are bound.

With European GMP, there is concern with both types of particle size. With the FDA, the primary focus is on the 0.5μm size.

The assessment of particles when classifying cleanrooms provides an indication that the physical aspects of the cleanroom operation, as found, are working correctly. For routine operations, particle counting is required to assess the impact of equipment and personnel upon the product and process.

The frequency of particle counting depends upon the nature of operations. For aseptic filling, the EU GMP and FDA guidance is for continuous particle counting. For other manufacturing activities, this is determined by risk assessment.

This may be by:

- Listing areas and operations that are not monitored (such as operations which are known to generate a high level of particles, such as, centrifugation. Some work may be needed to show that any particles in the room are generated from certain items of equipment, and are probably not microbial. This could be by determining the level of particles in the cleanroom

without people and equipment running; then with the equipment running and without people; and then with people and equipment running, and comparing the differences).
- Focusing on downstream operations such as purification, final formulation and primary packaging (component preparation)
- Determining a frequency for such monitoring (one approach is to start frequently and then justify decreasing to monthly on examination of satisfactory data).

The location for such monitoring could be determined from:
- The results of the particle count classification study, if one location was 'worst case'
- By means of examining the process flow and selecting representative tests of the operation
- By means of risk assessment. For example, does the operation in question impinge on a neighbouring cleanroom (perhaps one of a different grade)? In such an example a particle counter may be required to be placed in the adjacent area.

19.4.1 Particle counters
Particle monitoring is undertaken using particle counters. A particle counter is a device that draws air in using a pump at a controlled flow rate. The air is passed into a sensor area and through a light beam created by a laser diode. The amount of light reflected from each particle is measured electronically (as an electronic pulse). The larger the particle, then the larger the amount of reflected light (the greater the height of the light pulse). This allows the particle counter to 'count' the number of particles in a given volume of air (as the number of light pulses) and to assess the size of the particles counted.

Different particle counters have different flow rates. The flow rate is the rate at which air is drawn into a particle counter, and thus the time taken for the counter to measure a fixed volume of air. The long-standing flow rate has been 1.0 cubic feet per minute (equivalent to 28.3 litres per minute). This flow rate is the baseline for cleanroom certification. Readers are advised to refer to Chapter 20 for more information on particle counters and counting.

19.4.2 Particle sizes
For EU GMP, two particle sizes are assessed: 0.5μm (and greater) and 5.0μm (and greater). These sizes refer to the *diameter* of the particle.

19.4.3 Particle counting
Particle counts can be set for one of two counting modes:
- **Cumulative count**: where the counter is set to count the number of particles for the selected size and greater. For example, if a counter is set to count 0.5μm particles, it will count all particles at the 0.5μm and greater (such as 0.5, 0.7, 1.0, 5.0 and 10.0, depending upon the number of available channels on the counter). For cleanroom classification and for particle monitoring for EU GMP, the cumulative mode must always be used.
- **Differential mode**: where the counter is set to only count the number of particles of the selected size. For example, if a counter is set to count 0.5μm particles, it will only count particles of the 0.5μm size.

Many particle counters count at a rate of one cubic foot per minute In order to measure one cubic metre of air an equivalent sample of 35 minutes and 18 seconds of air is required[2] (in practice a thirty-six minute sample is taken). This time can be adjusted for particle counters which count at a faster rate (so, a particle counter which counts at two cubic feet per minute would require an 18 minute sample).

Before using particle counters it is typical for the counters to be purged in the dynamic state. This is in order to assess that the counter is operating satisfactorily and that any high value recorded particles

relate to a source other than the particle counter. Particles can remain inside a particle counter and collect on the laser diode and mirror. The act of regularly 'purging' a particle counter assists in keeping the sensor area free from particles and ensures that the measured counts are the 'true' counts.

A risk with the use of particle counters relates to the phenomenon of particle loss. Particle loss is minimised by the use of specialised tubing. The quality of the material used for particle counter tubing is important. In general, there are three types of tubing which may be considered:

a) Bev-A-Line tubing or Tygon tubing
   Bev-A-Line or Tygon tubing tubing is a co-extruded tubing consisting of a PVC exterior and a Hytrel interior. Its suitability as a tubing for particle counters relates to the smoothness of the interior wall
b) Stainless steel
   Stainless steel tubing is suitable for situations where particles in a hot air-stream require measurement (such as a dehydrogenation tunnel). The disadvantage of the tubing is its lack of flexibility
c) Polyurethane

Particle loss can also occur due to the tubing diameter. The recommended internal diameter of tubing for particle counters, by particle counter manufacturers, is 10-15mm. Furthermore, particle counter tubing lengths must be kept as short as possible. This is particularly important for avoiding particle loss for particles of a size of >1.0 micron. Research suggests that there is a 20% loss of 5.0 micron particle counts for tubing lengths of >3m (approximately 10ft). Particle counter tubing should not exceed 3m in length between the sampling head probe and the particle counter. This is to ensure the transportation and delivery of larger particles (such as 5.0μm) and to avoid 'drop-out'. Tubing should also be as straight as possible. Radial bends will result in the loss of particles. In addition, tubing must be changed at regular intervals (such as 3-monthly). All particle counter tubing will, over time, accumulate particles, particularly where particle counters are used for continuous monitoring. A phenomenon which can arise is the sudden release of particles (previously suspended on tubing walls) which may lead to an unusually high count or series of counts.

19.4.4 Isokinetic probes

For sampling Grade A environments and under any unidirectional airflow device, an isokinetic probe is always fitted to particle counter tubing.

19.4.5 Sample volumes

For cleanroom certification and for assessing particle counts according to the EU GMP guide a sample size of 1m^3 of air is required. This is equivalent to 1,000 litres.

ISO14644 sets out the required sample volumes for cleanroom classification. For continuous particle counting of batch filling, routine spot check sampling and for assessing rooms following maintenance or shutdown, a recommended practice is to sample 1m^3.

19.4.6 Particle count limits

There is a difference for particle count limits between EU GMP and ISO 14644. The FDA adopts ISO14644. Therefore, the limits applied will depend upon whether the cleanroom is described as an ISO class or as an EU GMP grade. The limits in the EU GMP Guide are almost identical to those in the ISO 14644 series and are expressed in particles per cubic metre of air. The difference relates to 'rounding down' of the EU GMP numbers.

The main difference, other than rounding, is the limit for the 5.0μm particle size at Grade A. ISO 14644 lists this as 29 counts per m^3, whereas EU GMP has a limit of 20 counts per m^3. This

changed from the issue of the revised EU GMP Guide in February 2008 (effective from 1st March 2009), where the limit was previously 1 count per m^3.

Tables 1 and **2** below give a comparison between EU GMP and ISO 14644 limits for the static and dynamic states:

Table 1: Static state equivalence

EU GMP Grade	Standard			
	EU GMP		ISO 14644	
	0.5µm counts per m^3	5.0µm counts per m^3	0.5µm counts per m^3	5.0µm counts per m^3
A	3,520	20	3,520	29
B	3,520	29	3,520	29
C	352,000	2,900	352,000	2,930
D	3,520,000	29,000	3,520,000	29,300

Table 2: Dynamic state equivalence

EU GMP Grade	Standard			
	EU GMP		ISO 14644	
	0.5µm counts per m^3	5.0µm counts per m^3	0.5µm counts per m^3	5.0µm counts per m^3
A	3,520	20	3,520	29
B	352,000	2,900	352,000	2,930
C	3,520,000	29,000	3,520,000	29,300
D	Not specified	Not specified	35,200,000	293,000

* The cleanroom user is required to set the level based on an examination of historical data.

For particle count limits, it is recommended that an alert level be set. This is either set based on historical data or by taking 50% of the action level.

19.5 Environmental monitoring programme: Viable counts

19.5.1 Microbiological environmental monitoring

Microbiological environmental monitoring involves the collection of data relating to the numbers of micro-organisms present in a cleanroom or clean zone. These micro-organisms are recovered from surfaces, air and people.

The main aim of microbiological environmental monitoring is to allow for the monitoring of trends over time and the detection of an upward or downward movement, within clean areas[17]. Individual results (whether of a high count or a low count) are rarely significant. However, for batch specific monitoring the data collected from a specific environmental monitoring session can be used to help with batch pass or reject decisions. Results are measured against recommended warning (or alert) and action levels. More ideally these levels should be user-defined based on a statistical review of the data[18]. In addition some trending of the micro-organisms recovered should be performed.

Analysing micro-organisms in cleanroom environments is traditionally performed according to clearly defined and detailed plans. Microbiological monitoring should be performed in the dynamic state, as opposed to the static state, as this represents the 'worst case' scenario (i.e. the clean zone with people present and performing processing). No microbiological monitoring programme can (or need) assess all microbiological contamination in a clean area. All programmes provide "snap shots" of an area at a particular time. These isolated sampling events can be placed together so that any adverse trends can be seen. When action levels are breached or adverse trends detected, the user should define appropriate measures to be taken in pre-defined and documented out-of-limits (OOL) procedures.

19.5.2 Sample types

The viable count aspect of environmental monitoring consists enumerating the numbers of micro-organisms present in a clean room by collection results by using the following sample types:

a) Passive air sampling: settle plates
b) Active air sampling: volumetric air sampler
c) Surface samples: contact (RODAC) plates
d) Surface samples: swabs
e) Finger plates
f) Plates of sleeves/gowns

Viable monitoring is undertaken using agar (a jelly-like growth medium) in different sized containers. The unit of measurement for viable monitoring is the Colony Forming Unit (cfu). This is a measure of viable bacterial or fungal numbers. It contrasts to direct microscopic counts where all cells, dead and living, are counted, for the 'cfu' is only a measurement of viable cells, where growth is dependent upon a specific culture medium and under specific growth parameters (time, humidity, atmosphere and temperature). The cfu only provides an estimate of the number of micro-organisms present in a given volume of air or from a surface. The main limitation in the context of environmental monitoring is that should two micro-organisms be present on skin that lands onto a settle plate, this will invariably appear as one countable cfu.

Each type of sample is examined below.

Settle plates

Settle plates are agar plates of either 9cm or 14cm diameter. Ideally they should be positioned and exposed either side of the testing environment and are designed to detect viable micro-organisms that may directly settle on or in the product (that is micro-organisms carried in the air-stream)[19].

The results from settle plates can either be assessed as the number of micro-organisms per plate or semi-quantitated by calculating the number of micro-organisms per four hours[20]. Guidelines such as EU GMP express alert and action levels as cfu per four hours.

When using settle plates, it should be noted that the use is required and accepted within Europe as a quantitative measurement; both the FDA guidance and USP <1116> consider them semi-quantitative and inferior to active air-samples (although still of use).

The length of time a settle plate can be exposed must be validated. This involves designing an experiment where plates are pre-weighed, exposed for a pre-defined time under the clean zone UDAF or a UDAF assessed as being similar to the clean zone of concern, and then challenged with a suitable range of micro-organisms pre- and post- incubation. This study is essential in order to determine if the degree of desiccation, which occurs during the exposure of an agar plate, does not cause excessive 'drying out' so that micro-organisms, which may deposit onto the surface will not grow.

Active air samples

Active (or volumetric) air samplers are a slightly different measure of micro-organisms in air than settle plates. The settle plate indicates the number of micro-organisms that may deposit onto a surface; the active air-sampler indicates the number of micro-organisms present in a given volume of air within the range of the air-sampler. This number of micro-organisms carried in the air may not have settled onto a critical surface. Therefore, data from an active air-sample may not be of direct relevance in terms of risk to exposed product.

The volume of air sampled is normally one cubic metre of air. Therefore, like the settle plate, the data can be quantified. Within a clean room environment the number of micro-organisms would be expected to be <1 cfu/m^3.

There are three main types of active air-sampler: impaction, filtration and centrifugal[21]:

- An impaction air-sampler functions by accelerating air, at an angle of 90°, through holes in the head of an air-sampler (often a 'sieve like' design) and impacting any micro-organisms onto an agar strip or plate.
- A filtration air-sampler draws the air through a filter, which means that it is possible to capture micro-organisms even at relative low concentrations, due to the fact that larger total volumes of air are able to pass the filter.
- A centrifugal air-sampler draws air into the sampler head through a rotating vane mechanism. The vane causes micro-organisms to be thrown out of the air and onto the agar by centrifugal force.

There are several developments with rapid methods that utilise the concept of Process Analytical Technology (PAT) in order to streamline air sampling. Technologies are now available which deploy fluorescence sensor technology to count both non-viable and viable particles. The prospect for 'real time' viable counting offers the potential to strengthen contamination control and avoid many of the concerns and limitations of conventional methods. An example is BioVigilant's IMD-A instruments that provide instantaneous microbial detection and also enables them to provide continuous monitoring and trending, which is a useful functionality not possible when using existing conventional methods and especially suitable for implementation of the FDA's Process Analytical Technologies (PAT) initiative by providing a process analyser tool for microbiological monitoring of cleanroom air[22].

Effective air-samplers must be able to precipitate particle sizes of at least 2mm. Although particles 5mm or larger are more meaningful (because most airborne micro-organisms are typically between 5-15mm and increase to 15-18mm for naturally occurring airborne particles which contain bacteria.

There is considerable debate within literature as to which type of air-sampler is the most efficient. This can be noted, but will not be explored further in this chapter. Furthermore, different models of air-sampler vary in their design. Some devices themselves can generate non-viable particle counts, and this should be considered in the design qualification of such devices. Other design considerations include the suitability of the sampler to any sanitisation procedure for equipment to enter the clean room and the sampler's operation as an isokinetic sampler (that is to match the airflow speed within the UDAF).

There are arguments against and in favour of active air samples in the Grade A/ISO Class 5 environment. This debate centres on the disruption of the air-flow caused by the operation of the air-sampler. The number of active air-samples taken during a session should be considered. The affect of the air-sampler can be examined through air-flow visualisations studies where the disruption of the air-flow can be visualised by smoke studies (at a time when the cleanroom is decommissioned).

Furthermore, the act of placing and removing an active air-sample is an intervention into the environment and this must be carefully practiced during media simulation trials or during practice sterility testing session. Where isolators are being monitored, many users have fitted the active air-sampler outside the isolator environment. This is achieved by feeding a length of tubing into the isolator, so that air can be drawn out and into the sampler. This is a similar concept to the placement of many particle counters and reduces the risks associated with intervention.

Surface samples: contact plates and swabs

Surface samples provide an indication of any contamination which may have settled onto surfaces as well as contamination which may be transferred by staff or equipment[23]. The various surfaces in a cleanroom and (or) a clean zone can be contaminated in different ways. Particles suspended in the surrounding air can sediment down on a surface; the operators can perform different types of interventions and thereby contaminate more or less critical surfaces. Even cleaning and (or) disinfection, especially if performed wrongly, can give rise to contaminated surfaces.

The two main techniques are contact (or RODAC: Replicate Organism Detection And Counting) plates and swabs.

The contact plate is superior to the swab and should always be used where there is a flat surface[24]. This is because the contact plate can be taken for a defined time using a set pressure. This can be achieved using commercially available activator devices. After use, the residue should always be wiped clean with a suitable sanitiser. A contact plate is an agar plate that, in contrast to a traditional plate, has been overfilled with agar. The overfilled agar will form a convex surface looking like an ink pad as shown in **Figure 2.**

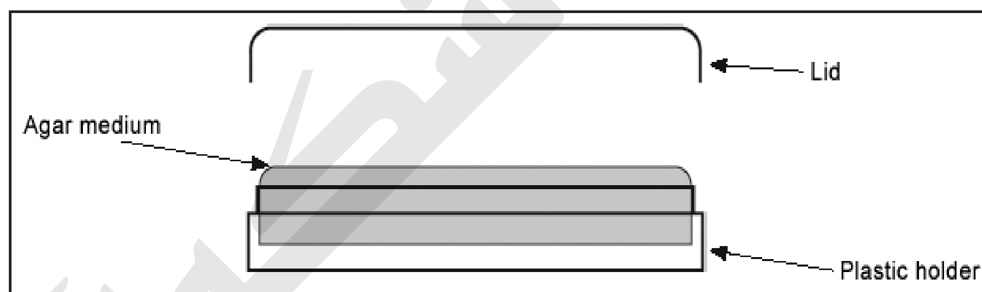


Figure 2: *The principle of a contact plate used for sampling micro-organisms on flat surfaces.*

The contact plate is pre-sterilised and equipped with a protective lid. The lid is removed and the exposed agar pressed towards the surface to be analysed. Compared with the techniques previously described for microbiological analysis of air, microbial surface sampling might be performed in different ways and subjected to variations dependent on how the sampling is performed. Differences in the outcome can be observed dependent on how hard the contact plate is forced towards the surface as well as the length of time that the plate is allowed to be in contact with the surface. The plate provides a ‘mirror image’ of the contamination and positional information.

A swab is a pin equipped with some type of porous material. Swabs are typically made up of sterile cotton tips. They are either contained within a transport medium or require pre-wetting with a suitable recovery medium (such as Ringer’s Solution; Phosphate Buffered Saline or sterile water). The swab is normally used to wipe a 10 cm^2 area. Swabs are used for sampling irregular surfaces or where high levels of contamination are expected (ie. ≥50 cfu). Generally, swabs provide only a

qualitative indication of a surface bioburden. Some disadvantages to this method are: (a) technique and sampling can affect results, and (b) requires manipulation to culture the sample[25].

Swabs are either designed to be sub-cultured on agar or dissolved and membrane filtered or placed into nutrient broth. Where swabs require sub-culturing, the swabs should be plated onto culture media inside the cleanroom environment rather than transported back to the laboratory as this reduces the risk of contamination. Swabs are less accurate than contact plates, as the recovery from a swab compared with a contact plate can be as low as 25%. However, swabs are useful for small areas or curved surfaces.

Due to the possibilities of performing the microbiological surface sampling in different ways, it is of vital importance to have written instructions outlining in detail how the sampling should be performed. The results obtained from microbial surface sampling become quite different if the contact plates have been pressed extremely hard towards a surface as compared to pressed only slightly. In some cases the written instructions state that a contact plate should be pressed towards a surface with a force corresponding to 200g and with a contact time of 20 seconds. Equipment is available in which the contact plate can be mounted so it always gives the same pressure during sampling. Many of these sampling aids also have built-in timers sounding an alarm when the test time is finished.

Both contact plates and swabs can be quantified. The typical surface area of a contact plate is $25cm^2$ and swabs are typically sampled using a template of a similar surface area. This allows the number of micro-organisms detected in a given area to be estimated and such data can be extrapolated for a larger surface.

Surface monitoring is further differentiated between floor surfaces and surface at working height. Surfaces at working height generally have tighter limits and are considered to be more critical. Surfaces at working height are horizontal surfaces. The differentiation between floors and surfaces with different limits is justified because no product touches the floor. Floor monitoring is a check of cleaning and basic hygiene.

Personnel samples

Samples from staff working in Grade A and B environments should be taken from their gowns in order to assess the bioburden they are carrying. For isolators, samples of parts of the isolator that have some form of human contact at the other side of the barrier should also be sampled. The key areas are gloves and sleeves, which are regarded as the most at risk areas for an isolator[26].

Gloves are sampled by finger-plates (or ‘dabs’) where each finger of the gloved hand is pressed onto the surface of an agar plate (9cm or larger). Sleeves are sampled by using contact plates. Therefore both sample types can be quantified as cfu per five fingers per hand or as cfu per $25cm^2$.

Such samples are taken at the end of the test session. The areas that have come into direct contact with the agar should be disinfected after use. Neither test will compensate for leak testing of gloves and sleeves *in situ*. A suitably designed test should be performed on a regular basis.

Culture media

Either one or two culture media are used for monitoring. For one medium, this is normally a general purpose one: tryptone soya agar (TSA), equivalent to the soyabean casein agar mentioned in the USP. A single medium is normally used at a dual incubation regime, such as: 30°C – 35°C for a minimum two days[c] and 20°C – 25°C for a minimum of five days[d] (incubation times should be altered in the event of weekends and bank holidays but the total incubation times will not be less than seven days). The dual incubation step is designed to encourage growth of any mesophilic aerobic bacteria and fungi that may be present

An alternative approach is to use two culture media, a general one for bacteria: eg. Tryptone Soya Agar (TSA) and a selective one for fungi, such as Sabouraud Dextrose Agar (SDA). With this regime, the TSA is incubated at one temperature (normally 30°C – 35°C) for three to five days and the SDA at another temperature (normally 20°C – 25°C for five days). Other agars or variations on the type of monitoring (such as anaerobic monitoring) may be undertaken for specific processes.

Before use, the culture medium must be validated[27]. Validation of culture media normally consists of testing articles of the medium at all the applicable test temperatures, against a range of micro-organisms. The micro-organisms used are those used to validate the sterility test (typed cultures from ATCC or a similar culture collection) and a number of environmental isolates from the cleanroom environments. The challenge inoculum is to be less than 100 cfu. The use of a low challenge is of particular importance because high numbers of micro-organisms will not be present in the cleanroom environment.

The type cultures will typically be: *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa* and *Aspergillus brasiliensis*.

It is important to perform validation work to confirm that the method of transferring the culture media into the cleanroom from an adjacent clean area or into an isolator does not cause inhibition of microbial growth. It may be, for example, that the hydrogen peroxide or peracetic acid used to sanitise loads into an isolator or the fumigant used to sanitise a clean room could cause inhibition if the sanitiser penetrated the bags containing the media. To counter this, the media can have neutralisers added to it in the event of an isolator sanitisation agent residing in the atmosphere (such as pyruvate to neutralise hydrogen peroxide). Where disinfectant residues are likely, the media used for surface samples (eg. RODAC plates) should also contain a suitable neutraliser.

19.5.3 Limits for viable environmental monitoring

Regulatory maximal values

With environmental monitoring, limits are required with which to compare the data gathered. Within ‘limits’ there are upper levels defined by regulatory authorities and warning and action levels set by the microbiologist.

With regard to regulatory limits and when setting maximal values for environmental monitoring, there are differences between the main international standards: EU GMP and the FDA. With USP chapter <1116> “Microbiological control and monitoring of aseptic processing environments”, reference to specific microbial limits was removed with the 2012 revision (for the 35th edition of the USP)[28].

The USP’s removal of specific limits was due to concerns with the limitations of environmental sampling methods, which included:

- That the methods are inaccurate,
- That the methods vary,
- Because personnel can contaminate them (a particular problem at ISO class 5),
- Because no single method can capture everything,
- Due to the methods being relatively poor at recovering damaged or stressed micro-organisms.

The argument in the USP was revised to indicate that treating a result of 4 CFU is not significantly different from one of 2 CFU. Instead, the USP argued that what was more important than limits was incident rates or the number of times that a non-zero event occurs. The chapter further argues that incident rates will vary according to the theoretical cleanliness of the environment.

To aid cleanroom microbiologists the USP recommends some incident rates for non-zero events,

although the chapter goes on to state that each cleanroom facility should set their own rates based on historical data and to trend these monthly.

The recommended incident rates are:

Class	Active air sample	Settle plate	Contact plate	Swab
Isolator or Closed RABS (ISO 5 or better)	<0.1%	<0.1%	<0.1%	<0.1%
ISO 5	<1%	<1%	<1%	<1%
ISO 6	<3%	<3%	<3%	<3%
ISO 7	<5%	<5%	<5%	<5%
ISO 8	<10%	<10%	<10%	<10%

It is important to stress, however, that the USP approach does not have universal regulatory acceptance and, within areas covered by EU and WHO GMP, the recommended limits apply.

The USP does not ignore high counts completely. The chapter uses a cut-off of 15 CFU and states that a plate with 15 CFU should happen very rarely. If such an event happens, a count of 15 CFU or more should be considered a significant excursion and subject to investigation.

In terms of recommended limits, as set out in the FDA guidance and EU GMP guide. The following tables compare the similarities and differences:

Active air samples (value per cfu/m^3)

Grade	FDA (2004)	EU GMP Annex I
A	1	<1 (average value)
B	10	10 (average value)
C	100	100 (average value)
D	Not stated	200 (average value)

Passive air samples (settle plates) (value per plate or per four hours)

Grade	FDA (2004)	EU GMP Annex I
A	1	<1 (average value)
B	5	5 (average value)
C	50	50 (average value)
D	Not stated	100 (average value)

Surface samples at working height (value per cfu/25cm^2)

Grade	FDA (2004)	EU GMP Annex I
A	Not stated	<1 (average value)
B	Not stated	5 (average value)
C	Not stated	25 (average value)
D	Not stated	50 (average value)

Surface samples (other e.g. floors) (value per cfu/25cm^2)

Grade	FDA (2004)	EU GMP Annex I
A	Not stated	Not stated
B	Not stated	Not stated
C	Not stated	Not stated
D	Not stated	Not stated

Finger-plates (value per hand)

Grade	FDA (2004)	EU GMP Annex I
A	Not stated	<1 (average value)
B	Not stated	5 (average value)
C	Not stated	Not stated
D	Not stated	Not stated

Suit gown plates (value per cfu/25cm^2)

Grade	FDA (2004)	EU GMP Annex I
A	Not stated	Not stated
B	Not stated	Not stated
C	Not stated	Not stated
D	Not stated	Not stated

It is noted with the above table that there are no regulatory guidance values for exit suit plates. Some cleanroom microbiologists adopt the same limits which apply to finger plates.

Notes:

a) EU GMP values are 'average values'

b) FDA 2004 Guide to Aseptic Filling states that alternative microbiological standards maybe established depending upon the nature of the operation and that "samples from class 100 [Grade A] environments are expected to yield no microbiological contaminants"

Alert and action levels

Alert and action levels have an important role in environmental monitoring. They indicate potential drift from normal operating conditions. A definition of alert and action levels is:

- *Alert level:* a level, when exceeded, indicates that the process may have drifted from its normal operating condition. Alert levels constitute a warning; they do not necessarily warrant corrective action but should be noted by the user.
- *Action level:* a level, when exceeded, indicates that the process has drifted from its normal operating range. This requires a documented investigation and corrective action.

Although compendia publish guideline alert and action levels for microbiological and particulate monitoring, regulators expect that, after a facility has been operating for a period of time, for monitoring levels to be set by the user to more closely reflect the actual environmental conditions. Maximum levels (action levels) for nonviable particles are defined in the various regulatory and compendial documents for each area classification. Microbial action levels are listed as recommendations in the various industry cleanroom standards.

To establish alert and action levels, 12 months of data are typically used. The most straightforward way to set levels is to analyse the data and to calculate descriptive statistics. From this, the second

and third standard deviations are adopted. The second standard deviation becomes the alert level and the third standard deviation becomes the action level. This approach only works if the data are normally distributed and most microbiological data are not (they are generally skewed with a large tail for the high counts, due to the majority of counts being zero). Attempts can be made to transform the data by converting them to $\log_{10}$ or by finding the square root. If the data, after transformation, do not conform to normal distribution, an alternative approach must be used. The best practices for the alternative approach are based on the data conforming to Poisson distribution. This is set either by looking at frequencies or using a percentile cut-off.

With these approaches, the alert level is an approximate of the 95th percentile and the action level at the 99th percentile. These levels would remain in place for approximately one year and then be recalculated. The assumption behind setting alert and action levels using historical data is that if the 12 months of data reviewed were 'normal', they will act as a good predictor for the next 12-month period. If this is not the case, then limits may need to be re-calculated with a justification. The regulators expect to re-evaluate Alert and Action Levels based on historical data periodically. Many companies re-calculate Alert and Action Levels annually based on the past data. Regardless of the frequency and method chosen, the expectation is that the calculated levels should go down with time and that adverse trends are timely detected and addressed.

19.5.4 Locations for monitoring

The number of environmental monitoring locations will depend upon the size of the cleanroom and the activities taking place. This involves a study of the room or process, as such monitoring sites will vary for different activities.

The locations selected should be representative of the cleanroom and each site should be justified as to why it has been selected. In taking into account locations for monitoring, the main areas of risk should be considered. The monitoring programme should acknowledge that people are the primary source of contamination in cleanrooms. Other areas can be chosen based on where there is potential for direct product impact: where microbial contamination would affect product quality and where contamination could spread through movement of samples, equipment or personnel.

Locations will include the air in the environment, critical surfaces and equipment, floors and walls and personnel. The programme should be based on risk assessment (see below). This will orientate monitoring to areas like routes of human traffic; areas which might become more heavily contaminated, such as door handles; where contamination is likely to spread or proliferate and transit routes; or focusing the programme on checking areas prone to be neglected like cleaning regimes. Sometimes the most appropriate location, during activities such as aseptic filling, will not be sampled because the act of sampling itself could cause contamination. In such cases this should be detailed in a rationale. Supporting evidence for the selected locations can be obtained from airflow studies.

The locations for viable monitoring can be selected in three ways:

a) Imposing an imaginary grid over the room and placing a sample at each location, approximately equidistant apart
b) Undertaking intensive or saturation monitoring for a period of time and then using the locations which provided the highest counts for routine monitoring
c) Using risk assessment tools and techniques to direct the monitoring towards the areas of greatest risk, such as open vessels, areas with a high personnel presence and so on[30].

Of the three approaches, a) is the most crude. It originates from the methodology used to classify cleanrooms using particle counts where the object is to obtain a homogenous sample of air, which

is best achieved by taking air from representative locations. The approach for microbiological monitoring is the same as the approach for particle counting whereby the square root of the surface area of the room is calculated. This is used to impose a grid over the room and samples are taken within each zone. The disadvantage with this approach is that it gives equal weighting to areas of the room that have negligible impact upon the product or process and no greater weighting to those areas of risk.

Approach b) is most often applied to newly built facilities. The approach is similar to that of c), whereby the process is studied and every area of risk monitored. After gathering a sufficient quantity of data, over the course of six months to one year, the number of locations and the frequency of monitoring can be reduced if the data are satisfactory and the samples for routine monitoring based upon those locations where the highest counts are recorded.

The most sophisticated approach is arguably c) as this orientates the monitoring to towards the actual process and product. Risk-based approaches include FMEA (Failure Mode and Effects Analysis); FTA (Fault Tree Analysis) and HACCP (Hazard Analysis Critical Control Points), all of which employ a scoring approach. At present, no definitive method exists and the various approaches differ in their process and the degree of complexity involved. However, the two most commonly used appear to be HACCP (which originated in the food industry) and FMEA (which was developed for the engineering industry.

These various analytical tools are similar, in that they involve:

- Constructing diagrams of work flows
- Pin-pointing areas of greatest risk
- Examining potential sources of contamination
- Deciding on the most appropriate sample methods
- Helping to establish alert and action levels
- Taking into account changes to the work process/seasonal activities

These risk assessment approaches are not only concerned with selecting environmental monitoring locations. They integrate the environmental monitoring system with a complete review of operations within the cleanroom to ensure those facilities, operations and practices are also satisfactory. The approaches recognise a risk, rate the level of the risk and then set out a plan to minimise, control and monitor the risk. Monitoring the risk will help determine the frequency and locations and the level of environmental monitoring.

One decision to be made is whether to rotate sampling locations or not. The argument in favour of this is that various areas of a cleanroom will be monitored, giving the data more range. The argument against this is that not rotating locations allows for greater consistency when the data is examined for long-term trends. The locations for monitoring should be justified in a report and indicated on a sampling map so that sampling is consistent and reproducible.

19.5.5 Sampling responsibilities

The environmental monitoring programme is normally controlled by the Microbiology Department who establish the appropriate frequencies and durations for monitoring based on a risk assessment approach. The sampling plan takes into account the cleanliness level required at each site to be sampled. The individuals who take the environmental monitoring samples should be clearly defined in a policy. Normally trained microbiologists take samples in Grade C and D cleanrooms. For Grade A and B samples there is a difference in thinking between the US and European approaches. In the US, the accepted approach is that all samples are taken by independent QC staff to counter against samples being taken by production staff, who may bias

the process. In Europe, the presence of additional staff in a cleanroom is seen as increasing the risk. Therefore, there is greater acceptance of process staff taking the monitoring samples for critical aseptic filling activities. A possible 'middle way' is to allow for process staff to take the majority of the samples for aseptic filling, but also for QC staff to monitor random fills (on a basis of 1 in 10).

Where process staff do take samples it is important that they go through the same rigorous training programme as QC staff and are trained in microbiological awareness.

19.5.6 Frequency of monitoring

The frequency of viable microbiological monitoring should be based on a criticality risk assessment of cleanrooms. For aseptic filling operations, monitoring is normally performed for each operating shift in Grade A zone and Grade B cleanrooms which are part of the batch fill or are associated with the batch fill. Each operating shift is defined as the duration of the batch fill and to cover all staff involved with the filling process. Therefore samples of air are taken at defined time intervals during the fill and surfaces samples taken at the end of the fill. Each individual present in the fill should also be monitored using finger-plates.

For lower grade cleanrooms, monitoring should be by risk assessment, which should be based on an examination of each cleanroom, based on different factors within the room. Such factors include:

a) Room activity (such as process, storage, office/administration, washing, sterilising, e.g. autoclave operation, sterile filtration, sterile filling)
b) Product risk/exposure time (such as none, enclosed, open momentarily but mostly enclosed, open plant assembly, e.g. centrifuges, open product)
c) Room temperature (cold, ambient or warm, e.g. room housing an autoclave)
d) Process stage (start, e.g. plasma stripping, mid, end e.g. pre-final filtration). This is based on the distance from the final formulation
e) Duration of activity (short, e.g. <30 minutes; medium 1 – 4 hours; long >4 hours)
f) Water present in room (absence, medium, e.g. water outlets in room or floor likely to be wet, high, e.g. wash-up area).
g) Drain present or not
h) Cleaning frequencies
i) Room occupancy
j) Equipment: fixed or mobile
k) Environmental monitoring history

Generally the frequency of monitoring increases the further the production process moves toward the bulk product.

Other sampling sessions may be performed, such as immediately after sanitisation so that the effectiveness of the sanitisation can be assessed and following maintenance. Other monitoring sessions can be added. These may include routine sweeps using selective agars (such as during seasons where fungi may be a concern).

19.5.7 Duration of monitoring

Viable monitoring for batch filling operations (within the Grade A zone and the Grade B room in which the fill takes place) must be at intervals during the duration of the fill (the intervals will be at a random time during the fill). It is not considered that any specific time represents "worst case" and therefore any given time is "equal case" and so random sampling is justified. For the types of samples previously discussed, a typical sampling regimen is:

- Settle plates are exposed for the duration of a fill (additional settle plates may need to be used if the fill exceeds the validated plate exposure time)
- Active air samples will be taken at the (near) start and (near) end of the fill
- Finger plates will be taken immediately after a connection activity, for any persons present during the fill at a random time during the fill, after a Grade A zone intervention and at any other time a microbiology technician believes it to be necessary
- Surface monitoring will take place immediately at the end of the fill. This is not performed during filling due to the invasive and disruptive nature of the techniques. Sterilised product contact surfaces and the filling needles are not monitored, because these areas should be zero growth, and held under Grade A conditions. Environmental monitoring is not designed as a surface sterility test
- Contact plates of gowns will be taken from all personnel immediately before they exit the Grade B area (Aseptic Filling Suite).

For Grade C and D process areas, viable monitoring will be for a defined period of time during an event or process. Active air samples should be run so that one cubic metre of air is sampled and settle plates must be exposed for more than one hour where possible. Exceptions include activities of a short duration. Surface monitoring is performed at some point during the dynamic state. It is not considered that any given time represents "worst case", whereas it is considered that a randomly sampled time represents "equal case" for a sample taken during the process.

19.5.8 Microbial identification

All micro-organisms detected above action level should be characterised to species level (where possible). Understanding the diversity of microflora is of importance for the pharmaceutical microbiologist. Knowing the potential origins of different micro-organisms can indicate the potential source of contamination. Knowing the species of a micro-organism can provide invaluable information as to its origins. For example, many Gram-positive cocci are part of human skin flora, Gram-positive rods can be transferred into clean areas via equipment and on footwear, and Gram-negative rods are often linked to water sources.

Such characterisations are important for various root cause analyses, such as linking contamination from surfaces to intermediate product, determining if contamination could have arisen from personnel intervention, or understanding if a sterility test failure is due to product contamination or is a false positive[29].

Furthermore, there is also considerable cGMP emphasis upon screening for objectionable micro-organisms. The impact of micro-organisms upon product quality attributes will depend on the product, its intended or potential application, method of manufacture, and subsequent treatment if applicable. In addition, monitoring for changes in microbial trend can indicate a possible problem emerging within the clean area environment, such as reduction in cleaning and disinfection standards.

Micro-organisms found in pharmaceutical ingredients, water for pharmaceutical use, the manufacturing environment, intermediates, and finished products are frequently identified ('speciated'). This is especially common if their numbers exceed alert and or action levels for the material or process environment tested. The method of identification is based on the needs of the microbiologist. In some circumstances, to know that a micro-organism is a Gram-positive coccus can be sufficient (where, the differentiation between a staphylococcus and a micrococcus) is of little relevance when evaluating what is found in a cleanroom. In other circumstances, a phenotypic identification is undertaken so that the microbiologist can understand the general profile for micro-organisms. In more specific circumstances, such as where a sterility test has failed, a close genetic match between the sterility test contaminant and cleanroom microflora may be sought.

Identification methods

Colony and cell morphology

The first step of most identification schemes is to describe the colony and cellular morphology of the microorganism. Colony morphology is normally described by directly observing growth on agar, where the colony will appear as a particular shape (such as raised, crenated, spherical, etc.) and the colony will have a particular pigment. Some microbiologists will attempt to identify the microorganism based on such visual identification. This is not normally encouraged as considerable experience is required to do this and the variety of microflora cannot be characterised with any degree of accuracy. Furthermore, the characteristics of a microorganism are often dependent upon the type of culture medium used. Nevertheless, a description of the morphology can assist with further stages of identification.

Cellular staining provides important information relating to the composition of the microbial cell wall, as well as the shape of the organism.

Gram stain

The primary staining technique used to differentiate bacteria is the Gram stain. The Gram stain method includes the four-step technique: crystal violet (primary stain); iodine (mordant); alcohol (decolouriser); safranin (counter-stain), or the three-step method in which the decolourisation and counter-staining step are combined. Done correctly, Gram-positive organisms retain the crystal violet stain and appear blue; Gram-negative organisms lose the crystal violet stain and contain only the counter-stain safranin and thus appear red. Common pitfalls in this method are that heat fixation may cause Gram-positive cells to stain Gram-negative and older cultures may give Gram-variable reaction; using too much decolouriser could result in a false Gram-negative result and not using enough decolouriser may yield a false Gram-positive result.

The Gram reaction is based on the differences in the cell wall composition for the two cellular ‘groups’. The bacteria that retain the stain (the Gram-positive bacteria) have a higher peptidoglycan and lower lipid content than those that do not retain the stain (the Gram-negative bacteria). The effect of the solvent is to dissolve the lipid layer in the cell wall of the Gram-negative bacteria, thereby causing the crystal violet to leach out; whereas for Gram-positive bacteria the solvent dehydrates the thicker cell walls, blocking any diffusion of the violet-iodine complex, which closes the pores of the cell and retains the stain. There are now several automated Gram stain devices available on the market that can reduce the labour requirement required when performing several multiple Gram stains and, possibly, improve accuracy.

In addition to the difference based on the cell wall, microscopic examination of the stains allows the cellular shape to be determined. Bacteria commonly fall into the categories of coccus (spherical), rod, vibrio (curved), spirilla (spiral) and Pleomorphic (variable)[31].

Spore stain

Some bacteria, such as species of bacillus, form endospores. Knowing if spores are present can help to characterise such organisms. Common spore staining methods employed include a two-step method: malachite green (primary stain) and safranin (counter stain). The fixed bacterial smear is stained with 7.6% aqueous malachite green solution for 10 minutes (sometimes this is facilitated by ‘steaming’ the stain), rinsed, and counter-stained with a 0.25% aqueous safranin solution for 10 seconds, rinsed, and blotted dry. This procedure stains the spores green, but the rest of the bacterial cell is stained red.

Fungal staining

Lactophenol Cotton Blue Stain is used in wet mounts for microscopically examining yeast and filamentous fungi and serves as both a mounting fluid and stain. Staining the specimen light-blue

allows subtle features such as septa, special mycelia and spore structures to be easily visualised by microscopy. The majority of fungal identifications are undertaken based on the morphological characteristics of the stain and growth on solid media.

Biochemical profiling
Once the cellular characteristics have been reported, the second stage of identification is to identify the genus and species of bacteria. The most common techniques used, based on their costs and long history, are biochemical tests.

Key biochemical screening tests, which aid the further differentiation of bacteria, include the oxidase test to separate Gram-negative rod-shaped bacteria into non-fementers (oxidase positive) and enteric (oxidase negative) bacteria; the catalase test to separate staphylococci (catalase-positive) from streptococci (catalase-negative), and the coagulase test to separate staphylococci into coagulase-negative (presumptively non-pathogenic) and coagulase-positive (more likely pathogenic) staphylococci (eg. *S. aureus*).

Phenotypic reactions typically incorporate reactions to different chemicals or different biochemical markers. These rely on more subjective determinations. The reliance upon biochemical reactions and carbon utilisation patterns introduces some disadvantages to the achievement of consistent (repeatable and reproducible) identification. However, these are mature technologies, such as the API strip, marketed by many companies as consistent, pre-packaged kits with well-established quality control procedures, often with instrumentation and with extensive databases to identify the most commonly encountered micro-organisms found in the pharmaceutical industry. The API strip is basically a pre-packaging of the standard methods of a series of test tubes into a convenient bubble-wrap design. This method was further refined with the VITEK automated systems which miniaturised the process. An alternative method is the Biolog, which uses a 96-well plate containing different carbohydrate sources and a reaction dye to produce a so-called ‘metabolic fingerprint’. Other variations include fatty acid analysis through the use of gas chromatography.

RNA and DNA homology
In contrast to the phenotypic methods, genotypic techniques are more accurate. This is because the microbial genotype is highly conserved and independent of the culture conditions, so the identifications may be conducted on uncultured test material--primary enrichments that increase the amount of nucleic acid available for analysis. Genotypic microbial identification methods based on nucleic acid analyses are less subjective, less dependent on the culture method, and theoretically more reliable because nucleic acid sequences are highly conserved by microbial species. These methods would include DNA-DNA hybridisation, PCR, 16s and 23s rRNA gene sequencing, and analytical ribotyping. An example is the Riobprinter, an automated Southern Blot device which uses a labelled ssDNA probe from the 16sRNA codon. Another rapid method is a PCR system which uses a form of ‘bacterial barcodes’ where the amplified genetic sequence is separated by gel electrophoresis and visualised to give a ‘barcode’ specific to that strain. This PCR technique utilises small amounts of samples to produce a high yield of the targeted DNA material.

These methods are more technically challenging for the pharmaceutical microbiologist and are more expensive in terms of both equipment and current testing costs. The methods are often used for more critical identifications, such as sterility test and media trial contamination, rather than for the routine characterisation of microflora.

19.5.9 Devising an effective environmental monitoring programme
All the various elements examined need to be combined together into an environmental monitoring programme. In constructing the programme, a number of elements need to be considered[32]. These can be summarised as:

Strategic questions

- What organisms are to be examined?
- What test methods are to be used?
- What is to be studied: cleanrooms, equipments, personnel?
- When is the analysis performed: in the operational and (or) the at-rest state?
- Are the tests used for routine or trouble shooting purposes?
- Are the tests performed as discrete sampling (a test here and now) for monitoring and (or) classification purposes?

Background detail questions

- Which cleanrooms are to be analysed?
- What takes place in the cleanroom when the analysis is performed? Such as the type of product produced, the number of operators, etc.
- Date and time for the analysis?
- Temperature and humidity?

Analytical questions

- How are the micro-organisms to be sampled? What equipment and what growth medium are used?
- How are the micro-organisms allowed to propagate? Temperature, time, humidity etc.?
- How are the final results collected? Counting of visible colonies on the growth medium, CFUs?

19.5.10 Data examination

Viable count data should be examined regularly and the findings reported to senior management. Individual excursions of action levels should be investigated using variations of an ‘out of specification’ procedure. However, individual excursions are rarely of value and do not indicate the correct picture of what is happening within the cleanroom. More meaningful data are provided by trend analysis[33].

More sophisticated trending is carried out with control charts that are used to differentiate between such variations to show:

a) Those variations that are normally expected of the process due to chance or common causes. These should be expected, to an extent

b) Those variations that change over time due to assignable or special causes. These often require some form of action.

The two major analysis charting tools commonly used are:

a) A control chart called a cumulative sum chart (or cusum), suitable for large quantities of low count data collected over time and where only a small shift in data is anticipated.

b) A control chart called a Shewhart chart (or X-Y chart), suitablefor larger numbers and/or for detecting larger shifts in the data.

Cusums function by displaying cumulative sums of the deviations of measurements or subgroup means from a target value. This can be an increase or a decrease away from the target. Cusum charts are theoretically more sensitive to shifts in the process mean than Shewhart charts. Cusums will show:

a) If changes have taken place.

b) Approximately when the change has taken place.

Shewhart charts have control limits. Data is plotted against the control limits, where the centre line

in the chart is the *grand average*. The power of Shewhart charts is that they can be studied for unusual events or patterns in the data. Applications include:

- Gaining an understanding of the variability of the process
- Being able to mark changes
- To determine if improved reactions occur following any changes
- To demonstrate effectiveness of any actions taken

19.5.11 Corrective and preventative actions (CAPA)

Environmental monitoring results which exceed the action level or where there is an upward trend relating to excursions of the alert level should be investigated through a formal documented system where the responsibilities and procedures for investigating these excursions should be clearly defined. These investigations may be described as:

- Out of specification
- Out of limits
- Out of trend
- Microbiological data deviations

Microbiological results are often difficult to interpret. This is for several reasons:

- Micro-organisms are ubiquitous in nature and common environmental contaminants
- The technician has the potential to introduce contaminating micro-organisms during sampling and/or testing
- Micro-organisms could not be homogeneously distributed within the sample or an environment or in water for pharmaceutical purposes. It is well established that micro-organisms follow a Poisson distribution in water samples, for example, an effect that is considerably enhanced with low populations of micro-organisms)
- Microbiological assays are subject to considerable inherent variability.

The investigation of an environmental monitoring level excursion should be covered by a Standard Operation Procedure (SOP) and formally documented. The SOP should contain decision trees to ensure that, where possible, the conclusions reached are consistent.

Before proceeding with a formal investigation, a check should be made in the microbiology laboratory to ensure that the result is not due to ‘laboratory error’. Such considerations may include whether the sample was taken and handled correctly; if the correct culture media was used; if the equipment was within calibration date (such as an active air-sampler); if the sample was incubated for the correct time and temperature; if there is any possibility of sample contamination; whether the result was read correctly and reported in the correct units of measurement.

There are many different approaches for examining out of limits events. In general, approaches include[34].

a) A description of the problem or event
b) Examination of trends
c) Data collection
d) Investigation
e) Risk assessment
f) Determination of the most probable root cause
g) Consideration of CAPA

h) Summary

Each of these areas is examined below.

a) Description of the problem or event

The contamination event or upward trend should be clearly defined. In describing the event,reference should be made to any similar incidents within a recent timeframe.

b) Examination of trends

The contamination event should be placed in context by examining recent trends. This is especially important for environmental monitoring data, for often individual results are of little significance and greater emphasis is placed on the direction that the data are taking.

c) Data collection

Often additional data are required to assess the impact of the contamination event. With environmental monitoring this often means additional samples taken from the same sample location and often from the same room in which the event occurred. Depending on the nature of the process, it may be necessary to take samples from adjacent rooms. As part of assigning a root cause, sampling is sometimes undertaken in the occupied and unoccupied states to distinguish between personnel, equipment and air handling system causes. Data collection may also lead to a period of intensive monitoring (at an increased frequency) for a period of time.

d) Investigation

The purpose of the investigation is to determine the root cause of the incident. Even if the incident leads to a batch rejected, the investigation is necessary to determine the source of the contamination to prevent recurrence (corrective and preventative actions, as part of a CAPA review) and to determine if the result is associated with other batches of the same drug product, other products (risk assessment is an important consideration), or reflects an on-going concern with personnel practice or clean area environmental operations.

Investigations into contamination events should consider why the action level was exceeded and why the contamination event has occurred. This involves determining the source of the contamination. For this, invaluable data come from understanding the microflora. The range of micro-organisms found in the cleanroom environment can be sub-categorised according to the source or their probable location. This can provide significant information when formulating corrective and preventative actions as resources can be directed to tackling the contamination source.

When undertaking investigations, there are a number of investigation tools which can be employed. These include flow charts, decision matrices, fishbone diagrams, contradiction tables, Failure Modes and Effects Analysis, etc.

There are a number of areas that can be considered when investigating environmental monitoring results:

- Check of cleaning and disinfection frequencies and methods
- Check of HVAC parameters, including air supply volume, air change rates, room pressure differentials, air flow patterns
- Review of utility monitoring data, such as compressed air and water
- Examination of staff behaviour and practices
- Linking any patterns with viable and particle monitoring data together

- Examination of equipment as sources of contamination (such as equipment generated particles)
- Visual observation of the process by QA staff

To be meaningful, the investigation should be thorough, timely, unbiased, well-documented, and scientifically sound.

e) Risk assessment

The use of risk assessment in the pharmaceutical industry is both an increasingly used tool and an expectation of regulatory authorities. Risk assessment forms part of the risk management programme. Risk assessment tools and techniques can be applied to every aspect of pharmaceutical processing and for microbiological investigations. An important part of this application involves an understanding of the process. Although the microbiologist should be at the forefront of any investigation into a contamination event, there is a great advantage from the use of a multi-disciplinary team[35].

With environmental monitoring, the most common risk assessment technique is Hazard Analysis and Critical Control Point (HACCP), a risk assessment approach that addresses physical, chemical and biological hazards. HACCP is designed so that key actions, known as Critical Control Points (CCPs) can be taken to reduce or eliminate the risk of the hazards being realised[36]. A typical risk assessment of a production process in a cleanroom might consist of:

- A route map (where the facility is drawn and the route indicated)
- Identification of hazards (which can be divided into biological, physical, equipment, transport and chemical). This will allow an assessment of existing control measures
- Process flow
- Assessment of environmental monitoring. This will determine if the activity is safe to proceed

f) Determination of the most probable root cause

The determination of the root cause is designed to provide the source of the contamination and an explanation as to why the contamination event happened. This is necessary in order to fully understand the problem and for assigning corrective and preventative actions.

Often the process of assigning a root cause involves a process of elimination, working through an investigation checklist to determine what 'is' or 'is not' a potential problem. The final conclusion may be a 'most probable' root cause based on no definitive cause but one which stands out following the elimination of all other potential issues.

g) CAPA

Corrective and preventative actions are the consequences of the investigation and describe the actions taken which are designed to lead to an improvement of process and environmental quality. Corrective actions relate to either things that can be done at the time or if any additional testing or monitoring can be performed. Preventative actions are designed to put in place measures to prevent the contamination event from reoccurring. Examples include:

- Staff training
- Additional cleaning
- Repairs to equipment
- Repairs to fabric
- Alterations to HVAC systems
- Changes to work practices and redesigning activities
- Further assessment of risk (such as conducting air-flow studies)

- Increased monitoring or review of monitoring locations

h) Summary

It is important that all investigations have a clear and succinct summary which briefly describes the origin of the contamination, the risk to the product and process and measures to prevent reoccurrence. Such summaries are useful for senior management and for showing to regulators.

Individual excursions and trends should be reported to senior management either directly or through summary reports (such as Quality Exception Reports).

19.6 Conclusion

This chapter has examined the best practice requirements for environmental monitoring of cleanrooms. The chapter has presented the techniques required and has discussed the approaches that can be taken for assigning locations for monitoring and for establishing the frequencies for monitoring. The chapter has included a section on data trending and results investigation. This latter section – investigation of out of limits results – is arguably the most important and overlooked area, for there is little value in gathering data and yet failing to react to problematic counts.

These elements of the microbiological monitoring programme have featured in several recent FDA warning letters and other regulator citations. In preparing this chapter, the authors examined some recent regulatory comments with regards to environmental monitoring. From this review, the key areas were:

a) Monitoring programmes being inadequate in terms of cleanrooms not being monitored for both viable and non-viable contaminants.
b) Similarly, critical areas of the production process were not monitored for contamination.
c) Failure to monitor all aspects, in relation to aseptic filling, for particles. These comments related to machine set-up, pre-fill periods, in-fill monitoring and post-fill disassembly.
d) Alert and action levels being set higher than regulatory guidance values with respect to the cleanroom class. In some cases action levels were not set at all.
e) Environmental monitoring programmes have been criticised for not being regularly reviewed.
f) In relation to viable monitoring, some comments have related to settle plates not being exposed for the recommended four-hour exposure time.
g) Furthermore, there have been comments about viable monitoring results being averaged, which meant that results that exceeded action levels were missed.
h) Some companies were not identifying all of the micro-organisms from the most critical areas (like Grade A/ISO class 5 areas).
i) Trend analysis has either been lacking or inadequate in that it failed to show the ‘true’ picture of the facility.
j) After taking finger plates, in one company, staff continued with their work without sanitising their hands.
k) Cleanroom gown monitoring has been criticised for being inadequate on several occasions. This relates to monitoring not being undertaken or to critical locations being missed.
l) Comments were made by regulators about the storage and incubation of environmental monitoring plates, such as plates being sealed (with inhibited aerobic growth) or stacked inappropriately.
m) In relation to incubation, one firm was found to be incubating environmental monitoring samples in the process area.
n) Some companies had failed to qualify their detergents and disinfectants and were not monitoring them for microbial contamination.

o) Also with regard to disinfectants, not all the environmental monitoring culture media were found to contain disinfectant neutraliser.

p) With some firms, environmental monitoring was not undertaken for sterility testing.

q) SOPs for environmental monitoring are regularly commented on as being unclear or nor being sufficiently comprehensive.

r) Microbiological investigations into out of limits results have been commented upon for not considering all root causes.

The above list indicates that the environmental monitoring of cleanrooms perennially is a regulatory 'hot topic' and an organisation's monitoring programme is likely to be a central feature of any inspection. The authors hope that this chapter will aid the reader in preparing for such inspections, as well as providing general information in relation to environmental monitoring.

Notes

[a] 'Laminarity' refers to a HEPA-filtered air stream moving within a confined space, along parallel flow lines, with uniform velocity.

[b] One cubic metre is equivalent to 35.18 cubic feet

[c] Plates moved on day three or thereafter

[d] Plates moved on day six or thereafter

19.7 References

1. Akers JE. Environmental monitoring and control: proposed standards, current practices. *PDA J Pharm Sci Tech* 1997; 51:36-47.
2. Food and Drug Administration. Guideline on Sterile Drug Products Produced by Aseptic Processing FDA, Rockville, MD: 2004.
3. Eudralex. The Rules Governing Medicinal Products in the European Community, Annex 1, European Commission, 2009.
4. Cundell AM. Microbial testing in support of aseptic processing,. *Pharmaceutical Technology* 2004: pp56-64.
5. Sandle T and Boschi F. *Environmental Monitoring Handbook*, Advanstar, Duluth, USA (HBEM06); 2006.
6. Sandle T. Environmental monitoring risk assessment. *Journal of GXP Compliance* 2006; **10** (2): 54-73.
7. Sandle T. Environmental Monitoring. In: Saghee MR, Sandle T and Tidswell EC (eds.). *Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices*, Business Horizons, New Delhi, 2011. 293-326.
8. Sharp J, Bird A, Brzozowski S and O'Hagan K. Contamination of cleanrooms by people. *European Journal of Parenteral and Pharmaceutical Sciences* 2010; **15**(3):73-81.
9. Ramstorp M. *Introduction to Contamination Control and Cleanroom Technology* 2000. Wiley VCH Verlag GmbH, Weinheim, Germany.
10. Whyte W and Hejab M. Particle and microbial airborne dispersal from people. *European Journal of Parenteral and Pharmaceutical Sciences* 2007; **12** (2):39-46.
11. Lee G and Midcalf B (eds). Isolators for Pharmaceutical Applications. 1994, HMSO, London.
12. Sandle T and Saghee MR. Advances in cleanroom technologies, Express Pharma, 16-30 September 2010
13. Farquharson G and Whyte W. 1992: The design of cleanrooms for the pharmaceutical industry. In:Whyte W (d) 'Cleanroom Design', John Wiley and Sons, Chichester
14. Whyte W. Cleanroom technology: fundamentals of design, testing and operation. Wiley, London. 2001, p15-16.
15. Sandle T. Selection and use of cleaning and disinfection agents in pharmaceutical manufacturing. In:Hodges N and Hanlon G 2003: Industrial Pharmaceutical Microbiology Standards and Controls, Euromed Communications, England.

[16] Ljungqvist B and Reinmuller B. Some observations on environmental monitoring of cleanrooms. *European Journal of Parenteral Sciences* 1996;**1**: 9 –13.

[17] Tetzlaff R. Investigational trends: clean room environmental monitoring. *PDA J Parenteral Sci Tech* 1992;46:206-214.

[18] Wilson J. Setting alert/action limits for environmental monitoring programs. *PDA J Pharm Sci Tech* 1997;**51**:161-162.

[19] Van Doorne H. Monitoring of aseptic drug preparation by fall-out plates. *Ziekenhuisfarmacie* 1996: pp 87-91.

[20] Whyte W. In support of settle plates. *PDA J Pharm Sci Tech* 1996; **50**:201-204.

[21] Meir R and Zingre H. Qualification of air-sampler systems: MAS-100. *Swiss Pharma* 2000;**22**:15 – 21.

[22] Miller MJ. Quality risk management and the economics of implementing rapid microbiological methods. *European Pharmaceutical Review 2009;***2**:66-73.

[23] Prout G. The nature and the environmental impact of control of floor level contamination. *European Journal of Parenteral and Pharmaceutical Sciences*, 2009;**14**(1):13-18.

[24] Tidswell E C, Bellinger M, McCullough D, Alexander A. Consecutive replicate contact plate sampling assists investigative characterisation of surface-borne bioburden *Eur. J. Parent. Pharma. Sci* 2005;**10** (4), 93-96.

[25] Lemmen S. Comparison of two sampling methods for the detection of gram-positive and gram-negative bacteria in the environment: moistened swabs versus RODAC plates. *Int J Hyg Environ Health* 2001;**203**:245-248.

[26] Sandle T. The use of a risk assessment in the pharmaceutical industry – the application of FMEA to a sterility testing isolator: a case study. *European Journal of Parenteral and Pharmaceutical Sciences*2003; **8**(2): 43-49.

[27] Noble NH. Validation of environmental monitoring methods and levels. *PDA J Parenteral Sci Tech* 1993;**47**:26-28.

[28] USP Chapter <1116> (2012). Microbiological control and monitoring of aseptic processing environments, United States Pharmacopoeia (35) and National Formulary (30): 697-707, May, 2012.

[29] Tidswell EC. Risk profiling pharmaceutical manufacturing processes. *European Journal of Parenteral and Pharmaceutical Sci*ences 2004; **9**(2),49 –55.

[30] Hyde WA. Origin of bbacteria in the clean room and their growth requirements. *PDA J Pharm Sci Tech* **1998**; 52:154-158.

[31] Cowan ST and Steel KJ. Manual for the Identification of Medical Bacteria, Cambridge University Press, Cambridge, UK; 1965.

[32] Vincent D. Validating, establishing and maintaining a routine environmental monitoring program for cleanroom environments: Part 1. *Journal of Validation Technolog* 2002;8(4):

[33] Hussong D and Madsen RE. Analysis of environmental microbiology data from cleanroom samples. *Pharm Technol. Aseptic Proc* 2004:10-15.

[34] USP Chapter <1117> Microbiological good laboratory practices. Pharmacopoeial Preview. *Pharmacopeial Forum* 2003; **29**(3):842-850.

[35] Sandle T. Risk Management in Microbiology. In: Saghee MR, Sandle T and Tidswell E. (eds). Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices, Business Horizons, New Delhi, India; 2011.

[36] World Health Organisation: Application of Hazard Analysis and Critical Control Point (HACCP) methodology to pharmaceuticals, *WHO Technical Report Series No 908, Annex 7, World Health Organisation*, Geneva, 2003. (www.who.int/medicines/library/qsm/trs908/trs908-7.pdf)

Cleaning and disinfection practices

Tim Sandle, Head of Microbiology, Bio Products laboratory, St Albans, UK
Madhu Raju Saghee, Assistant Manager – QA, Novartis India Ltd, Thane, India

20.1 Introduction

An important step towards achieving microbial control within a cleanroom is the use of defined cleaning techniques, together with the application of detergents and disinfectants. The objective of cleaning and disinfection is to achieve appropriate microbiological cleanliness levels for the class of cleanroom for an appropriate period of time. Thus the cleaning and disinfection of cleanrooms is an important part of contamination control[1].

This chapter is concerned with both detergents (which 'clean') and disinfectants (which remove or eliminate micro-organisms). Detergents are cleaning agents and are deployed to remove 'soil' from a surface. The removal of soil is an important step prior to the application of a disinfectant, for the greater the degree of soiling remaining on a surface, the lesser the effectiveness of disinfection. A disinfectant is a type of chemical germicide capable of eliminating a population of vegetative micro-organisms (although some disinfectants are sporicidal, a chemical does not need to be sporicidal to be classified as a disinfectant). A disinfectant that can kill spores is sometimes described as a sterilant or chemosterilant[2]. However, this term is open to confusion as a chemical disinfectant used to treat hard surfaces in a cleanroom cannot be proven to sterilise and achieve the same level of sterility assurance, which would be applied to a sterilisation device. Thus the term sporicidal disinfectant is less ambiguous).

Disinfectants vary in their effectiveness against different types of micro-organisms, a variation relating to both the intrinsic resistance of different micro-organisms and the range of different types and formulations of disinfectants. Furthermore, different disinfectants act in different ways depending upon their active ingredients.

The scope of this chapter is the cleaning and disinfectant practices for cleanrooms. This refers to the cleaning and disinfection of floors, walls, external surfaces of equipment and the practices undertaken to ensure that surfaces meet the cleanroom standards for permitted levels of viable micro-organisms. In addition, the chapter considers hand sanitisation practices (which is important given that personnel are the primary contamination sources within cleanrooms). However, this chapter does not cover the cleaning, sanitisation or sterilisation of vessels; neither does it discuss gassing systems, as this subject is discussed elsewhere in this book.

20.2 Cleaning and disinfection concepts

In order to examine different types of detergents and disinfectants, it is important to understand some key terms such as cleaning, detergent; and those for antimicrobial agents such as disinfectant, sanitiser and antiseptic. These are discussed below.

20.2.1 Cleaning

Cleaning, in the context of cleanrooms, is the process of removing residues and 'soil' (such as dirt, grease, protein residues, etc.) from surfaces to the extent that they are visually clean. This involves defined methods of application and often the use of a detergent. Cleaning steps are often necessary prior to the application of a disinfectant as it is essential for a surface or item of

equipment to be properly cleaned before the application of a disinfectant, so the disinfectant can work efficiently.[3]

Cleaning can arguably be seen as a form of disinfection in its own right as the cleaning process can remove or dilute microbial populations, and many detergents have chemical additives that can 'disinfect'. However, a cleaning agent will not meet the criteria for disinfection required by the European and United States standards for disinfectant validation in terms of reducing a microbial population to the required log reduction.

20.2.2 Detergent

A detergent is a chemical used to clean equipment or surfaces by removing unwanted matter (soil). Detergents generally work by penetrating soil and reducing the surface tension (which adheres soil to the surface) to allow its removal (in crude terms, a detergent increases the 'wettability' of water). Many detergents are surfactants (Surface Active Agents), which remove particles from surfaces by either capillary effects or electrostatic forces (many detergents contain differently charged ions that can cause micro-organisms to repel each other). This repulsion causes the micro-organisms to disassociate from the surface and become suspended. Suspended micro-organisms are easier to remove from the surface by the rinsing effect of the detergent (or a subsequent water rinse) or to be destroyed by the application of a disinfectant.

20.2.3 Disinfectants

A disinfectant is a chemical agent, from a very diverse group of products, that reduces the number of micro-organisms present either by removing or destroying them. The term disinfection is normally applied to an inanimate object, whereas the term antiseptic is used to describe the reduction of a microbial population on living tissue[4].

Various types of disinfectants with different spectrums of activity and modes of action have differing efficacies. Some are *bacteriostatic*, where the ability of the bacterial population to grow is halted. Here the disinfectant can cause selective and reversible changes to cells by interacting with nucleic acids, inhibiting enzymes or permeating into the cell wall. Once the disinfectant is removed from contact with bacteria cells, the surviving bacterial population could potentially grow.

Other disinfectants are *bactericidal* in that they destroy bacterial cells through different mechanisms. These include: causing structural damage to the cell, autolysis, cell lysis, or by the leakage or coagulation of the cytoplasm. Within these groups, the spectrum of activity varies, with some disinfectants being effective against vegetative Gram-positive and Gram-negative micro-organisms only, while other disinfectants are effective against fungi. Other disinfectants have a broader spectrum and are sporicidal in that they can cause the destruction of endospore forming bacteria. However, a chemical agent does not have to be sporicidal in order to be classed as a 'disinfectant' or as a 'biocide'[5]. The bacteriostatic, bactericidal and sporicidal properties of a disinfectant are influenced by many variables. These variables are examined later.

The process of disinfection can be performed using manual or mechanical (automated methods), for example, a Clean-in-Place (CIP) system used to process production equipment such as vessels. The chapter is primarily concerned with manual methods of disinfection.

20.2.4 Sanitiser

The term sanitiser is open to different interpretations. Within Europe, it is normally taken to be an agent that both cleans and disinfects (normally a disinfectant that contains a cleaning agent). Within North America (defined by the US Environmental Protection Agency), however, the term is normally applied to an antimicrobial agent for use on non-food contact surfaces. Sanitisation is a general description for reducing a microbial population. Disinfection is a more precise term in that

the chemical agent must reduce a known number of micro-organisms (a property demonstrated through validation).

20.2.5 Antiseptics

Antiseptic is a term applied to the use of a disinfectant on living tissue (ie. a substance which can be applied safely to the surface of the skin)[6]. In the context of this chapter, antiseptics for hands (‘hand disinfectants’) are examined. The use of hand disinfectants is part of the process of contamination control for personnel working in cleanrooms. Due to the association of antiseptics with the treatment of wounds, the term ‘hand disinfectant’ is used rather than ‘antiseptic’.

20.3 Regulatory requirements

The use of detergents and disinfectants, and the need to keep cleanrooms clean, is a regulatory requirement. The main regulatory documents relating to the use of disinfectants in pharmaceutical manufacturing and healthcare are:

- FDA Code of Federal Regulations: 21 CFR 211.56b, 21 CFR 211.56c and CFR 211.113b
- FDA Aseptic Processing Guide, revised 2004
- USP (General Chapter <1072> Disinfectants and Antiseptics)
- Annex 1 to the EC Guide to Good Manufacturing Practice

Although there are some differences between the regulations, a number of similar areas are covered. The regulations require:

- The need to have written procedures (CFR/EU GMP)
- Responsibilities for cleaning should be assigned (CFR)
- Staff must be trained in cleaning techniques and have a training record (CFR/EU GMP)
- Details of cleaning frequencies, methods, equipment and materials must be recorded in written procedures (CFR)
- The cleaning of equipment and materials must take place at regular intervals (CFR)
- In designing a disinfectant protocol for the sanitisation of floors, walls and surfaces, a pharmaceutical organisation will normally select two or three disinfectants for the same application. This is a requirement of regulatory bodies and the strongest pressure for it has come from Europe with the EU GMP Guide stating that “where disinfectants are used, more than one type should be employed” (Annex 1, paragraph 3714)
- Disinfectants should be rotated (EU GMP/FDA warning letters)
- Inspection of equipment for cleanliness before use should be part of routine operations (CFR)
- A cleaning log should be kept. The purpose is to keep a record of the areas cleaned, agents used and the identity of the operator (CFR)
- The microflora from environmental monitoring regimes should be examined for resistant strains (EU GMP)
- The monitoring for microbial contamination in disinfectant and detergent solutions should be periodically undertaken (EU GMP)
- The storing of disinfectant and detergent solutions should be for defined [and short] periods (EU GMP)
- Disinfectants and detergents used in Grade A and B cleanrooms should be sterile before use (EU GMP)
- Room use should be recorded after each operation (CFR/EU GMP)
- Disinfectants should be ‘qualified’ (validated) (CFR)

- There should be a technical agreement with the company who supplies the disinfectant. Ideally the disinfectants purchased should be lot-tracked (EU GMP)

20.4 Selection of detergents and disinfectants

When selecting both detergents and disinfectants there are scientific, regulatory and safety considerations which must be taken account of.

20.4.1 Criteria for the selection of detergents

There are two key considerations when selecting detergents. The first refers to the chemical composition of the detergent and the second to the compatibility of the detergent with the disinfectant. In considering the chemical nature of the detergent, it is typical that detergents are neutral and non-ionic solutions. Furthermore, it is preferred that the detergents used are non-foaming (non-ionic surfactants are low-foaming). For certain applications, such as in aseptic filling areas, the detergent should be sterile or subject to a sterilisation step through a terminal filter[7].

In terms of compatibility, it is important that any detergent used should be compatible with the disinfectants used, as some detergents with residues can neutralise the active ingredient in certain disinfectants thereby reducing the microcidal properties of the disinfectant.

20.4.2 Types of detergents

There are various types of detergents, each with differing modes of cleansing action. In addition to cleansing action, some detergents have additional antimicrobial properties due to their ionic nature (anionic (negatively charged) or cationic (positively charged). Some examples of commonly used detergents are:

a) *Soaps*

   Soaps are soluble or insoluble products of natural fats to which an alkali is added, such as, sodium or potassium.

b) *Anionic detergents*

   Anionic detergents are most commonly used in the industry. They are generally soluble, foam producing emulsifiers. An undue level of foaming can be a problem, and this tends to restrict the use of anionic detergents in cleanrooms.

c) *Cationic detergents*

   Cationic detergents are little used in the pharmaceutical industry due to incompatibility with the pH of commonly used disinfectants.

d) *Non-ionic detergents*

   Non-ionic detergents are used when pH is an important factor for sensitive surfaces. They are generally the most commonly used detergents in cleanrooms.

e) *Amphoteric detergents*

   Amphoteric detergents have the properties of both anionics and cationics, and often have some bactericidal properties.

f) *Alkali detergents*

   Chemical detergents of an alkali nature, such as sodium hydroxide (caustic), have some bactericidal properties. They can readily dissolve organic matter like protein.

g) *Acidic detergents*

   Chemicals of an acidic nature often include oxidants and have some bactericidal properties. They can work against organic and inorganic matter.

Additional chemicals are often added to detergents to enhance their performance. Such additives include surfactants, which act to disperse fat and to produce uniform wetting, and sequestrents,

which act against hard water and scaling. An assessment of compatibility between disinfectants and detergents must consider these chemical additives in relation to disinfectant efficacy. For example, quaternary ammonium compounds are inactivated by anionic surfactants[8].

20.4.3 Criteria for the selection of disinfectants

The purpose of a disinfectant solution is: "a chemical or physical agent that destroys or removes vegetative forms of harmful micro-organisms when applied to a surface" (USP <1072>). Behind this requirement there are a range of different disinfectants available with different modes of action[9]. Deciding the types of disinfectants to be used in cleanrooms is an important decision for the pharmaceutical manufacturer.

There are various types of disinfectant with different spectrums of activity and modes of action[10]. Pharmaceutical manufacturers, as the regulatory review above has shown, are expected to use at least two disinfectants with different modes of activity in order to confirm with current Good Manufacturing Practices[11] Whilst the two disinfectants do not need to be sporicidal, the use of a sporicidal disinfectant is recommended for aseptic areas, at least on an occasional basis, even where such a disinfectant does not form part of the standard set. Therefore, a third disinfectant is sometimes included and deployed at a lower frequency to the two primary disinfectants.

When selecting disinfectants, there are a number of different criteria to consider. These criteria are examined below[12]:

i) *A disinfectant must have a wide spectrum of activity*

   The spectrum of activity refers to the properties of a disinfectant being effective against a wide range of vegetative micro-organisms including Gram-negative and Gram-positive bacteria. In pharmaceutical grade cleanrooms, disinfectants should be bactericidal (that is rather than simply inhibiting microbial growth, they should be capable of killing bacteria). A separate decision to be made is whether the disinfectant is required to be sporicidal (these is discussed in relation to disinfectant rotation below). Furthermore, in some facilities the disinfectants should also be virucidal.

ii) *A disinfectant should have a fairly rapid action*

   The speed of action depends upon the contact time required for the disinfectant to destroy a microbial population. Thus the 'minimum' contact time is the time required, for the disinfectant to be effective after its application. For the time period of contact, the surface to which the disinfectant is applied must remain wet. This means that if the surface dries before the contact time has been achieved a further application of disinfectant is required in order to keep the surface 'wet'. The contact time is sometimes referred to as the 'action time'. Given the requirements of most pharmaceutical manufacturers and healthcare facilities a disinfectant should ideally have a contact time of 10 minutes or less, although certain sporicidal disinfectants may require longer contact times.

iii) *To be effective, a disinfectant must not be readily neutralised by organic matter, plastic materials or detergent residues.*

   Although detergents and effective cleaning practices can remove the majority of soil, including organic matter, some traces will remain. It is important these organic residues do not interfere with the active ingredient of the disinfectant and reduce its efficacy.

iv) *Environmental conditions*

   Some disinfectants require certain temperature and pH ranges in order to function properly. One type of disinfectant, for example, may not be effective in a cold room due to the lower temperature. The reason for this is the validation standards for disinfectants measure the bactericidal activity at 20°C, therefore the disinfectant may not be as effective at higher or lower temperatures.

v) *The disinfectant should not unduly damage the material to which it is applied*
If the disinfectant causes extensive abrasion of a surface, it will either degrade the material or cause cracks and recesses which can harbour micro-organisms. It is recognised, however, that the most efficacious disinfectants, especially those which are sporicidal, although repeated applications over time will cause some corrosion. A post-disinfection step to remove disinfectant residues, such as a Water for Injections rinse or wiping with a milder disinfectant such as 70% IPA, can minimise material surface damage.

The following are some additional factors that should be considered when choosing a chemical agent:

i) *Operator safety*
Many disinfectants are toxic or irritant and unpleasant for staff to use. Consideration must be given to national safety requirements, material safety data sheets, label information, the toxicity upon human health and to the protective measures required for staff to use them (such as avoiding contact with exposed skin or the need to use a disinfectant in a well ventilated area).

ii) *Compatibility with the surface to be disinfected*
Certain disinfectants may be less effective with certain materials or may cause excessive damage to certain materials, such as the reaction of chlorine dioxide against stainless steel.

iii) *Compatibility with detergents used*
As discussed earlier, it is important that the disinfectant and detergent are compatible and that detergent residues do not inactivate the active ingredients in the disinfectant solution.

iv) *Types of in-house micro-organisms in the aseptic facility*
This relates to the spectrum of activity, with the additional step of not only accounting for a range of micro-organisms but also taking into consideration, the types of micro-organisms found within the facility cleanrooms (the environmental isolates). If the facility recovers unusual types of micro-organisms outside the range used by the manufacturer of the disinfectant to validate the disinfectant[13],, either a different type of disinfectant may need to be sourced or a validation study should be performed in order show that the disinfectant is effective against the cleanroom microflora.

v) *Residual activity of the disinfectant*
Residual activity of the disinfectant may lead to resistant strains or cause problems when an alternative disinfectant is applied. It is good practice to remove disinfectant residues with a water rinse (as discussed in relation to cleaning practices below).

vi) *Whether a disinfectant is required to be sporicidal*
If isolates from the environmental monitoring programme include the recovery of endospore forming bacteria on a frequent basis or in high numbers, then the use of a sporicidal disinfectant is essential, with the frequency determined by a review of the environmental monitoring programme.

vii) *Range of formats available*
The cleanroom facility will require a disinfectant to be available in several formats. For example a type and formulation of disinfectant may be required in a ready-to-use format, as a concentrate, or an impregnated wipe, etc. so that the most convenient and effective method of cleaning can be used. The pharmaceutical manufacturer, when selecting cleaning agents, should aim for products produced in different presentations. For smaller spillages and the cleaning of process area surfaces and laboratory benches, sterile disinfectants in trigger sprays are the most effective design. With slightly larger areas, ready-to-use disinfectant solutions in bigger volumes are desirable. For cleaning larger areas, such as process area floors, it is more practical and cost effective for disinfectants to be supplied in the concentrate form (for this the disinfectant is prepared by cleanroom operators by dilution).

viii) Price

The calculation of cost needs to include not just the price of the disinfectant, but also other cost factors such as the time taken to prepare or apply the disinfectant, protective clothing requirements, wastage and the steps needed for the removal of residues.

ix) Health and safety

The safety aspects of a disinfectant are an important consideration and standard operating procedures should contain appropriate health and safety requirements for using detergents and disinfectants. These should include reference to appropriate PPE. In particular, contact to eyes, skin and mouth is to be avoided. Safety data sheets must be examined for all disinfectants and detergents and appropriate measures taken to ensure that they are applied properly, in well-ventilated areas.

A further safety consideration is that one disinfectant must never be mixed with another as this can create a chemical reaction potentially creating noxious gases that can harm the operator.

x) User's acceptance

It is important to know the manufacturer of the disinfectant and to be assured that the cleaning agents purchased are of an appropriate quality. High quality disinfectants and detergents should be manufactured under clean conditions and held in suitable packaging. It may be necessary to audit the disinfectant manufacturer and the manufacturing process. In evaluating the quality of the supplier, analysis of multiple lots of the disinfectant and an audit of the production capabilities should be considered. The ability of the supplier to provide stable products in a timely manner should also be evaluated[14].

20.4.4 Types of disinfectants

There are a number of disinfectants with *different* modes of activity and of varying effectiveness against micro-organisms. These disinfectants also have varying modes of action against microbial cells due to their chemical diversity. Disinfectant action against the microbial cell includes: acting on the cell wall, the cytoplasmic membrane (where the matrix of phospholipids and enzymes provide various targets) and the cytoplasm. Some disinfectants, on entering the cell either by disruption of the membrane or through diffusion, then proceed to act on intracellular components. There are *various* approaches to the categorisation and sub-division of disinfectants including grouping by chemical nature, mode of activity or by microstatic and microcidal effects on micro-organisms. This chapter discusses some of the more commonly used disinfectants employed in the pharmaceutical environment by categorising them according to their chemical properties. The two principal categories used are the division into oxidising and non-oxidising chemicals. The list below is not exhaustive.

A. Non-oxidising disinfectants

The majority of this group of disinfectants have specific modes of action against micro-organisms but generally have a lower spectrum of activity compared to oxidising disinfectants[14]. The most common types of non-oxidising disinfectants are alcohols, quaternary ammonium compounds and phenolics.

i) Alcohols

Alcohols have an antibacterial action against vegetative cells. The effectiveness of alcohols against vegetative bacteria and fungi increases with their molecular weight (therefore ethanol is more effective than methanol and, in turn, isopropyl alcohol is more effective than ethanol). Alcohols, where efficacy is increased with the presence of water, act on the bacterial cell wall by making it permeable. This can result in cytoplasm leakage, denaturation of protein and eventual cell lysis (alcohols are one of the so-called 'membrane disrupters'). The advantages of employing alcohols include a relatively low cost, little odour and a quick evaporation. Furthermore, alcohols have a

cleansing action. However alcohols are relatively ineffective against spore formers and can only inhibit spore germination at best[15].

ii) Aldehydes

Aldehydes include long-chain chemical compounds, such as, formaldehyde and glutaraldehyde. Glutaraldehyde is a very effective disinfectant (and sterilant), acting on cell wall proteins. It has a wide spectrum of activity and is effective against bacterial and fungal spores. However, it is rarely used today due to health and safety concerns. Formaldehyde and *o*-phthalaldehyde are slightly less effective due to a slower rate of reaction but possess an equally wide spectrum of activity. Aldehydes have a non-specific effect in the denaturation of bacterial cell proteins and can cause coagulation of cellular protein[16].

iii) Amphoterics

Amphoterics are acidic, having a relative wide spectrum of activity, but are limited by their ability to damage endospores. Amphoterics are frequently used as surface disinfectants. An example is alkyl di(aminoethyl) glycine or derivatives.

iv) Acid anionics

Acid anionics are weak acids with a relatively limited spectrum of activity and are very pH dependent. An example of this group is carboxylic acid. They are not effective against fungi or spore-forming bacteria. Their bactericidal properties arise from their ability to cause bacterial cell disruption through proton motive force where the balance of hydrogen across the cell is disrupted which, in turn, affects cellular division by disruption of oxidative phosphorylation.

v) Biguanides

Biguanides are polymers supplied in salt form, such as chlorhexidine, alexidine or hydrochloride. Biguanides have a relatively wide spectrum of activity with the exception of killing endospores. They work best on surfaces which are at an alkaline or neutral pH. They are less effective under acidic conditions. Biguanides affect the bacterial cell membrane, enter the cell through diffusion, and cause cell disruption and cytoplasm leakage.

vi) Phenolics

Phenols are produced from the fractionation of tar and are among the oldest scientifically evaluated disinfectants, dating back to Robert Koch's evaluation of phenol's bactericidal effect against *Bacillus anthraci* and Lister's application of phenol in hospital surgery units. The commonly used phenolic is basic phenol (carbolic acid), although synthetic variants are being widely used. Phenol can be made more complex by the addition of halogens such as chlorine (the bis-phenols and halophenols), to the phenol molecule (carbolic acid), to make compounds like triclosan and chloroxylenol. Phenols are bactericidal, and antifungal, but are not effective against spores. Some phenols cause bacterial cell disruption through proton motive force, others attack the cell wall and cause leakage of cellular components and protein denaturation[17].

Anionic surfactants are added to many phenolic disinfectants to provide a degree of cleaning ability and tend to be more efficient as an acidic formulation[18]. Care must taken to ensure that residues of phenolics are removed if phenolics are used in rotation with quaternary ammonium chloride products, which are cationic in nature, as the two disinfectants are chemically incompatible.

vii) Quaternary ammonium compounds (QACs)

QACs are cationic salts of organically substituted ammonium compounds and have a fairly broad range of activity against micro-organisms, albeit more effective against Gram-positive bacterium at lower concentrations than Gram-negative bacteria. They are considerably less effective against spore formers.

QACs are sometimes classified as surfactants and are available in both alkali and acidic formulations. An example is benzalkonium chloride. QACs are the most widely used of the non-oxidising disinfectants within the pharmaceutical industry. Their mode of action is on the cell

membrane leading to cytoplasm leakage and cytoplasm coagulation through interaction with phospholipids. As the disinfectant carries a positive charge it is attracted to the negatively charged bacterial cell wall, thereby leading to the absorption of the chemical.

B. Oxidising disinfectants

This group of disinfectants generally have non-specific modes of action against micro-organisms. They have a wider spectrum of activity than non-oxidising disinfectants, with most types able to damage endospores, but they pose greater risks to human health.

i) Halogens

Halogens are among the oldest identified disinfectants and include organic and inorganic varieties. They can be divided into chlorine releasing and iodophors. Both types have a broad spectrum of activity against a wide range of micro-organisms and are normally effective sporicides. Examples of chlorine releasing chemicals are sodium trichloroisocyanurate, sodium hypochlorite (bleach) and chlorinated trisodium phosphonate. Hypochlorites are one of the oldest commercial disinfectants. The mode of action of this group is not completely known. One concern with using such agents is the damage that they can cause to cleanroom surfaces through corrosion, especially at high concentrations (>1,000 parts per million). Such chemicals can also be respiratory irritants.

The most commonly used disinfectant in this group is chlorine dioxide. This disinfectant is normally produced as a two-component system (sodium chlorite and an organic acid like lactic acid). Chlorine dioxide is generated when the base (sodium chlorite) reacts with the activator (the organic acid). The shelf life of the solution is normally fairly limited.

Iodine is another disinfectant with a long history. Most commercial iodine-based disinfectants consist of iodine formulated with surfactants or polymers in an acidic solution. The effectiveness of iodine disinfectants is determined by the amount of free I_2. Iodine acts by iodinating tyrosine residues in cells. The disadvantages of iodine are its ability to stain and it gives an unpleasant odour.

ii) Oxidising agents

This set includes oxygen-releasing compounds such as peracetic acid and hydrogen peroxide. They are often used in the gaseous phase as surface sterilants for equipment. These peroxygens function by disrupting the cell wall causing cytoplasm leakage and denature bacterial cell enzymes through oxidation (from a powerful hydroxyl radical). These oxidising agents have advantages in that they are clear and colourless, thereby avoiding staining, but they do present some health and safety concerns particularly in terms of causing respiratory difficulties to unprotected users.

Hydrogen peroxide is a commonly used disinfectant, partly because of its having a low level of corrosiveness to surface metals and because it degrades to water and oxygen (and therefore leaves minimum residue). Hydrogen peroxide is used at a concentration range of 3%-10%. Peracetic acid is formed from the combination of hydrogen peroxide and acetic acid, where the reaction creates the oxidising agent. However, it will sometimes leave a residue on surfaces and has a pungent odour, and its use is more confined to gassing or fogging systems.

20.5 Disinfectant efficacy

There are a number of important criteria, connected to both the factors for selecting disinfectants (discussed above) and to the validation of disinfectants (to be discussed below), which affect the performance and efficacy of disinfectants. These factors are:

i) Concentration

Disinfectants are manufactured or validated to be most efficacious at a set concentration

range. The setting of this concentration range involves ascertaining the minimum inhibitory concentration (MIC). The MIC is the lowest concentration of the disinfectant that is shown to be bacteriostatic or bacteriocidal under experimental conditions. Experimental conditions are normally based on the examination of a disinfectant solution in suspension in the absence of soil. The MIC is measured through kinetic studies of the dilution coefficient. Kinetic studies demonstrate the effect of a change in concentration against cell death rate over time. The higher a disinfectant's concentration exponent, the longer it takes to kill cells. For example, if a disinfectant with a set concentration exponent was diluted by a factor of 2, the time taken for it to kill cells comparatively would double[19].

ii) Time

Time is an important factor in the application of disinfectants for two reasons: in relation to the contact time of the disinfectant and the expiry time of the disinfectant solution. Contact time is the time taken for the disinfectant to bind to the microorganism, traverse the cell wall and to reach the specific target site for the disinfectant's particular mode of action. Contact time is expressed generally for each disinfectant type at its optimal concentration range. The killing effect, for a constant concentration of a disinfectant, increases over time until the optimal contact time is established. In practical situations however many variables enter the equation which can alter the contact time. These include the type, concentration and volume of the disinfectant; the nature of the micro-organisms; the amount and type of material present that is likely to interfere with the active ingredient; the temperature of the disinfectant; and the surface that the disinfectant is applied to.

Another aspect relating to time is the deterioration of a disinfectant solution over time. This is more important where a solution of disinfectant is prepared 'in-house' from a concentrate than to ready-prepared solutions, which have been validated by the manufacturer and will come with an assigned expiration time. For ready-prepared solutions, an expiry time limit for the disinfectant solution should be established through chemical testing. As a rule, fresh solutions of a disinfectant should be used for each application.

iii) Number, type and location of micro-organisms

Different species of micro-organisms vary in their resistance to different disinfectants. These can be affected by: the numbers of micro-organisms present, their species and the community with which they are bound to.

a) <u>Number</u>

An antimicrobial agent, like a disinfectant, is considerably more effective against a low number of micro-organisms than a higher number or a population with a greater cell density. Similarly a disinfectant is more effective against a pure population than mixed grouping of micro-organisms. A routine disinfectant procedure will be unlikely to kill all micro-organisms present and a number will remain viable. Whether the surviving micro-organisms multiply in sufficient number is dependent upon the condition in which the surviving population remains, the available nutrients and the time between repeat applications of the disinfectant.

b) <u>Types of micro-organism and resistance</u>

Different types of micro-organism have varying levels of resistance to broad spectrum disinfectants as **Figure 1** shows. The increased resistance shown is primarily due to the cell membrane composition or type of protein coat.

The hierarchy of micro-organisms in **Figure 1** are placed in order of resistance. Resistance is either due to the natural genetic properties of the micro-organisms (intrinsic) as shown, or it is acquired through phenotypic or genotypic variations (similar to antibiotic resistance through the over-use of one type of disinfectant). Generally, innate sensitivity results in Gram-negative bacteria being more resistant to disinfectant

applications than Gram-positive bacteria. The reason for the greater resistance amongst the Gram-negative bacteria is due to the greater abundance of lipopolysaccharide (LPS), which is of a hydrophobic nature whereas the Gram-positive membrane is primarily made up of inelastic murein sacculi. In turn, for bacteria, endospores are the most resistant because of the relative impermeability to hydrophilic agents of the polypeptides that make up the spore coat.

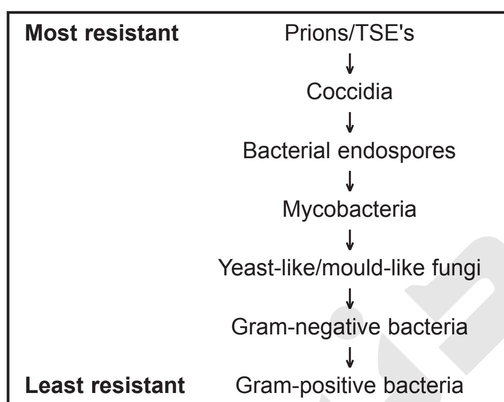


Figure 1: *Sensitivity of different micro-organisms to broad spectrum disinfectants.*

c) <u>Location of micro-organisms</u>

The location of micro-organisms influences the effectiveness of disinfectant treatment. Micro-organisms in suspension are easier to kill than those affixed to surfaces. This is due to the mechanisms of micro-organism attachment, such as bacteria fixing themselves using fimbriae or when a biofilm community develops. Such positioning impact upon the contact time required for the disinfectant to bind to the micro-organism, cross the cell wall and act at the required site. **Figure 2** illustrates the greater log kill achieved over time for bacteria in suspension compared to bacteria fixed to a surface.

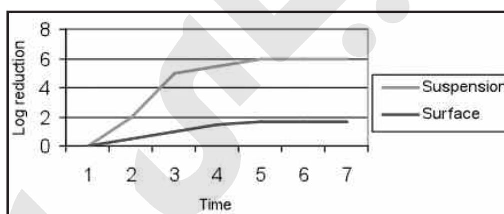


Figure 2: *Affect of a disinfectant against a bacterial species in suspension, or attached to a surface.*

iv) Temperature and pH

Each disinfectant has an optimal pH and temperature at which it is most effective. If the temperature or pH is outside this optimal range, the rate of reaction (log kill over time) is affected.

Generally, temperature influences the rate of reaction. Most disinfectants are more effective and kill a population faster at higher temperatures although many disinfectants, due to practical considerations, are manufactured for use at ambient conditions. Some disinfectants, particularly oxidising agents such as peracetic acid which has an optimal temperature of 40 - 50ºC, and sporicidal agents such as ortho-phthalaldehyde are more effective at temperatures higher than *ambient*. Disinfectants sensitive to temperatures other than *ambient* are normally assessed through the use of a temperature coefficient, or Q_{10} (which relates the increase in activity to a 10ºC rise in temperature)[20].

The effect of pH is important because it influences the ionic binding of a disinfectant to a bacterial cell wall thereby ensuring disinfectant molecules are bound to a high number of micro-organisms. Many disinfectants are more stable at a set pH range, for example, acid-based disinfectants can become less potent in alkaline conditions whereas a glutaraldehyde is more potent at a basic pH. The use of a disinfectant outside of its desired pH range results in reduced efficacy.

v) Interfering substances

The presence of different substances on the surface or in the equipment requiring disinfection can affect the efficacy of the disinfectant in various ways ranging from

increasing the contact time to complete inactivation. In order for a disinfectant to be effective, it must come into contact with the microbial cell and be absorbed into it. If substances, such as oil, dirt, paper or grease, act as a spatial barrier between the microbial cell and the disinfectant the efficacy of the disinfectant will be adversely affected. The presence of such substances (soil) halts disinfectant efficacy by either reacting with the disinfectant or creating a barrier. This effect is increased if the surface itself has defects and crevices which limit disinfectant penetration[21].

vi) Rotation of disinfectants

The earlier discussion, in relation to regulatory requirements, indicated that the rotation between two disinfectants of differing modes of activity was a regulatory requirement. The argument for rotating two disinfectants is to reduce the possibility of resistant strains of micro-organisms developing. Whilst the phenomenon of microbial resistance is an issue of major concern for antibiotics there are few studies that support development of resistance to disinfectants[22]. (For example, it is unlikely that resistance would develop when disinfectants are applied to dry environments, such as cleanrooms, for microbial replication, as the primary process for gaining resistance, is minimal[23]. The USP general information chapter <1072> states:

"The development of microbial resistance to antibiotics is a well-described phenomenon. The development of microbial resistance to disinfectants is less likely, as they are more powerful biocidal agents than antibiotics and are applied in high concentrations against low populations of micro-organisms, so the selective pressure for the development of resistance is less profound."

Building immunity to a disinfectant has never been documented in the cleanroom. Most reports of developed resistance are due to inadequate cleaning, improper use of the disinfectant, or poor application of the disinfectant to the surface for a specified contact time. Furthermore, antibiotics and disinfectants have different modes of action (with the former targeting specific sites and the latter typically having multiple modes of action). In short, there is no scientific evidence to support disinfectant resistance[24].

Nonetheless, rotation is a requirement needed to satisfy regulators and it is logical that the disinfectants used for rotation will have differing modes of activity. Policies for determining the frequency of rotation vary widely. Some manufacturers have adopted an even rotation while others rotate at different frequencies, sometimes to an extreme of three months for one disinfectant against one week for the alternate disinfectant. Other companies build up a case for only using one disinfectant on a day-to-day basis with a second used very infrequently. These manufacturers argue that two disinfectants only need to be employed if the environmental monitoring data indicate excursions from set limits and therefore the one disinfectant is not controlling surfaces.

The frequency of rotation needs to be defined by the user and supporting data can be supplied through field trials. When established, it is necessary to continue the detergent application between the changes of disinfectant types in order to remove residues. Once set, there may be a requirement to vary the frequencies of use, such as in response to an increase of microbial counts and as part of a formal investigation into a microbiological data deviation.

In addition, by rotating between two disinfectants with differing modes of activity, there are occasions when a third 'reserve' disinfectant should be considered. A third disinfectant might be applied in circumstances where there is a major contamination event, or the suspected development of a resistant strain, and under circumstances where the two primary disinfectants and any improvement to cleaning techniques or increases to cleaning frequencies do not appear to be effective.

20.6 Cleaning and disinfection techniques

The techniques of cleaning and disinfection are important. It does not matter how effective the purchased detergents and disinfectants are unless they are used in the correct way. Otherwise areas will not be cleaned effectively and unduly high levels of microbial contamination will remain.

20.6.1 Order of cleaning and disinfection

As indicated earlier, the phrase “cleaning and disinfection” not only distinguishes between cleaning agents and disinfectant, it also describes the order in which surfaces are treated. In order for a disinfectant to work effectively, ‘soil’ (such as grease and dust particles) must be removed first using a suitable cleanroom grade detergent.

20.6.2 Treatment of detergents and disinfectants

An important consideration for application is the need for sterile chemicals. Normally, agents for use in GMP Grade A and B/ISO class 5 and 7 (dynamic) environments must be sterile, whereas agents for use in GMP Grade C and D/ISO class 8 and 9 (dynamic) environments are not normally required to be sterile. However, with such lower grade areas it is recommended that the solutions should be qualified or periodically monitored for microbiological contamination and a time limit imposed on how long in-use dilutions be used for and controls in place so that In-use dilutions are discarded after use.

20.6.3 Preparation of detergents and disinfectants

When cleaning and disinfecting large areas, such as cleanrooms, ready-to-use solutions of cleaning agents are not often available and instead concentrates of cleaning reagents need to be prepared.

Detergents

The application of a detergent solution, made in hot purified water (>25°C), is the primary method of cleaning and removing particulate matter from floors and surfaces in clean rooms. In some areas, such as an aseptic filling area, hot water is unlikely to be available due to the restriction of water in such areas in order to maintain contamination control. Instead it is more common for detergents to be prepared using Water for Injections and filtered through a 0.22μm filter into the filling area.

It is important that the correct amount of detergent, as defined by the manufacturer’s recommendation, must be added to the appropriate quantity of hot purified water. Once prepared, detergents should have defined times from preparation to last usage.

Disinfectants

When preparing disinfectant concentrates as ready-to-use solutions, the correct amount of disinfectant, as defined by the manufacturer’s recommendation, must be added to the appropriate quantity of purified water or Water for Injections. For most disinfectants, hard water is not suitable for use as a diluent. This is because the ions in the water which cause hardness, such as magnesium and calcium, will cause interference with certain active ingredients in the disinfectant formulation. The water added should be of the correct temperature. If the water is too hot, it will cause certain disinfectants to be unstable (such as hydrogen peroxide, which becomes unstable if the water added is at a temperature of 60ºC or greater). A temperature range of 20-25ºC is suitable for most types of disinfectants,

Like detergent solutions, disinfectants should have defined times between preparation and usage. This is either a time applied to disinfectants prepared ‘In-house’, or the addition of an expiry time to pre-prepared disinfectants. For pre-prepared disinfectants (wipes, trigger sprays and hand sanitisers) the expiry time is one month after opening unless supporting data from the manufacturer is available.

20.6.4 Cleaning materials

For disinfectants and detergents used for floors, surfaces and walls, in the Aseptic Filling Area, these agents should be applied using non-particle shedding, cleanroom certified mop heads, cloths and wipes. These should be of a non-woven, non-particle shedding and lint-free variety (typically a hydro entangled polyester/cellulose blend), ideally with a large liquid holding capacity. For surfaces in Grade A/ISO class 5 and Grade B/ISO class 7 cleanrooms the solutions should be applied using certified cleanroom mop heads, cloths and wipes.

The wipes used should be effective at removing non-viable particulates, capable of applying a disinfectant, and capable of the subsequent removal of viable contamination. It is also important the wipe does not contribute to the bioburden or generate additional particulates. Transfer of such materials into an aseptic area should include an adequate decontamination step (for example, irradiated mop heads which have been multiple-wrapped). Similarly, buckets used for the application of detergents or disinfectants, in Grade B/ISO class 7 (dynamic) rooms should be autoclaved before use. For Grade C/ISO class 8 and Grade D/ISO class 9 (dynamic) rooms, buckets must be cleaned and dried before use. The mop heads, cloths and wipes should all be for single applications and should be discarded after use.

20.6.5 Cleaning steps

The cleaning and disinfection process consists of:

- Sweeping away dust and debris (if applicable)
- Application of a detergent solution through wiping or mopping
- Application of a disinfectant solution through wiping or mopping
- Removal of disinfectant residue through wiping or mopping with Water for Injections or 70% IPA

Areas to which a detergent is applied must be allowed to dry before the application of the disinfectant step.

During the cleaning process, care should be taken not to damage any HEPA filters, especially by wetting them with cleaning agents. Cleaning should commence with the ceiling followed by the walls and then floors. Benches, hatches, doors, cabinets and other clean room equipment should be cleaned by hand using low-linting wipes. The detergent clean should be repeated until the bucket at the end of the process is free from solid particles and the mop head does not look dirty[25].

Disinfectants are only to be applied to areas that are clean and have had an appropriate detergent wash as applicable. This is because poorly cleaned areas or areas where there is a detergent residue could give some protection to microbial populations and impair disinfectant efficacy. Furthermore, disinfectants must not be used for cleaning purposes. Disinfectants for surfaces are only to be applied to clean areas, such as after the application of a detergent. This is undertaken through mopping and wiping, which allows the target location to be treated and the amount of disinfectant solution added to the surface to be controlled.

When a disinfectant has been applied, the area must not be used until the contact time has elapsed. Different disinfectants have different contact times, based on manufacturer's recommendations, and these must be stated in local procedures. For the duration of the contact time, the disinfectant is to remain in contact with the surface. For floors, walls and ceilings this is 'visibly wet'. If drying out is noticed, additional applications must be performed.

After the application of a disinfectant it is normal practice to remove the residue. This is because residues, in theory, can lead to resistant microbial strains and often lead to discolouration or corrosion. Residues are removed using either WFI or IPA.

20.6.6 Cleaning techniques

The cleaning technique used for cleanrooms should be defined and standardised. It does not matter how effective the cleaning agents selected are if the cleaning technique practiced by cleanroom operators is poor. This partly relates to the techniques used and partly to the quality of the cleaning materials, as improperly cleaned areas where organic matter remains or areas which have a detergent residue, could potentially confer some protection to microbial populations and impair the efficacy of the subsequently applied disinfectant. In many ways, the physical treatment of a surface or item of equipment is as important as the chemical.

Figure 3: *Floor cleaning using the triple bucket method (image courtesy of Tim Sandle).*

Prior to the application of detergents, specialised equipment such as dry vacuum cleaners (with HEPA filters), steam cleaners and floor scrubbing machines (where a volume of detergent is applied to the scrubbing pad) can be used for collection or removal of visible items of waste from floors. Wet vacuum machines can be used to remove water, spillages or even excess detergent residues. However, such devices can damage floors and act to disperse contamination across an area rather than remove it. It is important for such machines to be carefully cleaned and stored after use, and never to be left damp in order to avoid building up biocontamination.

Detergents and disinfectants for use on surfaces (walls, floors) must be applied using the double or triple-bucket system to avoid cross contamination. The triple-bucket system uses two buckets of disinfectant or detergent and one empty bucket (where the technique should be outlined in local procedures). Furthermore, appropriate records should be kept of cleaning activities in process areas.

Both these techniques involve using a bucket of disinfectant and a bucket of water. In the "two-bucket" technique there is a "wringer" (for the mop) over the bucket of water. In the "three-bucket" technique there is a third bucket, empty except for having a wringer mounted over it. The two techniques involve preparing the detergent or disinfectant in the front bucket according to instructions.

Two-bucket technique

1. Dip the mop in the disinfectant
2. Mop the floor (preferably a specified number of square metres per "dipping")
3. Dip the mop in the bucket of water
4. Rinse the excess water off the mop head back into the bucket of water (this is to prevent carrying too much water back into the disinfectant and diluting it)
5. Dip the mop in disinfectant
6. Repeat

Three-bucket technique

1. Dip the mop in disinfectant
2. Mop the floor

3. Dip the mop in the bucket of water
4. Rinse the excess water off the mop head into the third (empty) bucket
5. Dip the mop in disinfectant
6. Repeat

To aid such techniques, commercial bucket and trolley systems are available. For aseptic filling areas it is important that these are manufactured from a material such as stainless steel, which can be autoclaved. In terms of the materials used for cleaning, appropriate mops and wipes should be used. Wipes should be sterile and also low particle-shedding. When using wipes for surfaces, the detergent or disinfectant should ideally be sprayed onto the surface rather than the wipe and left for the required contact time before wiping is undertaken[25]. With mops, the mop head should be cleanroom compatible (such as a microfibre-based yarn) and be single use. For Grade A and B areas, the mop heads should be sterile. Also of importance to such areas are the mops, which should be autoclavable. The design of the mop is a further consideration and must be easy for the cleanroom operator to use and allow for a uniform application of the disinfectant solution.

It is important that disinfectant residues are removed to avoid the potential for microbial resistance. The final stage in floor cleaning and disinfection is typically a rinse with sterile Water for Injections. For Grade A/ISO class 5 and Grade B/ISO class 7 areas, careful consideration is required as any water used needs to be sterile.

Cleaning and disinfection, using cloths and mop heads, is ideally performed by saturating the cleaning item and wiping the area using a series of parallel overlapping strokes (with an approximate one quarter overlap) and never in circular motions. The direction of the cleaning should be towards the operator (from top to bottom, from back to front). Only one application of the disinfectant or detergent should be applied to avoid over concentration. Cleaning and disinfection should begin with the visually 'cleanest' area first and towards the 'dirtiest' area last (typically the application of detergents or disinfectants involves starting from the far side of a room and towards the exit in a series of overlapping parallel strokes).

Cleaning Method Floor

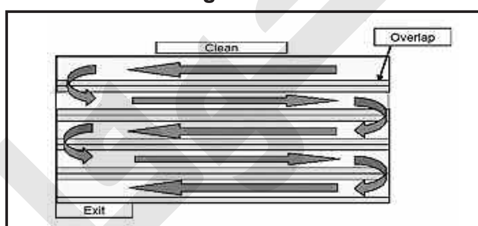


Figure 4: *Schematic approach to floor cleaning using parallel overlapping strokes.*

Cleaning Method Walls

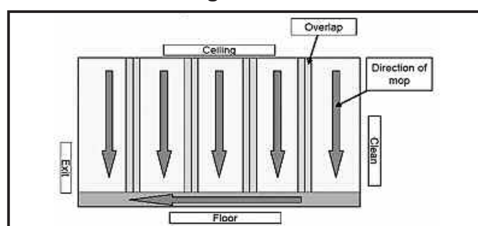


Figure 5: *Schematic approach to wall cleaning using parallel overlapping strokes.*

Application of detergent and disinfectants to surfaces at working height is often undertaken using ready prepared solutions in trigger spray bottles, with the use of a mop or wipe, and undertaking a similar cleaning technique, using parallel overlapping strokes, as applied to walls and floors.

As discussed above, when the disinfectant solution is applied, one of the critical aspects is the contact time. This can be achieved more easily when the disinfectant is applied in overlapping strokes. It is also important that the disinfectant does not become contaminated during its usage, such as, from aerosols during preparation. The use of single use containers can avoid such contamination, although this is more costly.

In addition to routine cleaning and disinfection, a major decontamination exercise is sometimes required for a cleanroom (such as following a HEPA filter failure or following maintenance work). There are different techniques used to decontaminate cleanrooms. Traditionally this has involved either manual cleaning and disinfection by operators using mops and buckets or, in more recent years, decontamination with fumigation units and chemicals such as hydrogen peroxide or peracetic acid (noting appropriate health and safety regulations). For this method, gas generators can be placed into cleanrooms at known locations. This takes some time and effort in mapping the cleanroom (which additionally requires some form of validation using chemical and biological indicators). Some VHP technologies can be fitted directly into HVAC systems to allow the gassing of cleanrooms through air inlets. The advantage of this is that the gas vapour is evenly distributed into all areas of the cleanroom.

20.6.7 Cleaning and disinfection frequencies

Cleaning and disinfection sessions are performed at different frequencies for different areas. This is based on the criticality of the area and will vary between facilities. The frequency is ideally established through risk assessment or based on empirical data, such as through a field trial.

Cleaning and disinfection frequencies are often expanded through different parts of the cleanroom being cleaned or disinfected at different times ('levels of cleaning'). That is, floors and working surfaces will often be cleaned at a higher frequency than walls or ceilings. An example of different cleaning levels is shown in the table below:

Criticality	Reason	Frequency
Level 1	Critical surfaces (all surfaces at working height and all areas used for product preparation)	High
Level 2	Less critical surfaces, e.g. walls	Medium
Level 3	Non critical, e.g. ceilings	Low

20.6.8 Fogging

Some disinfectants, normally sporicidal in nature, can be used to disinfect cleanrooms through gassing or fogging (sometimes referred to as fumigation). This process is suitable for surfaces that are not easily accessible by manual cleaning.

At one time the chemicals commonly deployed for this process were formaldehyde or glutaraldehyde, where a gassing device would heat up the chemical and create a vapour distributed through the cleanroom that caused disinfection on contact. Due to health and safety concerns, relating to the high toxicity and carcinogenic risks, these chemicals are no longer used.

Alternative gassing chemicals are used (such as peracetic acid). Many cleanroom managers prefer to use manual cleaning methods as gassing is more difficult to validate compared with manual cleaning techniques. The gassing process involves the aerosolisation of a liquid disinfectant that is dispersed onto a surface. The liquid is delivered by a gassing machine. Such machines vary in their capacity, dispersion pattern, range, droplet size, and operation. The use of these devices requires considerable validation, particularly in determining if the required quantity of disinfectant reaches a surface for the required contact time.

20.6.9 Cleaning and disinfection protocols

Appropriate protocols should be designed for the cleaning and disinfection of cleanrooms, and SOPs prepared. The SOPs should cover the following aspects:

- The type of detergents and disinfectants to be used (which are compatible)

- The frequency of rotation of disinfectants
- A list of suitable cleaning materials
- Cleaning techniques
- Contact times
- Rinsing
- Frequency of cleaning and disinfection
- Procedure for the transfer of cleaning agents and disinfectants into and out of clean areas (including the procedure for sterilisation of disinfectants)
- Holding times for detergents and disinfectants (for how long, after preparation, can a detergent or disinfectant be used?)

When writing cleaning procedures it is good practice to involve the staff who will be undertaking the cleaning so that the procedures are clear and easy to follow. As part of an on-going review of protocols and procedures, attention should be paid to environmental monitoring and the examination of trends as a measure of the effectiveness of a disinfectant or the cleaning/disinfection procedure. If adverse trends are seen, adjustment can be made to the cleaning and disinfection agents or the methods used, and the regulatory expectation is that such decisions will be documented.

20.6.10 Other considerations

There are other considerations to be taken into account when using disinfectants. These include the storage conditions for the disinfectants and the expiry dates. With storage conditions, the chemical stability of different disinfectants will vary and the effects of different storage temperature may affect disinfectant efficacy. With expiry time, the chemical agents will break down after a defined time period so the manufacturer's expiry times must be adhered to. The expiry time for the in-use dilution of the disinfectant is normally shorter than the expiry time for the concentrate. This is particularly important when disinfectants are provided at in-use concentrations in trigger spray bottles and the manufacturer should have undertaken a study to indicate not only the chemical stability but also the risk of microbial ingress into the trigger spray.

20.7 Qualification of disinfectants

Disinfectants used in pharmaceutical and healthcare facilities must be validated. This includes *in vitro* tests: an assessment of the disinfectant in solution and when applied to surfaces. Of these, the surface test is arguably the most stringent for it most closely reflects practical conditions as it uses micro-organisms that have been dried to a surface rather than in suspension, which simulates the actual state of micro-organisms found within a cleanroom[26]. In addition, *in situ* tests (field trials) should be conducted within the facility to establish the appropriate frequency of cleaning and disinfection.

Validation is undertaken in part by the manufacturer of the disinfectant and in part by the user, and both parts are a regulatory expectation. The user requirement should be conducted through controlled experiments and undertaken through a disinfectant validation programme. This section of the chapter summarises the different standards that are currently available to guide a microbiology laboratory through the process of disinfectant validation.

Whilst several standards are available as a guide through the validation process, these standards have not been written specifically for the pharmaceutical industry (more typically they have evolved from the food, cosmetics, healthcare, or environmental control sectors). It may be necessary for the pharmaceutical microbiologist to adapt the standards to suit practical situations (backing this up with a well-thought out rationale). Another point to be considered before embarking on such validation is whether all the standards are to be replicated by the pharmaceutical company, or

whether experimental work from some of the earlier standards can be provided by the manufacturer of the disinfectants. Here there is debate between reducing costs but also considering what is needed in order to meet regulatory expectations.

Approaches to disinfectant testing have varied over the past 50 years. This chapter examines the method validation approach commonly used across the European Union. It should be noted that within North America, the approach is similar but with subtle methodological differences as defined by the US Environmental Protection Agency (EPA) and the Association of Official Analytical Chemists (AOAC) methods[27]. The emphasis in North America is strongly on surface testing (the Hard Surface Carrier Method). The carrier test differs slightly in methodology from the European Surface Test. With the AOAC method the carrier is submerged in the test disinfectant for the duration of the experiment. With the European test, a quantity of disinfectant solution is applied directly to the test surface[28].

The beginnings of a unified approach for Europe was realised by the issuing of a standard by the European Committee for Standardisation Technical Committee 216 (CEN TC 216) in 1991 which offered guidance on disinfectant selection (EN 7152 24). But it was not until 1997 that the next European Standard was issued: BS EN 1276 for the quantitative suspension test, and several other standards followed[29]. These new standards replaced former methods for disinfectant validation, such as the once dominant Kelsey-Sykes test[30].

The standard European approach for disinfectant validation is divided up into three phases:

- *Phase 1* Basic suspension tests
- *Phase 2* Part 1: Suspension and surface tests to simulate practical usage: Bactericidal and fungicidal (sporicidal and virucidal)
- Part 2: Surface test
- *Phase 3* Field trial
- A separate phase exists for the validation of hand sanitisers

The basic suspension test is a simple time-kill test to determine if the test disinfectant possesses any antimicrobial properties (as shown by a significant level of microbial reduction). The quantitative suspension and surface tests are tests to determine the most effective concentration and conditions for the disinfectant as a simulation of practical conditions. The field trials show the effectiveness of a chosen disinfectant *in-loco* conditions (the pharmaceutical cleanrooms).

It is important to note that, as with any laboratory test established to mimic in-use conditions, the disinfectant methods are subject to variability and have problems in terms of reproducibility and repeatability. This can manifest itself from initial preparation of stock cultures to variations of abrasion on surfaces. These considerations aside, the European tests do offer a means to standardise the approach to disinfectant efficacy testing and to evaluate cross-laboratory studies[31].

In examining each one of these standards in turn:

Phase 1 – Basic Suspension Test (Standards EN 1275 and EN 1040)

A suspension test is a test designed to measure the efficacy of a disinfectant against selected micro-organisms in the planktonic state after a predetermined contact time. Two standards are published within Europe in order to examine this: EN 1040 to measure bactericidal activity and 1275 to measure fungicidal activity. The basic suspension test is a simple, limited test of the product, in order to determine minimum standards, and in many ways only serves to confirm the manufacturer's data within the testing laboratory.

Before undertaking the test, the selection of a suitable sterile neutraliser is required. Selection

involves spiking neutralisers of different activity with a range of micro-organisms and measuring the recovery. The neutraliser with the optimal recovery should be selected. Some neutralisers have general properties, eg. lecithin. Other neutralisers are compatible with specific disinfectants, eg. polysorbate-80 for biguanides and sodium thiosulphate for hypochlorites.

The test evaluates the activity of a disinfectant against a range of micro-organisms under conditions that simulate use. After challenging a disinfectant solution with a microbial population, the mixture is plated out, after the required contact time, and the surviving micro-organisms enumerated. No organic material is introduced to this test, unlike the quantitative suspension test described below.

Phase 2, Step 1 – Bactericidal suspension test (Standard: EN 1276: 1997) and *Fungicidal suspension test* (Standard EN 1650: 1998)
The purpose of the quantitative suspension test is to evaluate the activity of a disinfectant against a range of micro-organisms under conditions that more closely simulate practical use. The practical conditions make the test more advanced than the basic suspension test. The test consists of adding a test suspension of bacteria or fungi to a prepared sample of the disinfectant under test in simulated 'clean' and 'dirty' conditions. After a specified contact time, an aliquot is taken and the bactericidal/fungicidal action is immediately neutralised by the addition of a proven neutraliser (as identified in the basic suspension test). Following this, the number of surviving micro-organisms in each sample is determined and the reduction in viable counts is calculated.

To achieve neutralisation, the Standard recommends dilution but if this is ineffective then membrane filtration may be used where the filter may trap micro-organisms but filtration through the disinfectant by the application of rinse solutions, can also be employed. Dilution, addition of a chemical neutraliser and membrane filtration are the three standard methods for inactivation of antimicrobials[32].

The suspension test permits challenges of different concentrations of the disinfectant against a range of set test micro-organisms. The concentrations need to be chosen to cover the manufacturer's recommendations for the active and non-active ranges. The set organisms are: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecium/ hirae,* for the bactericidal test, and *Aspergillus niger* and *Candida albicans* for the fungicidal test. The bactericidal standard also makes provision for additional micro-organisms to be used in specific industries. These are: *Salmonella typhimurium* (used for the food industry), *Lactobacillus brevis* (used for breweries) and *Enterobacter cloacae*. To achieve a 'pass', the concentration of disinfectant, at a temperature of 20°C and a contact time of 5 minutes, must produce a minimum five log reduction of the challenge bacteria and a minimum of a four log reduction for the challenge fungi.

In addition to the standard, it would seem that many regulatory inspectors would expect the inclusion of environmental isolates found from the manufacturing environment. The addition of spore bearing micro-organisms can also be introduced to challenge disinfectants with sporicidal properties. Research from Payne *et al*[33] indicates that of all the test micro-organisms it is *Pseudomonas aeruginosa* that is generally the most resistant.

In addition to testing the differing concentrations, the Standard also requires that the disinfectant is made up in the 'worst case' condition by using 'water of standard hardness' (which contains ions like magnesium and calcium, as well as other salts). A further condition is the simulation of 'soiling', by the addition of bovine serum albumin (at 0.03%, representing 'clean' conditions and at a higher soil load of 0.3%, representing 'dirty' conditions). The greater the soil load the more difficult it is for the disinfectant to become inactive or destroy the microbial population. Some manufacturers will

also introduce an additional organic load, which is representative of residues likely to be found within their cleanrooms, as well as other in-use temperatures and variations to contact times from one to sixty minutes.

Phase 2, Step 2 – Surface test (Standards prEN 13713: 1999 and prEN 13697: 1999)
The second part of phase 2 is the surface test. The European Standards that describe the test are prEN 13713, for the basic surface test, and prEN 13697, for a quantitative surface test, which includes the presence of interfering substances. The Standards have been in 'draft' form since 1999 and are largely similar to previous German DGHM methods. The surface test is based on the suspension test with the use of interfering substances, temperature and contact time. However, the required log reduction differs from the suspension test in that it is a 4 log decrease for bacteria and a 3 log decrease for fungi. The required test organisms are identical to the suspension test: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus hirae, Aspergillus niger* and *Candida albicans.* For this test, fungi are incorporated within the one standard. The microbiologist will also consider the inclusion of environmental isolates and spore bearing micro-organisms. It is noted that there is a debate as to when an environmental isolate becomes a 'laboratory culture'[34]. However, this debate will not be explored further in this chapter.

The quantitative test evaluates test suspensions of bacteria and fungi in a solution of interfering substances, designed to simulate clean and dirty conditions, which are inoculated onto a test surface and dried. The test aims to acquire quantitative information about the ability of a disinfectant to kill micro-organisms attached to hard surfaces.

The test works by examining preparations of micro-organisms dried onto surfaces. To such a dried suspension a prepared sample of the disinfectant is added. The surface is then transferred to a previously validated neutralisation medium and tests performed to measure the reduction in viable counts. The test involves drying 0.05 ml suspensions of the micro-organisms (with interfering substances such as bovine serum albumin) onto different surfaces. The micro-organisms should have a population range of 1.5 – 5.0 x 10^8 for bacteria and 1.5 – 5.0 x 10^7 for fungi and are equilibrated to 25°C before use. Once applied to the surface the drying of the micro-organisms maybe accelerated using an incubator operating at 36-38°C. Disinfectant solutions (where disinfectants are made with Water of Standard Hardness, which increases the difficulty of the disinfectant to inactivate or destroy the microbial population) are added to the surfaces. After the specified contact time (five minutes is the target) the surfaces are transferred to the validated neutralisation medium and then pour plates are prepared for incubation and counting. An alternative method is available using a soaked swab step[35].

The surfaces selected should mimic the range of surfaces found in the clean room environment, such as, stainless steel and polyvinyl chloride (PVC). The use of different surfaces is important because the rates of inactivation on micro-organisms on different surfaces can vary considerably. One study demonstrated that bactericidal activity reduced on PVC compared with stainless steel. This was a factor both of the material type and the surface conditions, such as, the number of pores or ridges. Surfaces of the material can also differ depending upon the degree of finishing with smoother surfaces, like stainless steel or Formica, giving greater repeatability and reproducibility.

A variation of the surface test involves the use of mechanical action. Mechanical action is more akin to practical conditions (such as the application of a cloth or a mop). However, most efficacious disinfectants do not require any mechanical action when the disinfectant and the surface come into contact. It is preferable to evaluate a disinfectant without mechanical action and this aspect can be examined during the Phase 3 field trials. Furthermore, mechanical action is a very variable procedure and is difficult to evaluate.

In many ways, the surface test is more relevant than the suspension test because it is truer to practical conditions and theoretically, micro-organisms attached to a surface will be more resistant than those in a suspension, therefore this presents the greatest challenge. It may arise that the disinfectant concentration shown to be optimal for the suspension test needs to be increased to meet the requirements of the surface test. The suspension test has further weaknesses that it enhances the potential for small dilution errors made in the preparation of disinfectant solutions in relation to the final pass or fail result. The suspension test has been shown to be difficult to reproduce both between and within laboratories and often lacks precision. The suspension test can also pose problems when disinfectants with a high viscosity are challenged due to their distribution in the test suspension[36].

The surface test, however, cannot demonstrate the effect of a range of environmental factors like temperature, pH, detergent residues, mechanical stress and attachment. For these reasons a disinfectant which appears effective for the surface test can show marked variability when applied to practical conditions. The reasons for this are due to problems in drying and differences between surfaces. In terms of drying microbial suspensions, there is a marked loss in the viability of a population when dried onto a surface and attempts to speed up the drying process do not significantly reduce the variability of the actual number of micro-organisms challenged. Surfaces introduce another variation because surfaces, even of the same grade of material, are not truly identical and there have been marked problems in achieving reproducibility and repeatability for the surface test between laboratories particular in estimating the concentration of disinfectant required to be effective[37]. Some of these limitations can be addressed through field trials.

Phase 3 – Field trials (Standard is yet to be produced)
Only disinfectants that have satisfactorily passed the previous phases should be studied for field trials. The purpose of field trials is to test a disinfectant's efficacy in true In-use conditions: the working environment. Most researchers consider that a field trial is the only accurate test of a disinfectant, given the problems with repeatability and reproducibility associated with laboratory based tests. It is also because the trials examine a selected concentration on surfaces and equipment after the cleaning step has been applied. Field trials have an advantage because they test the disinfectant against a wide range of surfaces and with all the different types of interfering substances that may be present, as well as different physico-chemical conditions, like temperature and pH. The variable application of cleaning and disinfectant agents by different operators can also be considered. However, field trials can be costly to pharmaceutical companies both in terms of the cost of the study and the time taken to complete it.

Although no common European Standard exists, the common approach for field trials involves applying the disinfectant, at the selected concentration, to a selection of surfaces and equipment within the manufacturing facility. To these controlled areas, an intensive level of viable microbiological environmental monitoring is undertaken. Field trials are a test for both attached and suspended micro-organisms, as well as allowing evaluation of a range of variables like cleaning technique; recovery of damaged cells; temperature; contact time; interfering substances and so on[38].

In conducting a trial the areas should be monitored prior to the introduction of the disinfectant and shown to be satisfactory against test criteria, (normally the action levels for viable micro-organisms on surfaces for a given clean room, such as, the recommended levels in the EU GMP Guide or the FDA aseptic processing guide). This would be performed by selecting a representative number of sites in a room and monitoring these sites using appropriate methods, such as swabs or contact (RODAC) plates, daily over a four week period. It would be prudent for the microbiologist to have evaluated any neutralisers in the culture media used for environmental monitoring against the disinfectants under test at this stage. Following the establishment of a baseline the new disinfectant would be applied to the test areas and the level of environmental monitoring sustained. The two

sets of results would then be examined for any significant difference with the criteria being that the results after the application of the new disinfectant are equivalent to before or, ideally, better (that is, less micro-organisms recovered).

20.8 Hand sanitisation

For aseptic operations, even where cleanroom clothing is worn correctly and staff behave in ways which adhere to strict cleanroom disciplines, contamination control is achieved through regular gloved hand sanitisation. Hands, whether gloved or ungloved, are one of the main ways of spreading infection or for transferring microbial contamination[39]. The use of hand disinfectants is part of the process of good contamination control for personnel working in hospital environments, or those involved in aseptic processing and within cleanrooms.

Personnel carry many types of micro-organisms on their hands and such micro-organisms can be readily transferred from person to person or from person to equipment or critical surfaces. Such micro-organisms are either present on the skin not multiplying (transient flora, which can include a range of environmental micro-organisms like *Staphylococcus* and *Pseudomonas*) or are multiplying micro-organisms released from the skin (residential flora including the genera of *Staphylococcus, Micrococcus* and *Propionibacterium*). Of the two groups, residential flora is the more difficult to remove. For critical operations, some protection is afforded by wearing gloves. However gloves are not suitable for all activities and gloves, if not regularly sanitised or if they are of an unsuitable design, will pick up and transfer contamination.

Therefore, the sanitisation of hands (either gloved or ungloved) is an important part of contamination control either in hospitals, to avoid staff-to-patient cross contamination or prior to undertaking clinical or surgical procedures; and for aseptic preparations like the dispensing of medicines. Moreover, not only is the use of a hand sanitiser needed prior to undertaking such applications, it is also important that the sanitizer is effective at eliminating a high population of bacteria. Studies have shown that if a low number of micro-organisms persist after the application of a sanitiser then the sub-population can develop which is resistant to future applications[40].

There are many commercially available hand sanitisers with the most commonly used types being alcohol-based liquids or gels. As with other types of disinfectants, hand sanitisers are effective against different micro-organisms depending upon their mode of activity. With the most common alcohol based hand sanitisers the mode of action leads to bacterial cell death through cytoplasm leakage, denaturation of protein and eventual cell lysis (alcohols are one of the so-called 'membrane disrupters'). The advantages of employing alcohols as hand sanitizers include a relatively low cost, little odour and a quick evaporation (limited residual activity results in shorter contact times). Furthermore alcohols have a proven cleansing action.

20.8.1 Selecting hand sanitisers

In selecting a hand sanitiser the pharmaceutical organisation or hospital will need to consider if the application is to be made to human skin or to gloved hands, or to both, and if it is required to be sporicidal. Hand sanitisers fall into two groups: alcohol-based, which is more common, and non-alcohol based. Such considerations impact both upon cost and the health and safety of the staff using the hand sanitiser since many commonly available alcohol based sanitisers can cause excessive drying of the skin; and some non-alcohol based sanitisers can be irritating to the skin. Alcohol hand sanitisers are designed to avoid irritation through possessing hypoallergenic properties (colour and fragrance free) and ingredients that afford skin protection and care through re-fatting agents.

Alcohols have a long history of use as disinfectants due to inherent antiseptic properties against bacteria and some viruses. To be effective some water is required to be mixed with alcohol to exert

effect against micro-organisms, with the most effective range falling between 60 and 95% (most commercial hand sanitizers are around 70%)[41]. The most commonly used alcohol based hand sanitisers are Isopropyl alcohol or some form of denatured ethanol (such as Industrial Methylated Spirits). The more common non-alcohol based sanitisers contain either chlorhexidine or hexachlorophene. Additives can also be included in hand sanitizers in order to increase the antimicrobial properties.

20.8.2 Hand sanitisation techniques

With hand sanitisation is important that the validated technique is followed in practice. For this staff must use a minimum quantity of disinfectant and rub hands in a way which ensures that all parts of the hand are covered[42] and that the sanitiser selected is of a good quality: easy to apply, of pleasant odour and capable of eliminating transient bacteria like common skin commensurables like *Staphylococcus* and *Micrococcus.* Therefore the hand sanitisation technique is of great importance.

Before entering a hospital ward or clean area hands should be washed using soap and water for around twenty seconds. Hand washing removes around 99% of transient micro-organisms (although it does not kill them)[43]. From then on, whether gloves are worn or not, regular hygienic hand disinfection should take place to eliminate any subsequent transient flora and to reduce the risk of the contamination arising from resident skin flora.

The technique of hand sanitisation is of great importance as the effectiveness not only lies with the alcohol but also relates to the ‘rub-in’ technique. For example:

- Dispense a small amount of hand gel onto the palm of one hand
- Put hands together and proceed to rub the hand gel into both hands. Pay particular attention to the following areas:
  - Fingernails
  - Back of hands
  - Wrists
  - Between webs of fingers
  - Thumb
- Allow hands to dry, this should take no more than 60 seconds

Regular applications of the hand sanitiser are required and also prior to carrying out critical activities. This is because alcohols are relatively volatile and do not provide a continual antimicrobial action. Although micro-organisms are removed from material like latex more readily than from skin, a regular frequency of hand sanitisation should still be applied to gloves.

There are very few safety concerns with hand sanitisers and the occupational exposure is relatively low, although this can build up in enclosed spaces. Care should be taken when using sanitisers near naked flames (which can occur where gas burners are used in laboratories).

20.8.3 Validating hand sanitisation

Within Europe there is a standard describing the approach for the validation of hand sanitisers based on two norms: EN1499 (hygienic hand wash)[44], and EN 1500 (hygienic hand disinfection)[45]. It is more typical for the EN 1500 standard to be followed. This is described as a quantitative carrier test. The standards involve reducing the counts of bacteria on artificially contaminated hands. Each requires 12–15 volunteer subjects per test and comparison with a reference procedure. For North America, the common standard is CFR 333.470 which has a log-reduction requirement for a series of specific pathogens after three successive hand washes with the sanitiser on test[46].

Many commercially available hand sanitisers are surprisingly difficult to test against the European standard in terms of effectively reducing microbial populations and several types have compared unfavourably to straightforward hand washing with simple soaps[47] or when tested against a reference alcohol (2-propanol 60%)[48] or against ethanol[49]. The test for hand sanitisers can be applied to skin and to gloved hands, but one problem with its application to gloved hands is that the gloves themselves may either carry a microbial load or be prone to leaks. Some materials such as latex can trap micro-organisms onto the surface and these factors can reduce the reliability of the test results[48].

The test for hand sanitisers can be applied to skin and to gloved hands. One problem with the application to gloved hands is that the gloves themselves may either carry a microbial load or be prone to leaks. Some material, such as latex, can trap micro-organisms onto the surface. These factors can reduce the reliability of the test results. The European test determines if a hand sanitiser can reduce the number of transient microflora under simulated practical conditions. The hand sanitiser under test is compared against a reference standard (60% propan-2-ol) using 15 test subjects. Only one micro-organism can be used for the study on human skin for health and safety considerations. This is *Escherichia coli* strain K12 (ATCC 10538) which is a non-pathogenic Class I micro-organism under Directive 90/679 EEC. Even when using this bacterium the hands of the subjects must be inspected and those with cuts or abrasions rejected. For the study on ungloved hands a surrogate for Gram-positive pathogens was included (which is recommended to make for a more rigorous study)[43]. For this a suitable micro-organism, which was representative of skin flora, was selected. This was *Staphylococci aureus.* In relating back to the different types of microflora, *E. coli* was representative of transient skin flora and *Staphylococci aureus* representative of resident skin flora.

To be considered effective the test hand sanitiser must produce a 3 log reduction of the test micro-organism. The act of agitation and rubbing the hand sanitiser into the skin or into the glove presents the greatest variable of the test. This is partly overcome by the large number of subjects but difficulties exist in comparing different laboratories[50]. *In theory* there can be a significant variability in the survival of microflora depending upon the frequency of application, the degree of hand rubbing and the quantity of sanitiser applied[51].

The act of agitation and rubbing the hand sanitiser into the skin or into the glove presents the greatest variable into the test. This is partly, but not completely, overcome by the large subject size. In practice there is a significant effect on the survival of microflora based on the frequency of application, the degree of hand rubbing and the quantity applied[52].

Monitoring disinfectant efficacy

Once validation studies have been completed and cleaning protocols developed, the key assessment of on-going disinfection efficacy is derived from environmental monitoring data. Whilst environmental monitoring is covered elsewhere in this book, it is nonetheless advisable to have carried out some evaluations of cleaning and disinfection.

This monitoring could be of clean rooms before, during and after the cleaning and disinfection stages (as part of the field trials discussed earlier). Furthermore, monitoring the microbial content of the bucket after cleaning and disinfection is useful in validating the cleaning process. In addition, as part of the routine monitoring programme, a regular check of the microflora recovered should be undertaken. Such an assessment of the types and frequencies of bacteria recovered can indicate if the cleaning techniques are being followed or if the disinfectants are suitable. The appearance of endospore forming bacteria, like Bacillus species, would indicate a problem and should trigger a review. This relates to the regulatory requirement, mentioned earlier, that the results of an environmental monitoring programme are examined at regular intervals for the

development of resistant strains. If a resistant microorganism is suspected, then this may necessitate a change of disinfectant type.

For such assessments, the microbiological culture media used for environmental monitoring should contain appropriate neutralising agents in order to eliminate any disinfectant residues and thus allow any recovered micro=organisms to grow. Although some general neutralisers are available for culture media (normally a combination of lecithin (0.07%), Tween (polysorbate) 80 (0.5%), histidine (0.05%) and thiosulphate (0.05%)), the selection of the appropriate neutraliser is of great importance and its effectiveness must be demonstrated through validation studies. All micro-organisms should be identified to at least genus level to allow comparison with future monitoring isolates[52].

20.9 Conclusion

This chapter has examined cleaning and disinfection in cleanrooms, as an essential part of the contamination control programme[53]. In doing so, the chapter has shown that the selection of detergents and disinfectants represents an important choice. The range of different chemicals available is diverse and the variables that can affect performance, from contact time to temperature of use, are considerable. Furthermore, the chapter has demonstrated the importance of cleaning techniques and the requirement for a validated and standardised approach.

20.10 References

1. Sutton SVW. Disinfectant rotation in a cleaning/disinfection program for cleanrooms and controlled environments. In: Manivannan G. ed. Disinfection and decontamination: principles, applications and related issues. Florida, USA: CRC Press; 2008, pp165-174.
2. Gorman S, Scott E. Chemical disinfectants, antiseptics and preservatives. In: Denyer SP, Hodges NA, Gorman SP (eds). Hugo and Russell's Pharmaceutical Microbiology, 7th Ed, Oxford, UK: Blackwell; 2004, pp285-305.
3. Bessems E. The effect of practical conditions on the efficacy of disinfectants. *International Biodeterioration and Biodegradation* 1998; **41**:177-183.
4. Marcovitch H (ed). Black's Medical Dictionary, 42nd Ed, London: A&C Black; 2010, p193.
5. O'Leary WM (ed). *Practical Handbook of Microbiology*, 1977, second edition, CRC Press, Florida, USA.
6. Taylor DJ, Green NPO, Stout GW (eds). Biological Science 2: Systems, Maintenance and Change, 3rd Edition, Cambridge: Cambridge University Press; 1997.
7. Denny VF and Marsik FJ. Current practices in the use of disinfectants within the pharmaceutical Industry. *PDA Journal of Science and Technology*, **51**:227-228.
8. Collier L, Balows A, Sussman M (eds). Topley and Wilson's Microbiology and Microbial Infections Volume 2: Systematic Bacteriology, 9th Edition. New York: Arnold;1998, pp149-170.
9. Block S. Disinfection, Sterilisation and Preservation, 3rd Edition. Philadelphia, USA: Lea and Febiger; 1997.
10. Sandle T. Choosing Disinfectants. *Cleanroom Technology*, August 2010, pp11-13.
11. Sandle T. Selection and use of cleaning and disinfection agents in pharmaceutical manufacturing. In: Hodges N, Hanlon G (eds). Industrial Pharmaceutical Microbiology: Standards and Controls. Passfield, England: Euromed Communications, 2012.
12. Morton LHG, Greenway DLA, Gaylarde CC, Surman, S B. Consideration of some implications of resistance of biofilms to biocides. *International Biodeterioration and Biodegradation* 1998; **41:**247-259.
13. Sue C, Snyder A, Baston B, Disinfectants Efficacy Testing, Microbiology in Pharmaceutical Manufacturing, Edited by Richard Prince, Davis Horwood International, 2001, pp437-449.
14. Denny VF, Kopis EM, Marsik FJ. Elements for a successful disinfection program in the pharmaceutical environment. *PDA Journal of Pharmaceutical Science and Technology* 1999; **53**: 115-124.
15. *Denyer SP, Stewart GSAB*. Mechanisms of action of disinfectants. *International Biodeterioration and Biodegradation* 1998;**41**:261-268.

[16] McDonnell G, Russell A. Antiseptics and disinfectants: activity, action and resistance. *Clinical Microbiology Reviews*; 1999;**12**(1):147-179.

[17] Angelillo IF, Bianco A, Nobile CGA, Pavia, M. Evaluation of the efficacy of glutaraldehyde and peroxygen for disinfection of dental instruments. *Letters in Applied Microbiology* 1998; **27**: 292-296.

[18] Bergan T, Lystad A. Evaluation of disinfectant inactivators. *Acta Path Microbiol Scand* Section B 1972; **80**:507-510.

[19] Connor DE, Eckman MK. Rotation of phenolic disinfectants. *Pharm Technol,* Sept 1992: 148-162.

[20] Russell AD. Assessment of sporicidal efficacy. *International Biodeterioration and Biodegradation* 1998;**41**:281-287.

[21] Cooper MS. Biocides and preservatives. *The Microbiological Update* 2000;**18**(3):1-4.

[22] Frank JF, Chmielewski R. Influence of surface finish on the cleanability of stainless steel. *Journal of Food Protection* 2001; **64**(8):1178-1182.

[23] Anon. Bacteriotherapy: the time has come. *British Medical Journal*, 18th August 2001, 353-354.

[24] Madigan M, Martinko J, Parker J. Brock Biology of Micro-organisms, 8th edition. Prentice Hall; 1997, 408-409.

[25] Baird R. Cleaning and Disinfection in the Hospital Pharmacy. In: Collins *et al.* Disinfectants, their use and evaluation of effectiveness. Society Applied Bacteriology, TS16:154; 1981.

[26] Cooper DW. Applying a uniform layer of disinfectant by wiping. *PDA Journal of Science and Technology* 2000;54(1):44-58.

[27] Klingeren B, Koller W, Bloomfield SF, Bohm R, Cremieux A, Holah J, Reybrouck G, Rodger HJ. Assessment of the efficacy of disinfectants on surfaces. *International Biodeterioration and Biodegradation* 1998;**41**:289-296.

[28] Vina P, Rubio S, Sandle T. Selection and Validation of Disinfectants. In: Saghee MR, Sandle T, Tidswell EC. (eds.) Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices. New Delhi, India: Business Horizons; 2010, pp219-236.

[29] Reybrouck G. The testing of disinfectants. *International Biodeterioration and Biodegradation* 1998;**41**:269-272.

[30] Holah JT. Progress report on CEN TC 216 WG3: Disinfectant test methods for food hygiene – institutional and domestic applications. *International Biodeterioration and Biodegradation* 1997; **36**:355-365.

[31] Prince J, Deverill CEA, Ayliffe GA. A membrane filtration technique for testing disinfectants. *Journal of Clinical Pathology* 1975; **28**:71-76.

[32] Sandle T. Selection of Laboratory Disinfectants', *The Journal*, Institute of Science Technology, Summer 2006, pp16-18.

[33] Sandle T. Review of current approaches for the validation of disinfectants. *Pharmaceutical Microbiological Interest Group News*, No. 15, March/April 2004, pp10-15.

[34] Payne DN, Babb JR, Bradley CR. An evaluation of the suitability of the European Suspension Test to reflect *in vitro* activity of antiseptic against clinically chosen significant organisms. *Letters in Applied Microbiology* 1999; **28**:7-12.

[35] Russell D, Dancer BN, Power EGM. Effects of Chemical Agents on Bacterial Sporulation, Germination and Outgrowth, 1991, Society for Applied Bacteriology, reprint.

[36] van Kilgeren B, Koller W, Bloomfield SF, Bohm R, Cremieux A, Holah J, Reybrouck G, Rodger H-J. Assessment of the efficacy of disinfectants on surfaces. *International Biodeterioration and Biodegradation* 1998;**41**:289-296.

[37] Bloomfield SF, Arthur M, Begun K, Patel H. Comparative testing of disinfectants using proposed European surface test methods. *Letters in Applied Microbiology* 1993; **17**:119-125.

[38] Langsrud S, Sundheim G. Factors influencing a suspension test method for antimicrobial activity of disinfectants. *Journal of Applied Microbiology* 1998; **85**:1006-1012.

[39] Holah JT, Lavaud A, Peters W, Dye KA. Future techniques for disinfectant efficacy testing. *International Biodeterioration and Biodegradation* 1998;**41**:273-279.

[40] Larson E. A causal link between handwashing and risk of infection? Examination of the evidence. *Control Hospital Epidemiology* 1988;**9**:28-36.

[41] Ballantyne C. Do antibacterial soaps do more harm than good? *Scientific American,* February 2008, p96.

[42] Mittermayer, Rotter M. Vergleich der Wirkung von Wasser; Einigen Detergentien und Aethylalkohol auf die transiente Flora der Hande. *Zbl Bakt Hyg* 1985;**160**:163-172.

[43] Best M, Kennedy ME. Effectiveness of handwashing agents in eliminating *Staphylococcus aureus* from gloved hands. *Journal of Applied Bacteriology* 1992;**73**:63-66.

[44] Rotter M *et al.* Alkohole fur eine Standarddes-infektionsmethode in der Wertbestimmung von Verfahren fur die HygienischeHandedesinfektion. *Zbl Bakt Hyg* 1977; **164**:428-438.

[45] EN 1499. 1997; Chemical disinfectants and antiseptics. Hygienic handwash. Test method and requirements (phase 2/step 2).

[46] EN 1500. 1997; Chemical disinfectants and antiseptics. Hygienic handrub. Test method and requirements (phase 2/step 2).

[47] Anon. Effectiveness testing of an antiseptic handwash or healthcare antiseptic handwash *Federal Register* 1994,;**59**(116); 21 CFR333.470.

[48] Kramer A, Rudolph P, Kampf G, Pittet D. Limited efficacy of alcohol-based hand gels. *The Lancet* 2002; **359**:1489-1490.

[49] Kampf G. Intra-laboratory reproducibility of the hand hygiene reference procedures of EN 1499 (hygienic handwash) and EN 1500 (hygienic hand disinfection). *Journal of Hospital Infection* 2002;**52** (3):219-224.

[50] Goroncy-Bermes P. Hand disinfection according to the European Standard EN 1500 (hygienic handrub): a study with Gram-negative and Gram-positive test organisms. *International Journal of Hygiene and Environmental Health* 2001;**204**(2-3):123-126.

[51] Larson E, Mayur K, Laughon B. Influence of two handwashing frequencies on reduction in colonizing flora with three handwashing products used by health care personnel. *American Journal of Infection Control* 1989;**17**(2): 83–88.

[52] Namura S, Nishijima S, McGinley J, Leyden J. A study of the efficacy of antimicrobial detergents for hand washing: using the full-hand touch plates method. *Journal of Dermatology* 1993; **20**,88-93.

[53] Beaney AM (ed). Quality Assurance of Aseptic Preparation Services, 4th edition. London, UK: Pharmaceutical Press; 2006.

Cleanroom clothing

Matts Ramstorp, PhD, Biotekpro AB, Sweden

21.1 Introduction

"Cleanroom clothing and gloves are the primary barriers keeping contaminants, generated by personnel, from being emitted into the cleanroom and depositing on products".[1]

People are often considered as the greatest and most critical source of contaminants when it comes to handling products and (or) items having higher or increased demands of cleanliness and hygiene. Other sources of contaminants can also have an impact on the degree of cleanliness, but generally people are the predominant source. When looking at the various sources of contaminants, independently on where the actual handling is taken place, four major sources can be identified:

- Humans – in the form of personnel
- Air – in the surrounding environment
- Surfaces – in the surrounding environment
- Product and (or) process – the actual work undertaken by the personnel

All these four sources are interconnected within the room and (or) area in which the handling is taking place. **Figure 1** demonstrates schematically the interconnection of these four sources of contaminants[2].

The four sources shown in **Figure 1**: personnel, air, surfaces and product, are all connected (as indicated by the double arrowed lines). All four are equally important from a general point of view, but from a practical point, it is vital to distinguish the one (the ones) that has (have) the highest significance with respect to the work being undertaken.

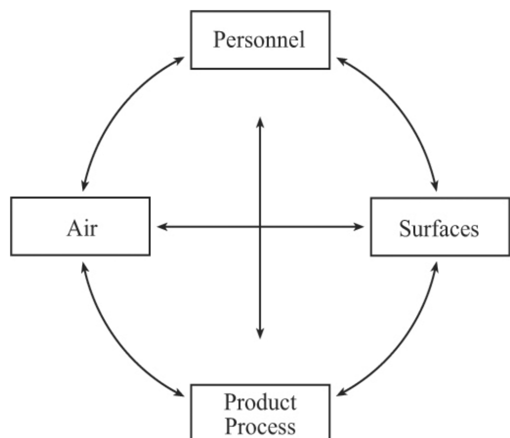


Figure 1: *The interaction between the four major areas of contamination control. The figure shows how personnel might affect the cleanliness of the surrounding air, surfaces in the room, products and/or the process handled in the room. The same general pattern also applies to how the cleanliness of personnel is affected by the cleanliness of the air, surfaces and/or the products, and vice versa.*

As can be seen from this figure personnel can contaminate the product or the production process through the surrounding air, with direct as well as indirect contact with different surfaces and also by direct or indirect contact with the product and (or) process equipment. The most important source of contamination derived from humans is through the natural process of continuous shedding of epidermal cells.

Cleanrooms, and other controlled environments, are used in order to protect the

product, the process and (or) the personnel from various contaminants, and are of vital importance. To properly design and build a cleanroom is not enough, the real challenge comes when introducing personnel and trying to perform production to the desired level of cleanliness.

When people are allowed into a cleanroom, the overall cleanliness of the room will change dramatically, because humans are enormous generators of particles (dead ones as well as micro-organisms). Particles from humans are to a large extent due to the replacement of the outer layer of the skin. The process of skin replacement takes place on a continuous basis, twenty-four hours a day. It normally takes approximately four days to renew the total skin surface of the body, indicating that the exchange rate of this process is quite rapid. The old skin, in the form of skin scales, becomes dry, breaks loose and leaves the body. Many of these skin scales will be stopped by and embedded in our clothes, but some of these particles will be released out into the surrounding air of the room. The human body will by this action release between 6 and 13 grams of skin scales per day. In a year this means that approximately 3.5kg of skin scales will be released from a human body. Men have a higher level of particle dispersion than women; this is mainly due to hormonal reasons. Also, younger persons have a higher level of skin scale dispersion as compared to older ones.

Even though the released skin scales comprise of dead material, they are considered to be serious contaminants when working with sensitive bio-activities, i.e. when handling material and production processes that can be contaminated by micro-organisms. This is because the skin surface of our body is more or less covered with micro-organisms as a part of our normal flora. In practice this means that even if the skin scales released are considered as "dead material", they must be looked upon as viable particles since the skin scale is probably carrying living micro-organisms.

How many particles does a human being actually create? Naturally, when moving fast, the number of particles will increase, but even when standing or sitting totally still we generate particles, as illustrated in **Table1**.

Table 1: The number of particles emitted by a person at different degrees of activity, wearing a snap smock[3].

Personnel activity	**Particle emission per minute** (Equal to and larger than 0.3µm)
No movement, sitting or standing	100 000
Light movement	500 000
Heavy movement	1 000 000
Change position	2 500 000
Slow walk 2.0mph (0.9m/s)	5 000 000
Normal walk 3.5mph (1.6m/s)	7 500 000
Fast walk 5.0mph (2.2m/s)	10 000 000

Most of the investigations and research within the field of contamination control and cleanroom technology ends back to the mid-60s of the last century. Much has happened since then. Our lifestyle has changed dramatically, we have adopted new behaviours, new and even more thorough ways to deal with the personnel hygiene, new materials in our clothes, new ways to wash our clothes etc, etc. All these factors must have a tremendous impact on the release of contaminants. The question is if it´s for better or for worse?

A study, undertaken by an MSc student at the University of Lund, Sweden showed the levels of contaminants arising from humans in cleanroom (**Table 2**).

Table 2: Strength of source, particles/minute, from a human being at different degree of activity and wearing different types of clothing. (aerodynamic diameter $D_a \geq 0.53 \mu m$)[4]

Activity	Laboratory garment	Underpants only	Cleanroom garment
Sitting, no movement	220 000	40 000	40 000
Sitting, arm movement	1 740 000	90 000	70 000
Standing, rotating torso	1 550 000	140 000	60 000
Walking on the spot	2 850 000	400 000	180 000

21.2 Particles generated by humans

Particles, including micro-organisms, can be released from humans in many ways, for example in the form of:

- Skin scales
- Micro-organisms
- Hair
- Dandruff
- Dried salt crystals from perspiration
- Product used for hair styling
- Remainder from hair shampoo and hair conditioner
- Remainder from washing powder and softener for textile materials
- Textile fibres
- Cosmetics
- Smoke particles
- Saliva
- Anal gases
- Nasal extracts

Due to natural reasons people release a considerable number of particles. Most of these arise from the outer layer of our skin surface, but particles can also be released into the surrounding air from the mouth and nose, for example when breathing, coughing, sneezing, laughing, singing and (or) shouting. Many of these latter particles are extremely contaminated with micro-organisms, as is illustrated in **Table 3**.

Table 3: The number of particles distributed through the mouth and nose in connection with various activities

Activity	Number of dead particles	Number of particles of microbiological origin
Sneezing	1 000 000	39 000
Coughing	5 000	700
Speaking loudly(100 words)	250	40

One very effective way to keep the level of particles arising from humans to a low level, and thereby reducing the risks of product contamination, is to keep the number of people in contact with the production process to an absolute minimum. It is also important to dress the personnel in specially designed cleanroom garments that will act as barriers (so-called "personnel filters"). The use of specially designed cleanroom garments will dramatically reduce the number of particles that will be dispersed out into the surrounding air. Normal cleanroom garments can, for instance, comprise of a coverall, long-legged boots that are fixed below the knee, barrier gloves, a hood and facial

protection (face mask) and in some cases, eye protection (goggles). Eye protection in the form of goggles will reduce the possibility of particles and droplets generated from the eye region entering the surrounding air and, due to gravity, sediment down towards the material handled.

Another activity that is associated with personnel and the creation and deposition of contaminants is the physical contact by personnel with different surfaces, using their hands and/or other parts of their body. By the act of touching particles, fats and other liquid material will be deposited on various critical surfaces. This type of contamination can be avoided by, first, reducing the need to touch critical surfaces; and, secondly, by using different types of clean barrier gloves and (or) tools if there is an absolute need, for example move an object or touch a surface.

21.3 The human microbial flora

Many billion micro-organisms exist on and in some parts of our body. These organisms are called the normal flora of people. Since all of these micro-organisms live on and inside us they can be considered as parasites, although we gain much from their presence. This means that we as humans live in harmony and thereby in symbiosis with the normal flora. The major question to be asked is: What is the object of the interaction between people and the normal flora? The function of the normally occurring micro-organisms on and in our body is for example:

- To hinder pathogenic organisms, i.e. micro-organisms capable of creating sickness on our skin and our mucous membranes
- To produce vitamins in the large intestines
- To produce lactic acid in the vagina of fertile women, that adjusts the pH in order to avoid infections

When studying the human body for micro-organisms in detail, it turns out that different parts of the body show considerable differences both in presence as well as in numbers of micro-organisms per unit area. These differences normally depend on the varying presence of food, moisture as well as temperature and oxygen. In a symbiotic relationship there is always a state of equilibrium. The normal flora of humans can be divided into:

- Skin flora
- Nose flora
- The flora of the eye
- Mouth- and throat flora
- The flora of the gastrointestinal tract
- The urinary tracts flora
- The vaginal flora

21.4 Cleanroom Garment Demands

The general reasons for using cleanroom garments are protection, particularly with regard to:

- Surrounding air
- Product
- Process
- Personnel
- Authorities

The overall aim to use specially designed cleanroom garments is to provide protection. The general purpose when working in cleanrooms and other controlled environments is to protect the surrounding environment, i.e. the surrounding air in the cleanroom, and to minimise the possibility of the product handled from becoming contaminated. The same goes for the process in which the product is produced. The process can differ quite a lot. It can be a large production process, with large vessels, pipes, pumps and valves, but it can also comprise of a very small process, i.e. a test

tube to which a small sample is to be transferred from another test tube. Despite of the size of the process, the product is normally quite unprotected when inside the process, which indicates the need to protect, not only the product, but also the process.

In some cases there is an additional need to protect the personnel working with the product and (or) process, for example when dealing with toxic and (or) pathogenic material, i.e. dangerous micro-organisms. This type of protection must also be considered when choosing the proper garment in a cleanroom, but this will not be covered in this chapter.

Cleanrooms are used in many different types of industrial and other types of critical activities, ranging from microelectronics, pharmaceuticals, medical devices to food and beverages and hospitals. These users all have the same purpose for using cleanrooms – protection, in some way or another. But what are these needs based on? Microelectronic manufacturing is solely based on profit, if the cleanliness is not good enough, the products will not have the desired quality and the economic profit will not be obtained. Within the pharmaceutical, as well as other bio-industries and activities, there is an additional need to protect the products so that these will do no harm for the end-user and (or) consumer. This is the reason for the requirements by regulatory authorities in regard to level of cleanliness in a cleanroom together with how the personnel should be dressed when entering the cleanroom.

Despite the industrial activity it is important to recognise that a cleanroom will not achieve the required levels of cleanliness with personnel working in it, if they not are properly dressed before entering the cleanroom[5,6].

One of the most fundamental questions stated is how much of each person is to be covered, i.e. dressed by cleanroom garments when entering the cleanroom? In the 1990s some pharmaceutical companies stated that the lower the classification of the cleanroom, the more coverage of the personnel is needed. This discussion was due to the fact that lower classification cleanrooms had much more ineffective ventilation systems, and thus needed to be better protected from particles generated by humans. However, it is not wise to demand that the personnel should be too heavily dressed when entering a cleanroom. This is because of two principal disadvantages: the practical point of view in regards to personnel comfort, as well as the cost of all the items put on and worn. When dressing for the cleanroom, the personnel should only be covered with cleanroom garments enough for the work being undertaken, not more nor less.

21.4.1 The European GMP and Cleanroom Garment Demands

The European Good Manufacturing Practice (EU GMP), volume 4, annex 1[7] divides the various cleanrooms used in the pharmaceutical industry into four grades: A, B, C and D. These cleanrooms are used for different purposes[8]:

- *Grade A* is a local zone used for high risk work, i.e. a filling zone for open ampoules and flasks that after filling are closed aseptically. This clean zone often comprise of different types of LAF- (UDAF-) units or safety benches.
- *Grade B* is a room, a cleanroom, for aseptically filling and (or) aseptic work, in which the local zone (Grade A) is present
- *Grade C and Grade D* are cleanrooms, in which less critical process steps are performed, in connection with the manufacture of sterile products
- *Grade C* is used for manufacturing of products at risk for contamination and for filling of ampoules and flasks that normally are sterilised after closure, for example in an autoclave. Grade C is also used during aseptic manufacturing of solutions that are sterile filtered prior to filling
- *Grade D* is used during manufacturing of solutions and preparation of various process components that are to be used during filling. This type of cleanroom is also used during

the manufacture of tablets and handling of components, for example after washing. Grade D is also used as final step during bulk manufacturing

The recommended garment systems according to the EU GMP are as follows:

- *Grade D* – Hair and/or beard should be covered. General protective clothing and appropriate shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination from entering the cleanroom
- *Grade C* – Hair and/or beard should be covered. A single or two-piece trouser suit, gathered at the wrists and with a high neck and appropriate shoes or overshoes should be worn. They should shed virtually no fibres or particulate matter
- *Grade B* – Headgear should totally enclose hair and/or beard; it should be tucked into the neck of the suit; a face mask should be worn to prevent the shedding of droplets; sterilised non-powdered rubber or plastic gloves and sterilised or disinfected footwear should be worn; trouser legs should be tucked inside the footwear as well as garment sleeves into the gloves. The protective clothing should shed virtually no fibres or particulate matter and retain particles shed by the body.

21.4.2 Recommended Practices and Cleanroom Garments

IEST[a] provide standardised procedures based upon pre-approved applications of environmental technology. All documents are formulated by IEST Working Groups. Two documents are available in regard to garment systems, IEST-RP-CC-003 with the title: Garment System Considerations for Cleanrooms and Other Controlled Environments[9], and IEST PR-CC-005 with the title: Gloves and Finger Cots Used in Cleanrooms and Other Controlled Environments[10].

21.4.3 General requirements

The material used for cleanroom garments must conform to three major requirements:

- It must offer and provide an adequate barrier between the user and the outer environment, i.e. it must be a good enough filter
- The material of the garment parts must have a low level of particle release, i.e. it should not by itself be a contamination hazard
- It should be as comfortable as possible for the user, i.e. it must not be uncomfortable or restrictive to wear

These three requirements must be discussed together since they are, in some sense, interrelated. The best possible filter is actually a plastic bag, through which no particles or gases can pass, but this will create a massive disturbance for the user since it will not be comfortable! There must be a balance between the filtration efficiency and the experienced comfort for the wearer, which has been one of the fundamental developments for the producers of cleanroom garments for the last decade. The term comfort with regard to cleanroom garments is explained later in this chapter.

21.4.3.1 Disposable or reusable cleanroom garments

Cleanroom garments can be either disposable or reusable. Some sterile facilities use disposable garments due to contamination concerns relating to reusable garments returned from laundering facilities. Some other companies use disposable garments at some locations and reusable at others. In most cases disposable garments are used for visitors, engineer undertaking repair and maintenance etc.

Traditionally disposable cleanroom garments have been made from flash-spun polyethylene fabric, which provides a good filtration efficiency for submicron sized particles as well as micro-organisms. This material has also proven suitable for light splash protection from non-hazardous liquids. Some consider disposable garments easier to manage, from a cost standpoint. Reusable garments often carry cost such as to cover pick-up, delivery, laundering and sterilisation.

Table 4: shows a shorter version of the recommendations found in the older version of IEST- RP-CC 003.

US Fed Std 209 D US Fed Std 209 E ISO 14644-1	Class 100 000 Class M 6.5 ISO 8		Class 10 000 Class M 5.5 ISO 7		Class 1000 Class M 4.5 ISO 6	
Cleanroom type	Non-LAF[1]	Mixed	Non-LAF[1]	Aseptic	Non-LAF[1]	Aseptic
Frock	R	R	R	NR	AS	NR
Two-piece suit	AS	AS	AS	NR	AS	NR
Coverall	AS	AS	AS	R	R	R
Shoe cover	R	R	R	NR	AS	NR
Boots	AS	AS	AS	R	R	R
Hair cover	R	R	R	R	R	R
Hood	AS	AS	AS	AS	AS	R
Facial mask	AS	AS	AS	R[2]	AS	R[2]
Powered headgear	AS	AS	AS	AS	AS	AS
Woven gloves	AS	AS	AS	NR	AS	NR
Barrier gloves	AS	AS	AS	R	AS	R
Inner suit	AS	AS	AS	AS	AS	AS
Changes	2/week	2/Week	2/week	Per entry	2-3/week	Per entry

US Fed Std 209 D US Fed Std 209 E ISO 14644-1	Class 100 Class M 3.5 ISO 5		Class 10 Class M 2.5 ISO 4		Class 1 Class M 1.5 ISO 3	
Cleanroom type	Non-LAF[1]	Mixed	Non-LAF[1]	Aseptic	Non-LAF[1]	Aseptic
Frock	NR	NR	NR	NR	NR	NR
Two-piece suit	AS	NR	AS	R	AS	NR
Coverall	R	R	R	R	R	R
Shoe cover	NR	NR	NR	NR	NR	NR
Boots	R	R	R	R	R	R
Hair cover	R	R	R	R	R	R
Hood	R	R	R	R	R	R
Facial mask	R	R[2]	R	R[2]	R	R[2]
Powdered headgear	AS	AS	AS	AS	AS	AS
Woven gloves	AS	NR	NR	NR	NR	NR
Barrier gloves	AS	R	R	R	R	R
Inner suit	AS	AS	R	R	R	R
Changes	1/day	Per entry	Per entry	Per entry	Per entry	Per entry

Table 4. Recommendations for cleanroom garments according to IEST-RP-CC-003.2:
US Fed Std = US Federal Standard. It should be noted that this standard was withdrawn in 2001 and that the principal cleanroom classification standard is ISO 14644.
R =Recommended; NR = Not Recommended; AS = Application Specific
[1] Conventionally-ventilated cleanroom
[2] Surgical masks recommended

Modern disposable garments are made from spun-bond-melt-blown – spun-bond (SMS) fabric, which is composed of an outer layer of spun bond polypropylene, giving strength and cloth-like comfort, a middle layer composed of a matrix of micro-fibers, which creates a torturous filter path for particles and liquid to pass through.

Reusable cleanroom garments are today typically made from woven polyester blend fabric, which will degrade after multiple laundering and sterilisation cycles.

21.4.3.2 Comfort

Beyond the aspects of protection there is a general requirement for all types of working clothes, including cleanroom garments, to be both functional for its user as well as comfortable to wear. The term 'comfort' is a subjective matter and can be divided into three major components:

- Climate
- Movement
- Feeling

Comfort in regard to climate depends on how well the heat equilibrium between the wearer, i.e. the operator, and the surrounding environment works. There are several ways by which energy in the form of heat can be expelled from our bodies:

- Humid heat transfer
  - Sweating
- Dry heat transfer
  - Breathing
  - Radiation
  - Convection
  - Physical transfer

The heat equilibrium, i.e. the interaction between a human being and the surrounding environment, can be studied and followed in climate laboratories[11,12].

Some studies indicate that a person standing naked in a climate research laboratory, without any movement of the surrounding air and at approximately 28°C, has a temperature at the perimeter of the body of approximately 32°C and the air close to the skin surface has a humidity of 30% RH. This temperature and relative humidity is stated as the optimal thermal condition of a human being. However, when becoming physically active, and also due to the various degree of heat production which differs from person to person, differences between the personnel will be noted.

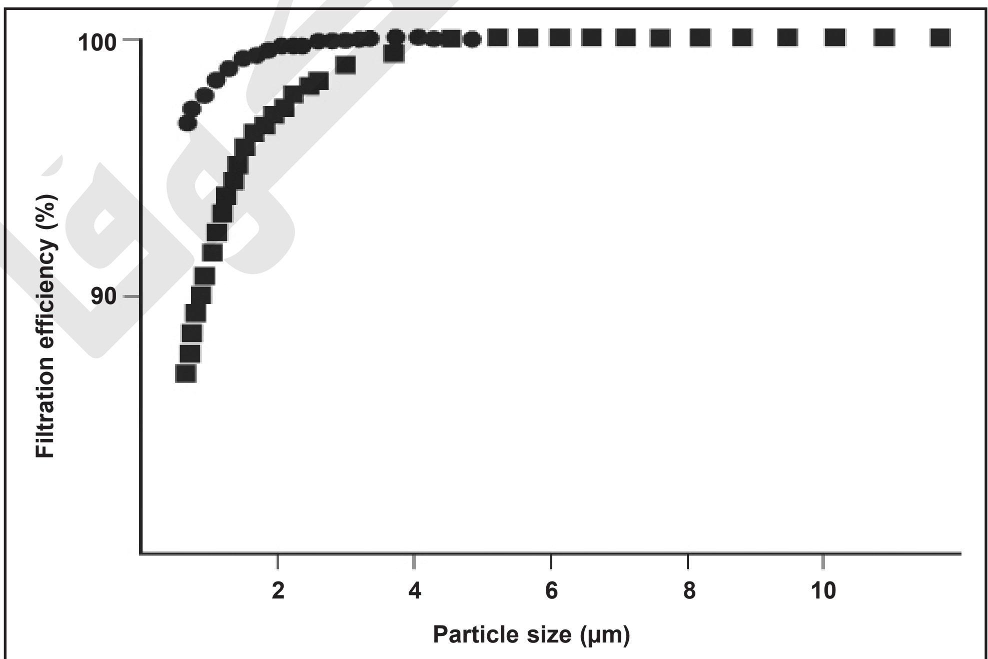
Comfort with regard to movement can be achieved by ensuring that there is no discomfort or restriction during movement caused, for instance, by garments that are too tight. All cleanroom garments should be large enough for all movements performed by the operators.

To ensure comfort with respect to feeling the garment, the textile fabric as well as all seams, should be such that it will not irritate the skin on contact. Some years ago the standard way of using cleanroom garments, for example within the pharmaceutical industry, was that when dressing for the cleanroom, all personal clothing, except for underwear was removed. A coverall was then donned without anything, except for the underwear, worn underneath. This led to complaints from some operators: some said that they felt naked underneath the coverall; some complained about the plastic feeling they experienced from the polyester material of the coverall. In order to cope with these problems, cleanroom clothing manufacturers developed specific cleanroom undergarments, which from the start where intended to be used solely as comfort garments. However, it was later shown that these special cleanroom undergarments act as pre-

Figure 2 *(above): Photo of cleanroom underwear shirt in 100% polyester. Published with kind permission from Fristads AB, Sweden.*

Figure 3 *(right): Photo of cleanroom underwear trousers in 100% polyester. Published with kind permission from Fristads AB, Sweden.*

Figure 4 *(below): The difference in filtration efficiency between a coverall alone as compared to a coverall together with specially designed cleanroom undergarment in polyester: Coverall alone (■) and combined coverall and underwear (●).*



filters, stopping quite a lot of particles. This combination of undergarment and coverall results in a far more effective filter for particles generated by the operators when wearing the complete garment system, as is illustrated in **Figure 4**.

21.4.3.3 Filtration efficiency

The tighter the material of cleanroom garments the better filtration, i.e. the better protection of product, process as well as the surrounding environment of the cleanroom. In order to fulfil the demands of comfort the material of the garment must be "open" to a certain extent, especially to allow water vapour to be removed as the operator perspires. However, the more open structure of the garment material the less filtration will be obtained. The photographs in the following figures, **Figure 5** to **Figure 9**, show magnifications of textile materials and illustrate the difference between relatively open and closed structures.

When producing textile materials for cleanroom garments, there are two ways of creating an effective filtration capability. Firstly the weave is constructed of threads that comprise a large number of filaments. The number of filaments constituting a thread is important for the larger the number the better the filtration efficiency. However, this also relates to how these threads are weaved together to form the textile fabric. The weave itself can be produced in several ways. **Figure 8** shows a weave that is called "one over one". The weave in **Figure 9** is called "two over two". There is a fundamental difference between these two weaves. The one over one tends to give a more stiff feeling for the user, whereas the two over two will feel much softer and more comfortable. The one over one is, from a theoretical point of view, much higher in filtration efficiency, depending on how hard the weave is produced; whereas the two over two theoretically has decreased filtration efficiency.

21.4.3.4 Particle release from the garment material

Due to natural reasons the material of the garment system should not create a risk for contamination when used in the cleanroom. This is the reason why garment systems created from natural material are commonly not used in cleanrooms and why 100% polymeric material, such as polyester, is preferred when producing textile fabrics for cleanroom use. Returning to the European GMP, Volume 4, and the **Table** in Annex 1, the recommendations for Grade D cleanrooms does not state anything about the material used in cleanroom garment, whereas for Grade C, as well as for Grade B, the annex states that the material should be chosen to "shed virtually no fibres or particulate matter". This is why many pharmaceutical companies use blended materials, polyester blended with cotton, in their cleanroom garments.

Figure 5: *Microscopic image of fibres from a cotton T-shirt. 180 x magnifications.*

Figure 6: *Microscopic image of fibres from a man´s cotton shirt. 180 x magnifications.*

Figure 7: *Microscopic image of fibres from 100 % polyester undergarment. 180 x magnifications.*

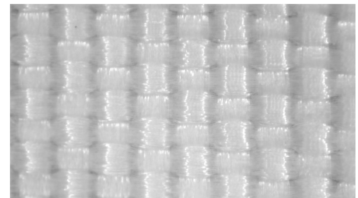


Figure 8: *Microscopic image of fibres from 100% polyester cleanroom garment, weave one over one. 180 x magnifications.*

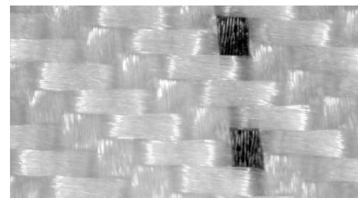


Figure 9: *Microscopic image of fibres from 100% polyester cleanroom garment, weave two over two. 180 x magnifications.*

21.4.4 Private clothing worn underneath cleanroom garments

Even though the cleanroom garment is aimed towards being a good enough filter to be able to filter out fibres and particulate matter from the wearer, there is an ongoing discussion whether or not to allow private clothing underneath. About 10 to 15 years ago some companies allowed private clothing underneath, for example a cleanroom coverall, especially if the operators felt cold. The operators, in these cases, could take whatever domestic clothes to use underneath, which in some cases led to problems with cleanliness.

Companies allowing private clothing underneath the cleanroom clothing will not have full control over control of cleanliness. Another problem that might arise is in the laundry step of the clothing. A pharmaceutical company did not allow any private clothing underneath (except for private underwear) and introduced cleanroom undergarments. These were manufactured from 100% polyester. However, they were disliked by the operators and in response the company changed the undergarments to a type manufactured from natural material, i.e. 100% cotton. Following the use of the cotton garments no problems where initially observed in the actual cleanrooms. However, problems with fibres and other particulate matter were later identified in the airlocks where the cotton underwear was exposed to the surrounding air. Furthermore, the contracting company handling the laundry, noticed after some time that the polyester coveralls where all covered with cotton fibres which proved extremely hard to wash away during a normal laundry process.

From the above it can be seen that private clothing as well as clothes produced of natural material, except for personal underwear, should never be introduced in cleanrooms with strict requirements for cleanliness and hygiene!

21.5 Cleanroom clothing parts

This section examines the components of cleanroom clothing, which consist of:

- Trousers
- Jacket
- Tunic
- Underwear
- Coverall
- Hood
- Goggles
- Boots
- Socks
- Shoes
- Shoe covers
- Face masks
- Beard cover
- Hair cover
- Gloves and finger cots

21.5.1 Body covering parts

Trousers, jackets, tunics, underwear, coveralls, hoods and boots are, in many cases, manufactured from textile materials, although single use, disposable items also are available.

The major purpose of the body covering parts of a cleanroom garment is to provide a suitable degree of coverage of the operator. Depending on the criticality of the work performed, i.e. if the product, process and (or) personnel, respectively, is to be protected, the parts of the garment may

vary. This is demonstrated quite clearly in the European GMP, previously discussed, when looking at the various grades of cleanrooms. In Grade D, for example, the coverage required is much smaller as compared to Grade C and grade B.

When performing less critical work it might be sufficient to use trousers and frocks, whereas when the demands increase, i.e. when the contaminants arising from the personnel might have much higher impact, the personnel must be more thoroughly dressed, i.e. using a coverall with hood together with long legged boots, gloves, mouth protection, and even goggles (**Figures 10-12**).

Boots are traditionally used with a hard sole of a shoe that has been welded together with the textile tube of the boot. The cleanroom boot is to be used with a cleanroom shoe placed inside to obtain a two layer sole. The boot is secured in different manner, however, all boots are secured under the knee of the coverall and straps are normally used to fasten the sole in a secure way. The straps used to secure the foot always touch the floor when dressing for the cleanroom, which makes it hard to dress in a completely clean way. Some companies use a different type of boots, where the sole has been taken away, leaving only a sock, made from the same material as the coverall. Since the footwear does not have to be sterile, according to the European GMP, Volume 1, annex 4, only disinfected, operators put on the sterilised textile boots, and place the foot covered with the sock inside the pre-disinfected shoes. The sock is then secured to the coverall under the knee.

21.5.2 Shoe covers

Shoe covers (sometimes called overshoes) are mainly used to cover either private shoes or facility shoes when passing from and into a cleaner area or zone, when there is no need for a traditional shoe change. Shoe covers come in a vast number of variations, ranging from simple plastic bags equipped with rubber elastics, to specially designed and cleanroom accepted plastic boot like items that are tied around the lower part of the trousers. When using shoe covers it is important to first of all determine the criticality of the area to be entered. Secondly, to determine the risk if such a shoe cover disintegrates and exposes the private shoes worn in the outside environment.

Figure 10: *(left). Photo of cleanroom coverall in 100% polyester. Published with kind permission from Fristads AB, Sweden.*

Figure 11: *(above). Photo of cleanroom hood in 100% polyester. Published with kind permission from Fristads AB, Sweden.*

Figure 12: *(above). Photo of cleanroom boot. Published with kind permission from Fristads AB, Sweden.*

21.5.3 Face masks

Face masks are used in higher classification cleanrooms and in other environments where there is a need to protect the surrounding air, the product, the process and (or) the personnel performing the work. The purpose of a face mask is twofold: firstly to filter out particulate matter in the air when inhaling as well as exhaling, secondly to decelerate the speed of air movement, especially when exhaling.

The efficiency of a face mask differs quite remarkably, depending on whether the wearer is inhaling or exhaling. When exhaling through a face mask, the over pressure created during exhaling will force the face mask to leave the surface of the face. Only a small portion of the exhaled air will be filtered through the material of the face mask, and a larger portion of the air will pass more or less freely into the outer environment, in an unfiltered state upwards, downwards as well as to the sides of the face mask. The situation is totally different when inhaling. During inhaling a negative pressure is created forcing the face mask to glue to the skin of the face. During this action a very large portion of the air is forced and thereby filtered through the face mask material before entering the mouth and nose of the wearer.

Even though the efficiency of a face mask can be disputed, the fact that the speed of air flow will be dramatically decreased when passing through the material is a very good reason for wearing this protective device. A person breathing and talking will have an airflow directed out from the face that will be approximately 0.0 to 1 metre long. Using a face mask the length of this air flow will be dramatically reduced to approximately 10 centimetres.

The need, as well as the efficiency, of face masks has been disputed for many years. Some investigations have shown microbial breakthrough on the outer surface of the mask material even after such periods as short as 15 to 30 minutes.

21.5.4 Beard cover

Beard covers are used in lower classification cleanrooms in order to keep the hair collected under the protective material. The use of beard covers differs from company to company. Some companies state that if men are not newly shaved when coming to work, beard covers are to be used. Other companies have rules stating that personnel do not have to shave for two to three days before beginning to use beard covers. Whereas some companies allow a certain length of the beard, for example 2 – 3 millimetres, before beard covers must be used.

A beard cover is traditionally made from a single layer of non-woven synthetic material, with quite a large porosity, which means that it will not function as a face mask, filtering out large portions of particles. Loose hair as well as small drops of saliva and dried skin scales will, to a large extent, pass a beard cover.

21.5.5 Hair cover

Hair covers are also called bouffant covers and are intended to be used in order to collect all hair in a secure way. Even if a hood is used, for example as an integrated part of the coverall or as a separate item, a hair cover must be used to keep the hair in a secure way so that it will not be accidentally exposed to the cleanroom environment. Hair covers are not used because of their filtration efficiency; the porosity of a hair cover is vast, which in practice means that loose hair as well as dandruff might quite easily pass the non-woven material, (**Figure 13**).

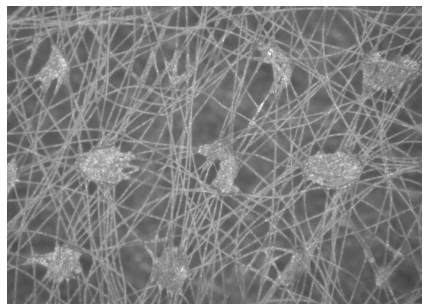


***Figure 13:** Microscopic image of the fibres of a hair cover. 180 x magnifications.*

21.5.6 Gloves and finger cots

Gloves and finger cots are used in cleanrooms both to ensure the safety and cleanliness of the operators and other personnel, and to preserve the cleanliness of the product, the process and other critical surfaces within the cleanroom itself.

Gloves and finger cots can be made from any material as long as it satisfies the requirement of the user. However cotton, wool, asbestos, or any other material that is likely to release and generate particles should not be used in an exposed state within a cleanroom. Powdered gloves and (or) gloves lined with flocking are also not recommended. If conductive fibres or fibres made conductive by the addition of conductive materials are used it must be totally clear that they are compatible with the chemicals used within the cleanroom and (or) in the process.

The length of the gloves is also important, since the gloves should cover the cuff of the cleanroom garment, overlapping by 25mm or more.

Cleanroom gloves fall into three categories:

- Barrier gloves
- Knitted or woven gloves
- Heat-resistant gloves

Barrier gloves are typically constructed from materials such as latex rubber, polyvinyl chloride, neoprene and polyurethane. The major aim, when choosing the glove material, is to see that it is compatible with the chemicals expected to be used. This includes not only the product handled itself, but also all chemicals used for cleaning as well as disinfection.

Figure 14: *Photo showing the different outcome of glove prints on a clean window, using powdered gloves as well as non-powdered gloves. Using this window imprinting method is quite easy in order to rapidly detect if the gloves are totally unacceptable or not.*

Knitted or woven gloves should preferably be made from continuous filaments, in the same way as for the garment covering major parts of the body of the operators. Traditionally 100% synthetic fibre material such as polyester, polyamide, acrylic and polyolefins, are used. The porosity of the material is also important and it is recommended by IEST that the minimum denier[b] used in the construction of knitted and (or) woven gloves should be 60. When using filament with a lower denier number, a more open structure will be obtained, leading to a greater potential of transfer of particles, salts, acids and oils through the weave or knit. Cuffs of knitted and woven gloves should be turned under and stitched with a minimum of 12 stitches per inch (2.5cm). Basted cuffs are unacceptable. All seams should be free from loose treads and all threads should be made from 100% synthetic, continuous filaments with controlled, minimum shrinkage.

Heat-resistant gloves are generally made from high-temperature polymers, such as aromatic polyamides and silicones.

Within the "Recommended Practice IEST-RP-CC-005" methods are provided for testing two categories: physical attributes and cleanliness. It must be recognised that cleanroom gloves and finger cots can be major sources of contamination. The IEST standard cited above includes tests for particles, extractable material and inorganic ash.

IEST-RP-CC 005 covers various tests on gloves and finger cots:

- Test for mechanical structural and thermal properties of gloves
  - Strength
  - Chemical compatibility
  - Barrier integrity
  - Bonding of a barrier palm to a knitted or woven glove
  - Permeability to liquids
  - Resistance to heat
- Test for particle release
- Test for extractable matter
- Ashing
- Test for hydrogen sulphide
- Test for micro-organisms
- Tests for contamination and corrosion of surfaces caused by contact with gloves and finger cots
- Testing and inspection
- Packaging

21.6 Maintenance of cleanroom garments

Cleanroom garments will become contaminated during use. If the garment is reusable it must be cleaned and also, if needed, be sterilised before use[13].

Laundering, i.e. washing, drying as well as packaging, must be performed with great care. The general rule is that the facility responsible for laundering must have access to cleanrooms, with cleanliness levels equal and (or) superior to those of the cleanroom in which the garments are to be used. This demand means that the laundry facility must have the same or more stringent cleanliness level as compared to the end user of the garment!

Many of the cleanroom laundries wash the garment at raised temperature, ≥60°C, sometimes up to 72°C, which will not only remove dead particles, but the temperature will also have a major effect on pre-sterilisation bioburden by reducing their numbers on and in the textile material

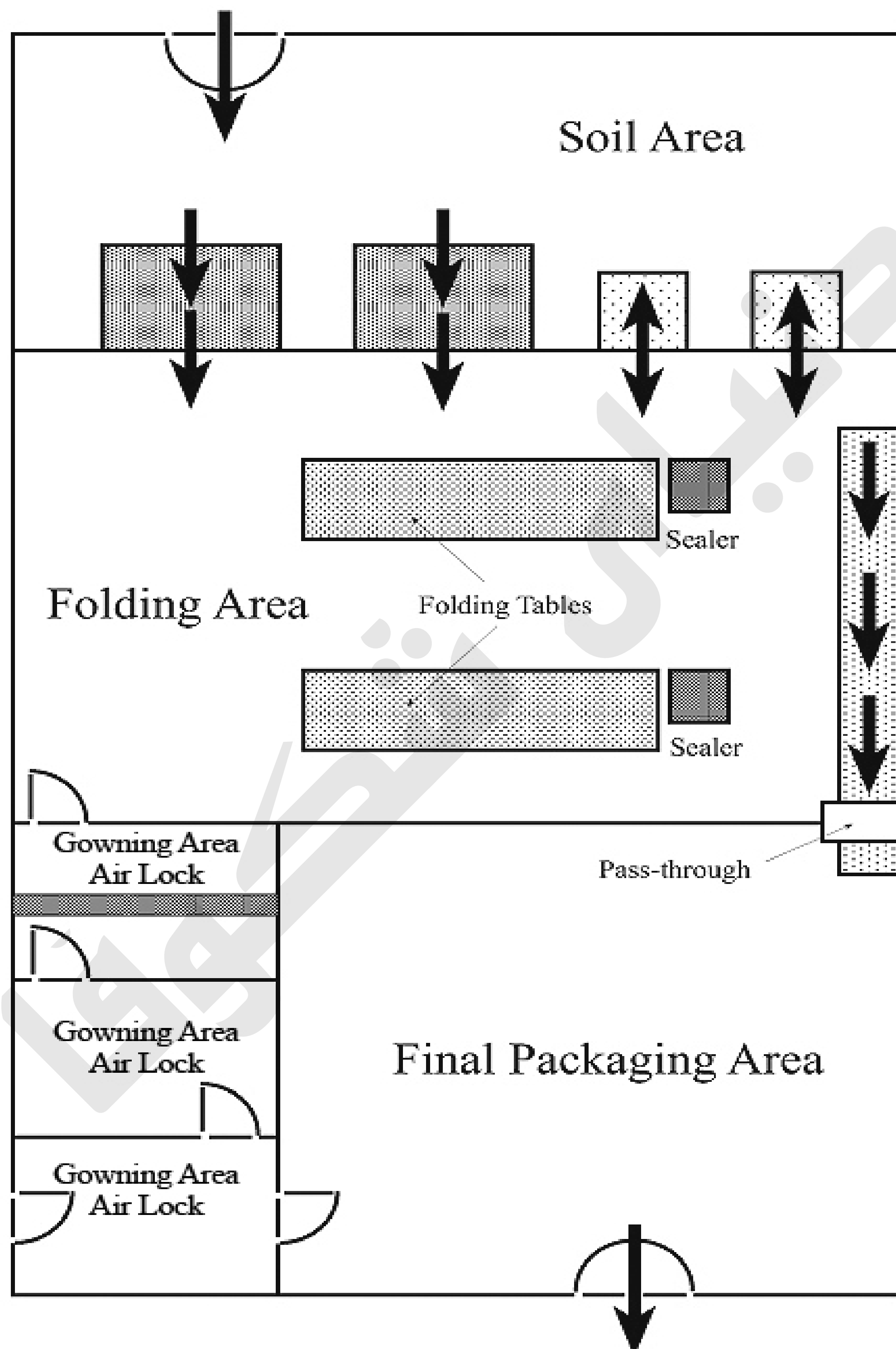


Figure 15: *Schematic representation of the layout of a cleanroom laundry facility.*

washed. This means in practice that a laundry process at this elevated temperature will be very effective in disinfecting the garment.

Cleanroom laundry facilities are built solely for processing cleanroom textile material, such as cleanroom garments as well as reusable wipers and mops. The design of a laundry facility, **Figure 15**, comprise mainly of a loading room, with quite low demands on cleanliness. In the loading area, the sorted cleanroom garment is loaded into pass-through washing machines that are placed in the wall between the loading room and the processing cleanroom. The garments are treated in the washer after which the washer is opened in the processing cleanroom. The operators working in this cleanroom, themselves dressed in cleanroom garments, remove the washed garments from the washer into a tumble drier, equipped with HEPA-filters. After drying the operators take the dried goods out of the drier to fold and pack the garment items into their respective packaging materials (**Figure 16**). The packaging material differs depending on whether the garments are to be further processed, i.e. sterilised for example in an autoclave, or just transported to the end user.

In some industrial applications, for example within the pharmaceutical industry, there is not only a need to clean or even reduce the concentration of micro-organisms. If micro-organisms are considered to be critical contaminants in the cleanroom, the laundry has, after drying and packaging, to be sterilised.

Washing, especially at elevated temperature as well as sterilisation, will have an impact on the shelf life of the garment. Washing is much gentler towards the textile fabric and many cleanroom laundries work to discard, for example, a cleanroom coverall after 150 washing cycles. Sterilisation, on the other hand, is an extremely harsh treatment. Sterilisation is performed by autoclaving or by radiation. In this case many laundries use no more than 50 sterilisation cycles before the garments are discarded.

The frequency of changes of garment varies and is mainly based on the cleanliness of the cleanroom as well as what type of production is taking place in the room. This latter statement is further dependent on the intended use of the product being produced. In general, the more sensitive the operation carried out in the cleanroom, the more frequent the garment has to be changed for cleaner ones.

Figure 16: *Cleanroom operators folding and packing cleanroom garments in a cleanroom laundry facility. Published with kind permission from Berendsen Textil Service, Sweden.*

21.7 Testing cleanroom garments

Due to the importance of cleanroom garments it is important to know that the garment works as intended, i.e. that the textile fabric as well as the complete garment system, including its maintenance, has the capability to minimise particle release. Even though cleanrooms have been in use since the mid-1960s, each and every manufacturer has adopted their own standards for testing fabrics as well as ready-made cleanroom garments. In the information leaflets provided by the manufacturer, you will see that the material is suitable for use in for example ISO

class 5 or Grade A, but you will not find any type of correlation in the information to a standardised comparison to justify the statement. In many cases, the information provided by the manufacturer is based on actual testing on site in a cleanroom with the classification mentioned, i.e. the recommendations are mainly based on trial and error. A situation that is quite surprising in regard to the importance of the garments used!

Many different standards are available and used when producing fabrics, as well as garments used in cleanrooms. Some manufacturers utilise these and some have made modifications to the tests, which makes it quite difficult to compare garments from different manufacturers.

Some of the most fundamental tests are presented below, together with some new systems that have been developed in Sweden at the University of Lund, in collaboration with the Textile University of Borås.

21.7.1 Test methods on textile fabrics as well as cleanroom garments

Several tests are available for the textile fabric itself as well as ready made cleanroom garments and (or) parts thereof. The following test methods are the most commonly used and are discussed below:

- The particle penetration test
- The bubble point methods
- Releasable large particles test
- The Helmke Drum test
- The microbial penetration test

21.7.1.1 The particle penetration test

A section of garment fabric is placed in a holder and controlled, particle challenged air is passed through the fabric under controlled conditions. The ability of the fabric to filter out particles from the air is determined by testing the air on both sides of the holder, with the aid of an automatic particle counter, **Figure 17**.

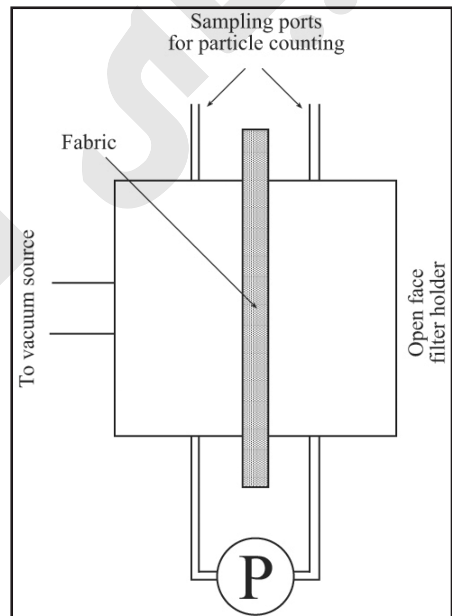


Figure 17: *Schematic representation of the test layout for performing the particle penetration test.*

The holder, equipped with the garment fabric, is placed in an occupied room where a high concentration of airborne particles is generated by people that are present in the room.

The removal efficiency of the fabric is calculated as the removal efficiency between 0.5µm and 5µm and larger than 5µm particles using the following equation:

$$\text{Removal efficiency} = 1 - \left(\frac{\text{average particle concentration in front of the fabric}}{\text{average particle concentration behind the fabric}}\right) \text{x } 10$$

21.7.1.2 The bubble point method

This test is also called equivalent pore diameter test. A piece of fabric or a part of a cleanroom garment is placed in a holder, **Figure 18**. The textile material is then wetted with a suitable liquid of known surface tension and air pressure is applied on one side of the holder. The air pressure is

then increased in order to determine the bubble point pressure of the fabric.

The holder has an opened face making it possible to view the wetted fabric surface. The air pressure is increased until bubbles are observed on the exposed surface of the fabric. According to IEST-RP-CC-003.3 the same procedure is performed on, in total, five pieces of fabric after which the average bubble point pressure is determined. The equivalent pore diameter D of the fabric is calculated, using the equation:

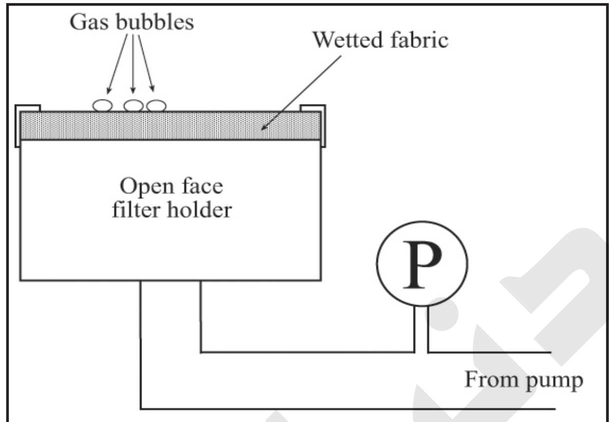


***Figure 18:** Schematic representation of the test set-up for performing a bubble point test.*

$$\textit{Equivalent pore diameter } (\mu m) = \frac{4 \times T \times 10^6}{Z \times P \times g}$$

Where:
T = is the surface tension of the wetting liquid used (mN/M) at the temperature of the test
Z = is the density of the wetting liquid at the temperature of the test (mg/mm^3)
g = is the acceleration due to gravity (mm/s^2)
P = is the bubble point pressure (mm water gauge)

21.7.1.3 The releasable large particle test
A section of the fabric or the garment item to be tested is placed over a screen and vacuumed, **Figure 19**. The vacuum air is then filtered through a membrane filter collecting particles on the filter for further microscopic analysis.

The result from the microscopic analysis is then compared to the particle and fibre count limits in a table, specified in IEST – RP-CC-003.3.

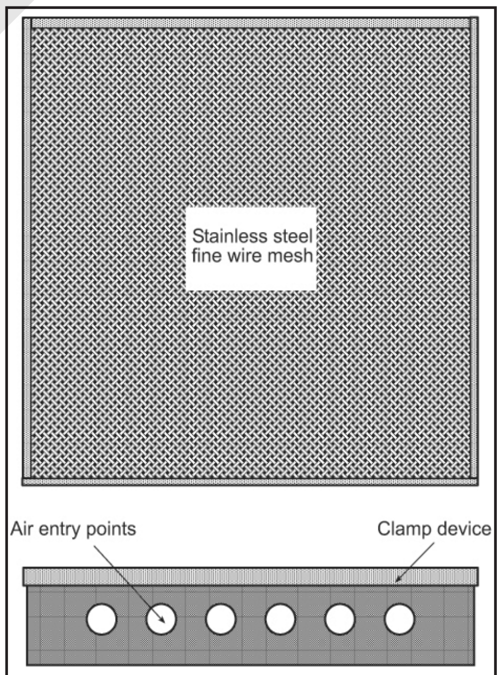


***Figure 19:** Schematic representation of the frame used for performing the released large particle test.*

21.7.1.4 The Helmke Drum test
This test is named after George Helmke, who developed the test together with Dick Yiech[14]. The method is also referred to as the tumbling or rotating drum method.

The Helmke drum test[15,16] is used to quantify particles dislodged from garments as well as wipers by applying mechanical energy under dry conditions, in order to simulate particle shedding from the surface of the material tested. The material to be tested is tumbled in a rotating drum, in order to release particles from the material under controlled conditions,

Figure 20. A standard optical particle counter, traditionally used in cleanroom technology, is monitoring the air within the drum to determine the average particles concentration of the air during the first ten minutes of the test. The result from the particle counter is then used to calculate a particles emission rate, which then is compared with a table to determine garment cleanliness classification of the type of garment studied.

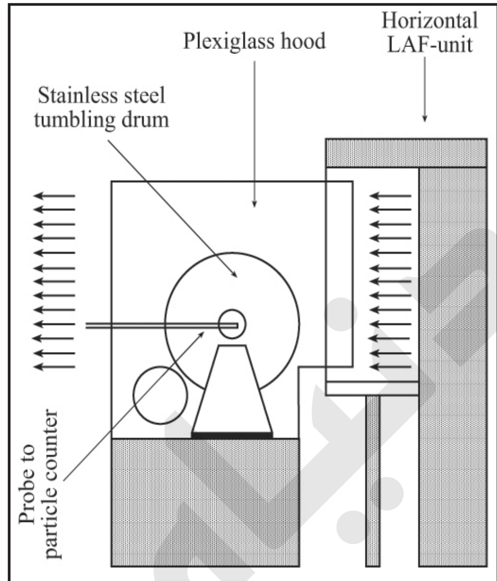


Figure 20: Schematic representation of the set up for performing the Helmke drum test.

21.7.1.5 The microbial penetration test

This test can be used to determine the microbial penetration and to study the performance of fabrics, as well as entire garment items, in preventing the passage of particulate contaminants including micro-organisms.

The test should be performed in a class II safety cabinet and the fabric to be tested is placed in a filter holder. Test organisms are aerosolised from an aqueous suspension and subsequently challenged to the fabric in the holder. Downstream of the holder the organisms penetrating the fabric are collected with the aid of a slit-to-agar sampler equipped with agar plates containing suitable growth media, **Figure 21**.

The penetration of microbial particles is calculated using the following equation:

$$\text{Microbial penetration} = \frac{\text{CFU recovered (test fabric in holder)}}{\text{CFU recovered (no fabric in holder)}}$$

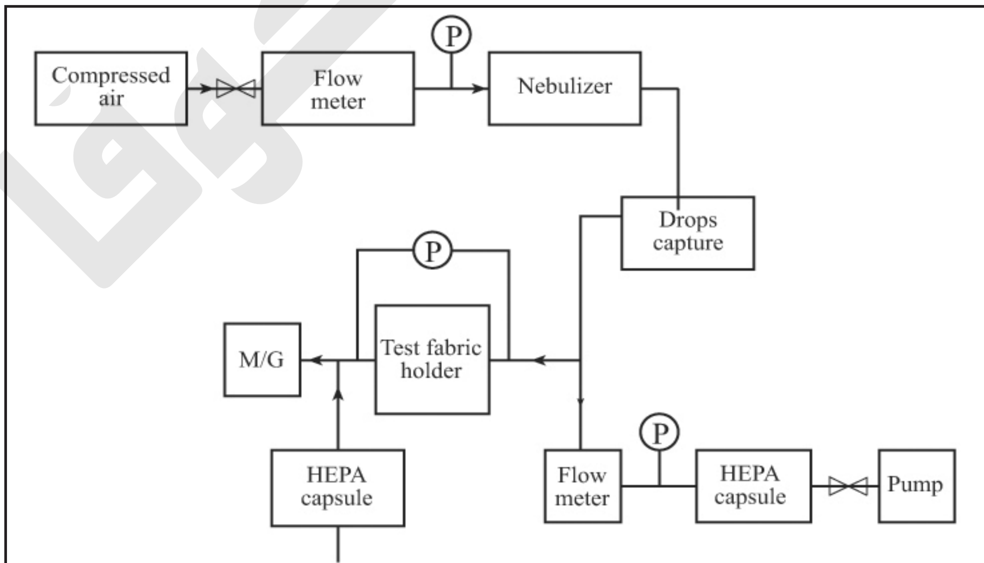


Figure 21: Schematic representation of the microbial penetration test.

$$\text{Filtration efficiency of the fabric} = \left(1 - \frac{\text{CFU recovered (test fabric in holder)}}{\text{CFU (no test fabric in holder)}}\right) \times 100$$

Performing the microbial penetration test, in combination with the particle penetration test, as well as the Helmke drum test, is often considered a comprehensive evaluation of the barrier and shedding properties of cleanroom fabrics as well as cleanroom garment items.

21.8 Modern approach to testing cleanroom garments

Testing cleanroom garment can be performed in several different ways. One of the properties that is of most interest is the filtration effect of the garment. This is of interest not only to study the filtration efficiency of a new garment but also the time effect, especially in regard to the number of washing cycles and subsequent sterilisation. As stated earlier in this chapter, washing, and especially sterilisation, will have quite a long-term adverse impact on the textile fabric and therefore also on the filtration effect.

In many cases a dispersal chamber, or a so called "body-box", is used to study the cleanroom protection efficiency[17,18], **Figure 22**. A specially designed dispersal chamber equipped with HEPA-filtered air supply and

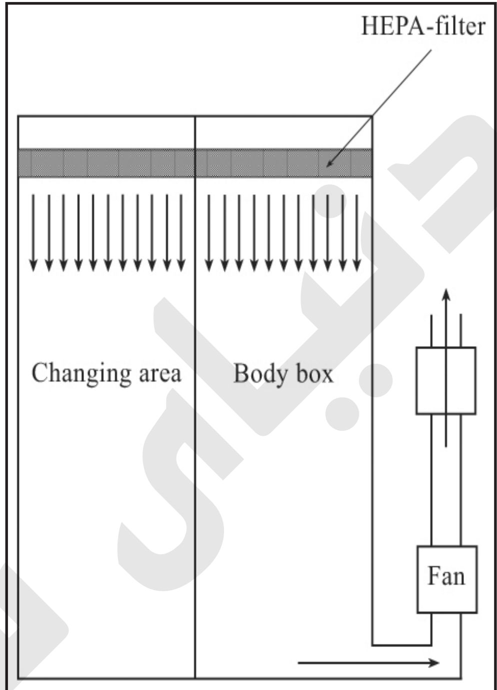


Figure 22: *Schematic representation of a standard "Body-box".*

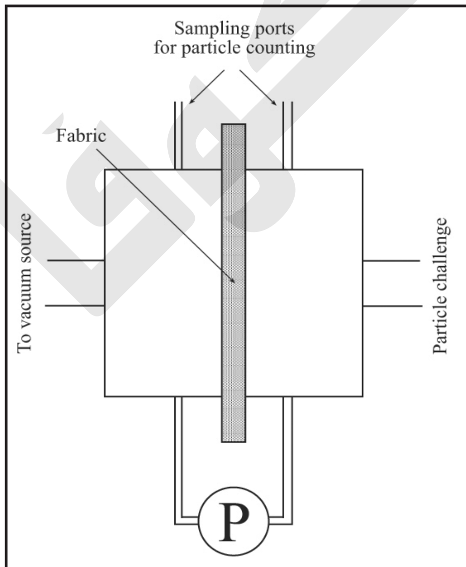


Figure 23: *Schematic representation of a modified "body-box".*

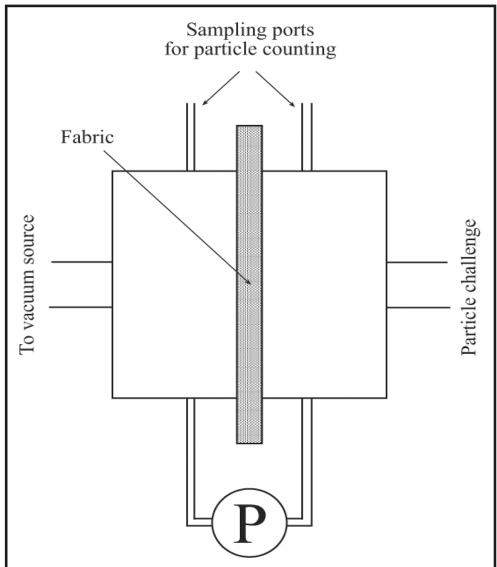


Figure 24: *Schematic representation of the test layout for performing the particle challenge test.*

separate exhaust air, has been qualified for the evaluation of cleanroom clothing systems[19]. This system has also been used at KTH, Sweden, in order to study newly developed cleanroom garment systems[20], **Figure 23**.

In the body-box-test of cleanroom garments, the individuality of the test person dressed in the cleanroom garments and performing various movements cannot be neglected.

Another test set-up was used during an MSc thesis work performed at the Swedish School of Textiles, University of Borås, in association with LTH, the Faculty of Engineering at the University of Lund. In these tests the textile fabric and (or) parts of cleanroom garments were placed in a specially designed set-up and particles were challenged to the fabric, under specific test conditions. Particle measurements were performed on the air, before as well as after passing the textile fabric, **Figure 24**, by the aid of an aerodynamic particle counter, measuring particles in

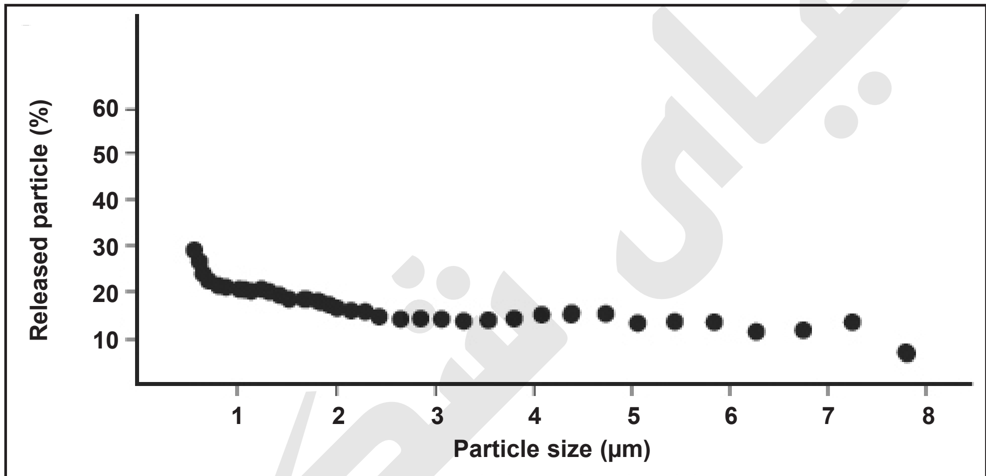


Figure 25: *Particle penetrating of a cleanroom fabric in percentage of the total challenge. The fabric has been washed and sterilised through 20 cycles.*

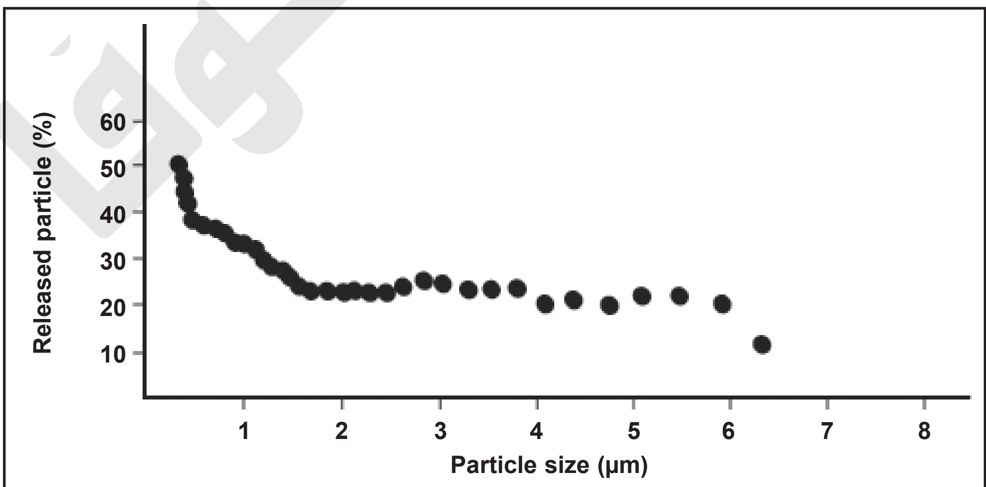


Figure 26: *Particle penetration of the same cleanroom fabric as in* ***Figure 19****, in percentage of the total challenge. The fabric has been washed and sterilised through 80 cycles.*

the particle size range of 0.7μm and up to 14μm. It is quite interesting to compare one and the same textile fabric, for example after 20 and 80 washing and sterilising cycles, respectively, **Figure 25** and **Figure 26**. The graphs presented in these figures have been changed from particle filtration efficiency to particle permeability capability.

The advantage of this test method is that it is possible to follow the filtration efficiency of the fabric or, for that part, even a ready-made cleanroom garment over the full range of particle sizes, thus creating a better understanding of the differences between different fabrics.

The bubble point method is, according to the author, a method that has both advantages and disadvantages. The method can be used to study the overall porosity of the fabric, but cannot be used for studying the filtration efficiency. **Figure 27** shows the equivalent pore diameter as well as the tearing strength of a cleanroom fabric that has been washed and sterilised up to 80 cycles. In this figure it is clearly demonstrated that the equivalent pore diameter will increase, whereas the tearing strength of the fabric will decrease as the number of washings and sterilisation increase.

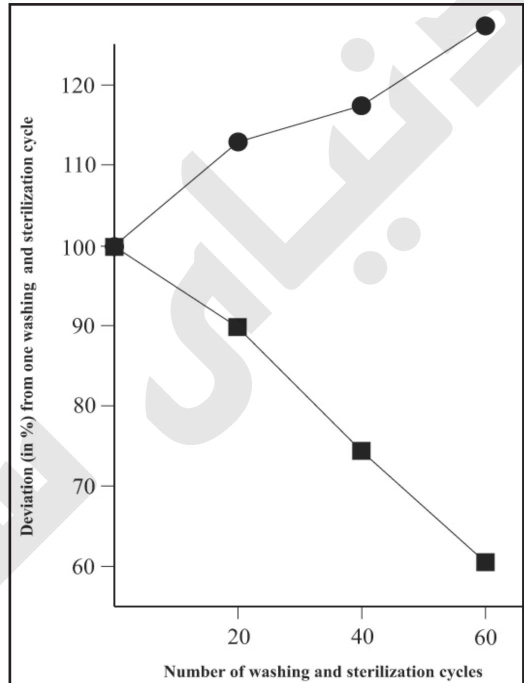


***Figure 27:** The equivalent pore diameter (•) as well as the tearing strength [n] of a cleanroom fabric as a function of the number of washing and sterilisation cycles.*

Although the bubble point method is commonly used, not only when analysing textile fabric but also in the world of microfiltration, there are some drawbacks that must be emphasised. The method results in what is called the equivalent pore diameter which, if not understood correctly, could be quite misleading. With a filter, either a microfilter for filtration of process fluids or cleanroom garment fabric filtering out particles generated by operator, the major aim is to stop particles from passing the filter material. It is a well known fact that a filter will become much more efficient if the depth through which the fluid should pass is increased. In practice this means that if the filter thickness will for example be doubled the filtration effect should increase. If a test method is used in order to give a valid explanation of the filtration capability of a textile fabric, it should show that when tested. Several tests have been performed with the aid of the bubble point method at the Textile University of Borås, studying single layer fabrics as well as double layer fabrics. The results are shown in **Table 5** (overleaf):

As is seen from this table, it is very hard to draw any straight conclusion from the different results obtained when testing single layer and double layer fabrics, respectively.

Another drawback of the bubble point method is that the result is given as equivalent pore diameter. Looking at the results given in **Table 5**, the numbers are so high that there might be a possibility to sort the fabrics out due to the size of the pore diameter, i.e. the result might give the impression that the fabric is not good enough.

Table 5: Equivalent pore size (µm) measured for single and double layer fabric, respectively, for seven different textile materials[21].

Textile	Number of layers	Intended use	Stated pore size (µm)	Measured pore size (µm)
A	Single	Cleanroom	20	30.9
	Double	Cleanroom	20	26.1
B	Single	Controlled Environment	27	54.8
	Double	Controlled Environment	27	49.1
C	Single	Controlled Environment	Not specified	49.8
	Double	Controlled Environment	Not specified	50.6
D	Single	Inner garment	Not specified	78.2
	Double	Inner garment	Not specified	74.5
E	Single	Cleanroom	Not specified	41.6
	Double	Cleanroom	Not specified	39,4
F	Single	Cleanroom	16	48.8
	Double	Cleanroom	16	43.6
G	Single	Cleanroom	Not specified	8
	Double	Cleanroom	Not specified	8

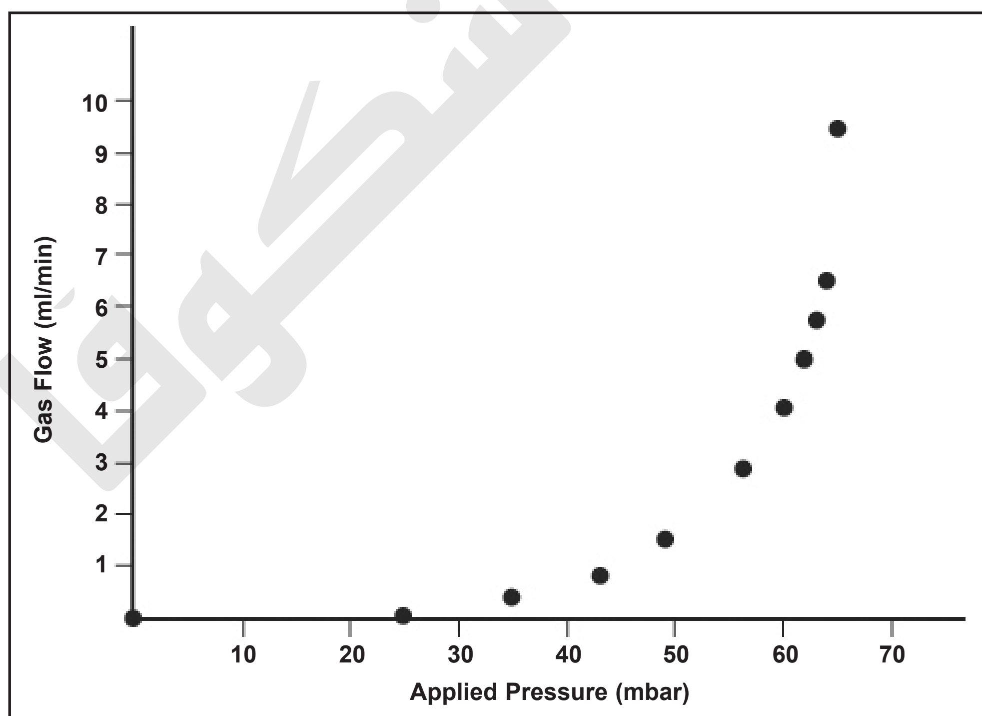


Figure 28: *The diffusion flow of pressurised air through a wetted fabric used for cleanroom garments, as a function of the air pressure. The fabric tested has quite an open texture as compared to the one in* ***Figure 29****.*

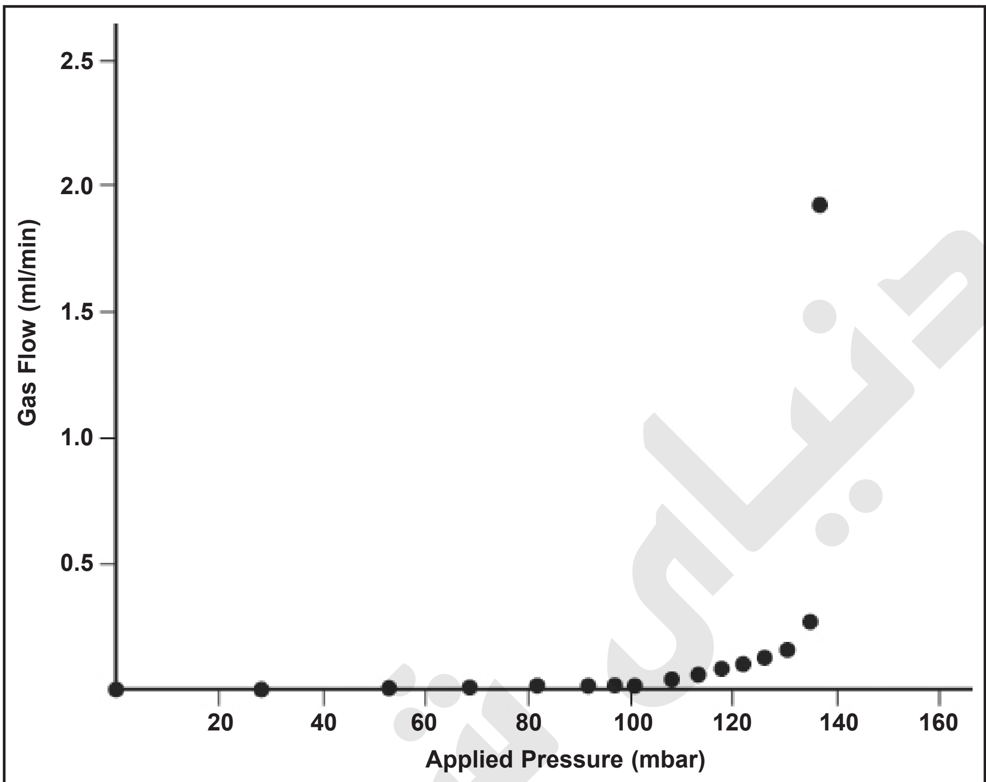


Figure 29: *The diffusion flow of pressurised air through a wetted fabric used for cleanroom garments, as a function of the air pressure. The fabric tested has quite an open texture as compared to the one in* ***Figure 28.***

A new test method has been developed at the University of Lund. Instead of testing the fabric with the bubble point method they utilised another test method that today is commonly used for process filters. The test method utilises the diffusion of gas through a wetted fabric that is placed under low overpressure. This method is used in industry for testing sterility grade filters and is called the diffusion test and (or) the pressure hold test[22]. The new test method[23] needs quite a lot of further development, especially in order to set the limits for pass or failure. **Figures 28** and **29** shows some test results obtained with this diffusion test on a standard fabric for cleanroom garments.

The fabric of cleanroom garments are intended to act as filters. It might be wise in the future to look at the tests performed on, for example, HEPA- (High Efficiency Particulate Air) and ULPA- (Ultra Low Penetrating Air-filters) in order to find new and more easily understood tests. At the University of Lund the MPPS (Most Penetrating Particle Size) was analysed at some cleanroom fabrics. **Figure 30** shows the results from one of these tests. The test was performed using both an optical particle counter (OPS) for the larger particle sizes and a Condensation Nucleus Counter (CNC) for the smaller particles.

21.9 Use of cleanroom garment

Various activities, including industrial, laboratory as well as hospitals, utilise cleanrooms and thereby also cleanroom garments. Depending on the background environment of the cleanrooms, cleanroom garments are different from activity to activity. Within bio activities, i.e. pharmaceutical, medical device, food and beverages, and hospitals, there is a need to control microbiological

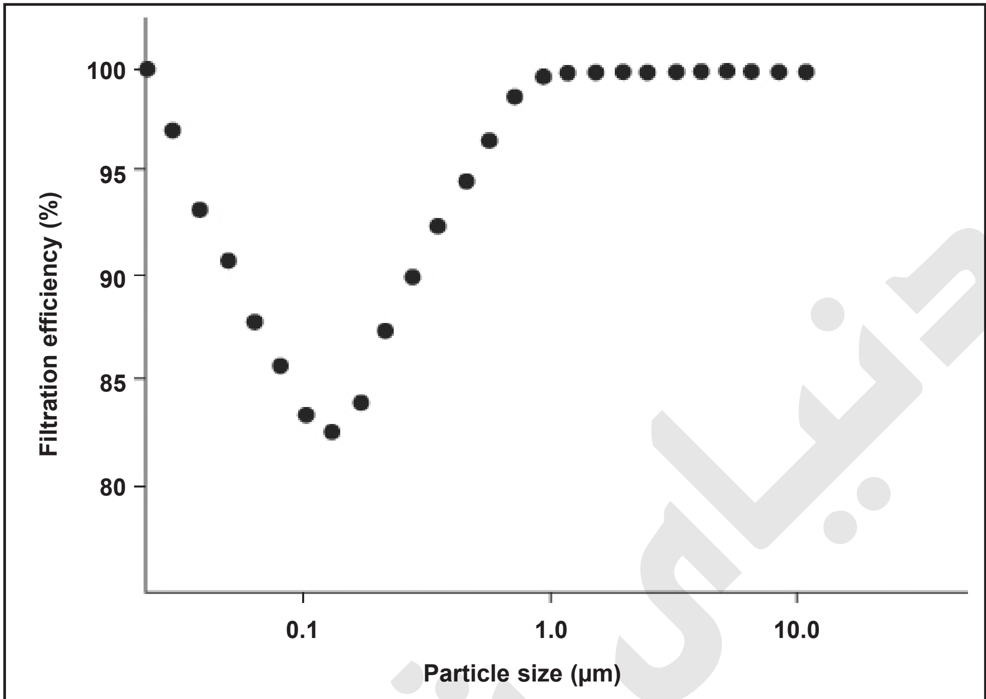


Figure 30: *Determination of the MPPS (Most Penetrating Particle Size) of a cleanroom fabric.*

contaminants; in contrast, within the non-bio-activities, i.e. microelectronics, micromechanics and optics, generally all types of particles are considered as contaminants. Due to natural reasons there is a difference between the two major activities taking place in cleanrooms and other controlled environments.

21.9.1 Bio-activities

With products produced or items handled within the pharmaceutical, medical device, food and beverages and the hospital sector, the major focus is placed on contaminants, especially of microbiological origin, that can degrade the product, damage the products or the items handled (cells and tissues, within the hospital sector, for example), and by doing so create a risk to the person (i.e. patient). These bio-activities have the most stringent demands since the garment should not only be cleaned, but in many cases also sterilised, and used during one entry in a cleanroom. After that specific use the garment, if reusable, will be sent for laundering and subsequent sterilisation before being used once again.

21.9.2 Non-bio-activities

The products produced and (or) handled within this sector need to be produced in a good enough filter condition, in order to remove particles generated by the operators. The major aim is to eliminate particles. The background for this situation is that the products produced and (or) handled will not function properly if contaminated with particles. Micro-organisms are also particles but will not have the same impact on the production within this sector, as compared to the bio-activities, since the products or the process do not generally support microbiological growth.

The difference between these two activities, bio- and non-bio, is also distinct when it comes to garment change frequencies. From a general point of view the bio-industries has a much higher

exchange rate of the cleanroom garments in comparison with the non-bio ones. It must be recognised that there are no hard and fast rules when it comes to cleanroom garments in regard to the differences between bio- and non-bio activities. Each and every activity must determine its own regime when it comes to cleanroom garments, and the degree of coverage of their personnel.

21.10 Changing into cleanroom garment

Due to the different needs, together with the overall cleanliness demands, changing into cleanroom garments can be performed in many ways. For example, when working with aseptic production within the pharmaceutical industry, everything brought into the cleanroom must be pre-sterilised. This also means that the cleanroom garment used by operators must be pre-sterilised and put on in a manner not compromising the sterility of the garment. On the other hand, working within microelectronic industry, the focus is on cleanliness in regard to particulate matter but without demands on microbiological cleanliness.

These two opposite situations, pharmaceutical and microelectronic, will have different demands in regard to microbiological cleanliness, which result in different viewpoints in regard to dressing for the cleanroom as well as time span to use one single item of cleanroom garment.

In this context dressing for the cleanroom will be visualised with an example of gowning procedure used within the pharmaceutical industry, and stricter gowning for aseptic production. It should be brought in mind that the procedure described below is only an example and that variations might be found in different facilities.

- Leave outside, everyday, private clothing in lockers when entering the facility
- Remove, if any, jewellery
- Wash off, if any, cosmetics
- Wash hands thoroughly. In some situations it is permissible to use an odourless humidifying agent in order to avoid dry skin. If this type of agent is used it must be done with great care, not contaminating the hands with dangerous contaminants
- Put on factory clothing
- Move further into the production site
- Chose the correct garment items, if these are placed outside the airlock leading further to the cleanroom
- Enter the air lock to the next cleanliness area
- Remove the factory clothing
- Wash hands and, if desired, disinfect hand
- Put on the specialised cleanroom undergarment
- Enter the next airlock and perform the routines stated, i.e. hand washing, disinfection etc
- Place the cleanroom clothing on the step-over bench between the less clean and the clean areas of the airlock
- Put on specialised dressing gloves
- Put on sterile socks and swing legs over to the cleaner side of the step over bench
- Open the autoclave pack and remove the coverall
- Put on the coverall in a careful way, making sure that the garment is not touched on its outside and that it does not touch any surfaces of the airlock, for example the wall and (or) the floor
- Put on the integrated hood of the coverall and zip close the coverall
- Put on the face mask and secure it

- Put on footwear
- Put on working gloves on top of the dressing gloves
- Finally, disinfect the working gloves, after which entrance to the cleanroom is allowed

Since the dressing and gowning for the cleanroom, described above, is a general description, some points need to be highlighted.

If the coverall does not have an integrated hood, the hood must be put on before putting on the coverall. Face masks may be worn under or over the hood. There is no strict way of doing this. Some recommendations state that the mask is more effective when worn underneath, but many companies state that it should be used over the hood. Using the face mask over the hood is much more practical, for example when just needing to change mask without changing coverall.

Dressing gloves are today mandatory in almost all companies working with cleanrooms with elevated cleanliness standards. The gloves used from robing must be at the same level of cleanliness as to the garments put on. Putting on a pre-sterilised coverall using non-sterile, or even gloves used for domestic purposes, will present a hazard to the cleanliness of the coverall later on in the cleanroom.

Some companies follow the routine described above, where sterile working gloves are put on, on top of the dressing gloves. Other remove the dressing gloves in the last air lock before entering the cleanroom and put on sterile working gloves onto the naked hands. This latter way of using gloves might give rise to contamination problems due to the possible release of dead particles as well as micro-organisms when removing the dressing gloves.

Exiting from the cleanroom is in some cases as important as when entering. This is the case when the personnel are to use their cleanroom garment more than once. When working in aseptic cleanrooms a newly washed and pre-sterilised garment is used only once when entering the cleanroom. However, when working in cleanrooms with less cleanliness demands, in the pharmaceutical as well as other industries, i.e. microelectronics, medical device etc, the garment might be used during the entire day or even for several days without changing into washed garments.

If the garment is to be used more than once it is of vital importance to undress as carefully as when dressing, in order not to contaminate the outside of the garment. It is also important to store the garment in a way as not to allow contaminants from other garments to become a contamination risk.

21.11 Risk factors associated with cleanroom clothing

When choosing as well as using cleanroom garments there are quite a lot of factors to consider. Some of the factors relating to cleanroom garments will have an impact on the operation carried out and (or) the quality of the cleanliness of the environment in a cleanroom:

- The overall cleanliness demand of the cleanroom
- The criticality of the product and how this is handled by the operator(s)
- Authority demand
- The required level of containment, i.e. the items of clothing chosen to cover the operator
- The performance of the garment material, both in regards to the filtration efficiency as well as the comfort of the wearer, together with the risk of particle release
- Design and construction of the garment system
- Whether the garment is washable or disposable

- The practical way to put on as well as to remove the garment
- Nature of personal clothing worn under cleanroom clothing
- Time interval or the number of uses before laundering is required
- The choice of laundry facility for the garment
- Packaging, storage and distribution of clothing
- Time interval or the number of laundry cycles before clothing should be disposed
- The frequency by which disposable items are changed, i.e. gloves, facemasks, etc.

Each company, and even each activity within a company, should have its carefully written procedures covering all aspects of cleanroom garments. Since the personnel in most cases are considered the worst and most critical source of contaminants, not only the behaviour of the personnel, but also the garment coverage together with the way the garment is used, are of crucial importance.

21.12 Standards

Many standards and recommendations are available with regard to gloves and finger cots as well as cleanroom garments and the fabric by which these are produced. Some of the most useful documents are listed below, together with short summaries of their respective scopes.

21.12.1 Gloves and finger cots

21.12.1.1 ASTM

ASTM is formerly known as the American Society for Testing and Materials. ASTM International is a globally recognised leader in the development and delivery of international voluntary consensus standards. Further information is available at www.astm.org.

ASTM D412 – 06ae2 (2006), “Standard test Methods for Vulcanised Rubber and Thermoplastic Elastomers – Tension”[24]. These test methods cover procedures used to evaluate the tensile (tension) properties of vulcanised thermoset rubbers and thermoplastic elastomers.

ASTM D573 – 04 (2010), “Standard Test Method for Rubber – Deterioration in an Air oven”[25]. This test method describes a procedure to determine the influence of elevated temperature on the physical property of vulcanised rubber.

ASTM D882 – 10, “Standard Test Method for Tensile Properties of Thin Plastic sheeting”[26]. This test method covers the determination of tensile properties of plastics in the form of thin sheeting, including film (less than 1.0 mm in thickness). ASTM D991 – 89 (2010), “Standard Test Method for Rubber Property Volume Resistivity of Electrically Conductive and Antistatic products”[27]. This test method covers the determination of volume resistivity of rubbers used in electrically conductive and antistatic products

ASTM D3577 – 09e1, “Standard Specification for Rubber Surgical Gloves”[28]. This specification describes certain requirements for packaged sterile rubber surgical gloves used in conducting surgical procedures.

ASTM D3578 – 05 (2010), “Standard Specification for Rubber Examination Gloves”[29]. This specification describes certain requirements for natural rubber gloves used in conducting medical examination and diagnostics and therapeutic procedures.

ASTM D3772 – 01 (2010), “Standard Specification for Natural Rubber Finger Cots”[30]. This specification covers the requirements for finger cots made from natural rubber latex.

ASTM F739 – 07, “Standard Test Method for Permeation of Liquids and Gases through Protective Clothing Material under conditions of Continuous Contact”[31]. This test method measures the permeation of liquids and gases through protective clothing materials under the condition of continuous contact.

21.12.1.2 ISO

ISO (**I**nternational **S**tandardization **O**rganisation) is the world's **largest developer** and publisher of **International Standards**.

ISO 12127-1:2007, **"Clothing for protection against heat and flame – Determination of contact heat transmission through protective clothing or constituent materials, Part 1: Test method using contact heat produced by heating cylinder"[32].** This standard specifies a test method for the determination of contact heat transmission.

ISO 12127-2:2007, **"Clothing for protection against heat and flame – Determination of contact heat transmission through protective clothing or constituent materials, Part 2: Test method using contact heat produced by dropping small cylinders"[33].** This standard specifies a test method designed to evaluate the heat transfer and the behavior of materials used for protective clothing when such materials are struck by high temperature metal particles, especially when these are trapped in the folds of the garment in working situations.

ISO 10282:2002, **"Single-use sterile rubber surgical gloves – Specification"[34].** This standard specifies requirements for packaged sterile rubber gloves intended for use in surgical procedures to protect the patient and the user from cross-contamination.

ISO 12243:2003, **"Medical gloves made from natural rubber latex – Determination of water-extractable protein using the modified Lowry method"[35].** This standard specifies a method for the determination of the amount of water-extractable protein in natural rubber (NR) gloves for medical use.

ISO 21171:2006, **"Medical gloves – Determination of removable surface powder"[36].** This standard specifies methods for the determination of readily removable powder on the surface of gloves for medical use.

ISO 11193-1:2008, **"Single-use medical examination gloves – Part 1: Specification for gloves made from rubber latex or rubber solution"[37]. This standard** specifies requirements for packaged sterile, or bulked non-sterile, rubber gloves intended for use in medical examinations and diagnostic or therapeutic procedures to protect the patient and the user from cross-contamination.

ISO 11193-2:2006, **"Single-use medical examination gloves – Part 2: Specification for gloves made from poly (vinyl chloride)"[38].** This standard specifies requirements for packaged sterile, or bulked non-sterile, poly (vinyl chloride) gloves intended for use in medical examinations, and diagnostic or therapeutic procedures, to protect the patient and the user from cross-contamination.

ISO 25518:2009, **"Single-use rubber gloves for general applications – Specification"[39]**

21.12.2 Cleanroom garments

21.12.2.1 AATCC

American Association of Textile Chemists and Colorists. AATCC is the world's leading not-for-profit association serving textile professionals. Further information is available at: www.aatcc.org.

AATCC Test Method 22 (2010), "Water Repellency: Spray Test"[40]. This test method is applicable to any textile fabric, which may or may not have been given a water-repellent finish. It measures the resistance of fabrics to wetting by water. It is especially suitable for measuring the water-repellent efficacy of finishes applied to fabrics.

AATCC Test Method 118 (revised 2007, "Oil Repellency: Hydrocarbon Resistance Test"[41]. This test method is used to detect the presence of a fluorochemical finish, or other compounds capable of imparting a low energy surface, on all types of fabrics, by evaluating the fabric's resistance to wetting by a selected series of liquid hydrocarbons of different surface tensions.

AATCC Test Method 127 (2008), "Water Resistance: Hydrostatic Pressure Test"[42]. This test method measures the resistance of a fabric to the penetration of water under hydrostatic pressure. It is applicable to all types of fabrics, including those treated with a water resistant or water repellent finish.

AATCC Test Method 76 (2005, "**Electrical Surface Resistivity of Fabrics"[43].** The purpose of this test method is to determine the electrical surface resistivity of fabrics. The surface electrical resistivity may influence the accumulation of electrostatic charge of a fabric.

21.12.2.2 INDA

International **N**onwovens & **D**isposables **A**ssociation. INDA is the trade association representing the nonwoven fabric industry since 1968. Further information is available at www.inda.org.

STANDARD TEST: WSP 80.8 (2005), "Standard Test Method for Alcohol Repellency of Nonwoven Fabrics"[44]. This method is used to measure the resistance of nonwoven fabrics to wetting and penetration by alcohol and alcohol/water solutions.

21.12.2.3 ASTM

ASTM D257 – 07, "Standard Test Methods for DC Resistance or Conductance of Insulating Materials"[45]. These test methods cover direct-current procedures for the measurement of insulation resistance, volume resistance and surface resistance.

ASTM D737 – 04(2008)e2, "Standard Test Method for Air Permeability of Textile Fabrics"[46]. This test method covers the measurement of the air permeability of textile fabrics.

ASTM D1777 – 96(2011)e1, "Standard Test Method for Thickness of Textile Materials"[47]. This test method covers the measurement of the thickness of most textile materials.

ASTM D2261 – 07ae1 (2007), "Standard Test Method for Tearing Strength of Fabrics by the Tongue (Single Rip) Procedure (Constant-Rate-of-Extension Tensile Testing Machine)"[48]. This test method covers the measurement of the tearing strength of textile fabrics by the tongue (single rip) procedure using a recording constant-rate-of-extension-type (CRE) tensile testing machine.

ASTM D3776/D3776M – 09ae1 (2009), "Standard Test Methods for Mass per Unit Area (Weight) of Fabric"[49]. These test methods cover the measurement of fabric mass per unit area (weight) and is applicable to most fabrics.

ASTM D3786/D3786M – 09 (2009), "Standard Test Method for Bursting Strength of Textile Fabrics-Diaphragm Bursting Strength Test Method"[50]. This test method describes the measurement of the resistance of textile fabrics to bursting using a hydraulic or pneumatic diaphragm bursting tester. This test method is generally applicable to a wide variety of textile products.

ASTM D3884 – 09 (2009), "Standard Guide for Abrasion Resistance of Textile Fabrics (Rotary Platform, Double-Head Method)"[51]. This test method covers the determination of the abrasion resistance of textile fabrics using the rotary platform, double-head test (RPDH).

ASTM D3885 – 07a (2011), "Standard Test Method for Abrasion Resistance of Textile Fabrics (Flexing and Abrasion Method)"[52]. This test method covers the determination of the abrasion resistance of woven or nonwoven textile fabrics using the flexing and abrasion tests.

ASTM D5034 – 09 (2009), "Standard Test Method for Breaking Strength and Elongation of Textile Fabrics (Grab Test)"[53]. This test method covers the grab and modified grab test procedures for determining the breaking strength and elongation of most textile fabrics. Provisions are made for wet testing.

ASTM D5035 – 11 (2011), "Standard Test Method for Breaking Force and Elongation of Textile Fabrics (Strip Method)"[54]. This test method covers raveled strip and cut strip test procedures for determining the breaking force and elongation of most textile fabrics. Provision is made for wet testing.

ASTM D6193 – 11 (2011), "Standard Practice for Stitches and Seams"[55]. This practice covers the requirements and characteristics of stitches and seams used in the fabrication of sewn items.

ASTM E96/E96M – 10 (2010), "Standard Test Methods for Water Vapor Transmission of Materials"[56]. These test methods cover the determination of water vapor transmission (WVT) of materials through which the passage of water vapor may be of importance, such as paper, plastic films, other sheet materials, fiberboards, gypsum and plaster products, wood products and plastics.

ASTM E284 – 09a (2009), "Standard Terminology of Appearance"[57]. This terminology standard defines terms used in the description of appearance, including but not limited to color, gloss, opacity, scattering, texture, and visibility of both materials (ordinary, fluorescent, retroreflective) and light sources (including visual display units).

21.12.2.4 ISO

ISO 13937-1:2000, **"Textiles – Tear properties of fabrics – Part 1: Determination of tear force using ballistic pendulum method (Elmendorf)"[58].**

ISO 13937-2:2000, **"Textiles – Tear properties of fabrics – Part 2: Determination of tear force of trouser-shaped test specimens** (Single tear method)"[59].

ISO 13937-3:2000, **"Textiles – Tear properties of fabrics – Part 3: Determination of tear force of wing-shaped test specimens** (Single tear method)"[60]**.**

ISO 13937-4:2000, **"Textiles – Tear properties of fabrics – Part 4: Determination of tear force of tongue-shaped test specimens** (Double tear test)"[61]**.**

ISO 13934-1:1999, **"Textiles – Tensile properties of fabrics – Part 1: Determination of maximum force and elongation at maximum force using the strip method"[62].**

ISO 13934-2:1999, **"Textiles – Tensile properties of fabrics – Part 2: Determination of maximum force using the grab method"[63].**

ISO 12947-1:1998/Cor 1:2002, **"Textiles – Determination of the abrasion resistance of fabrics by the Martindale method – Part 1: Martindale abrasion testing apparatus"[64].**

ISO 12947-2:1998/Cor 1:2002, **"Textiles – Determination of the abrasion resistance of fabrics by the Martindale method – Part 2: Determination of specimen breakdown"[65].**

ISO 12947-3:1998/Cor 1:2002, **"Textiles – Determination of the abrasion resistance of fabrics by the Martindale method – Part 3: Determination of mass loss"[66].**

ISO 12947-4:1998/Cor 1:2002, **"Textiles – Determination of the abrasion resistance of fabrics by the Martindale method – Part 4: Assessment of appearance change"[67].**

ISO 13936-1:2004, **"Textiles – Determination of the slippage resistance of yarns at a seam in woven fabrics – Part 1: Fixed seam opening method"[68].** This standard is intended for the determination of the resistance offered by thread systems of woven fabric, to slippage at a sewn seam.

13936-2:2004, **"Textiles – Determination of the slippage resistance of yarns at a seam in woven fabrics – Part 2: Fixed load method"[69].** This standard is intended for the determination of the resistance offered by thread systems of woven fabric, to slippage at a sewn seam.

ISO 13936-3:2005, **"Textiles – Determination of the slippage resistance of yarns at a seam in woven fabrics – Part 3: Needle clamp method"[70].** This standard describes a method for the determination of the resistance offered by the yarns of a woven fabric to slippage while being held in a needle clamp under conditions of stress.

21.13 Acknowledgment

I would like to express my gratitude to Mrs Camilla Dahl for all the help supplied in finishing this manuscript.

21.14 References

[1] Lieberman, A. (1992) "Contamination Control and Cleanrooms – Problems, Engineering Solutions and Applications", Van Nostrand Reinhold, New York, USA

[2] Ramstorp, M. "Introduction to Contamination Control and Cleanroom Technology" (2000) Wiley-VCH, Weinhem, Germany

[3] Austin, R.P. and Timmerman, S.W. (1965) "Design and Operation of Cleanrooms", Business News Publishing Co., Detroit, USA

[4] Ramstorp, M., Gustavsson, M. And Gudmundsson, A. (2005) "Particle Generation from Humans – A method for experimental Studies in Cleanroom Technology", J Indoor Air, 15 (Suppl. 11) Blackwell Munksgaard

[5] Austin, R.P. (1995) "Encyclopaedia of Cleanrooms, Bio-cleanrooms and Aseptic Areas, Contamination Control Seminars", Brookfiled, Michigan, USA, Chapter 33, pp632 – 657

[6] Spector, B.R. (1999) "Fundamentals of Cleanroom Garments" Institute of Environmental Sciences and Technologies

[7] "The Rules Governing Medicinal Products in the European Union, Volume 4 – Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use", "Annex 1 – Manufacture of Sterile Medicinal Products"(2008), the European Commission, Brussels, Belgium

[8] Ramstorp, M. "Microbial Contamination Control in Pharmaceutical Manufacture" (2010) Microbiology and Sterility Assurance in Pharmaceuticals and Medical devices, Eds. Saghee, M.R., Sandle, T. And Tidswell, E.C., Business Horizons New Deli, India

[9] "Garment System Considerations for Cleanrooms and Other Controlled Environments" IEST – RP – CC – 003.3 (2008) Institute of Environmental Sciences and Technologies (IEST), USA

[10] "Gloves and Finger Cots Used in Cleanrooms and Other Controlled Environments", IEST PR-CC-005.3 (2003) Institute of Environmental Sciences and Technologies (IEST), USA

[11] ISO 8996:2004 "Ergonomics of the thermal environment – Determination of metabolic rate"

[12] ISO 9920:2007 "Ergonomics of the thermal environment – Estimation of thermal insulation and water vapour resistance of a clothing ensemble"

[13] Austin, R.P. (1995) "Encyclopedia of Cleanrooms, Bio-cleanrooms and Septic Areas", Contamination Control Seminars, Brookfiled, Michigan, USA, Chapter 36, pp689 – 699

[14] Helmke, G.E. (1982) "A Tumble Test for Determining the Level of Detachable Particles Associated with Cleanroom Garments and Cleanroom Wipers" Proceedings of the 28th Annual Technical Meeting of the Institute of Environmental Sciences. Mount Prospect, Illinois: IES(T), pp218 – 220

[15] Elion, J.M., Ensor, D.S. Berndt, C.W., Bovino, M., Diener, R., Ely, G., Eudy, J., Giroux, R.L., Rataj, M. and Witt, J. (2001) "Improving the Repeatability and Reproducibility of the Helmke Drum Test Method", Journal of IEST, 44, no 4

[16] Ensor, D.S., Elion, J.M. and Eudy, J. (2001) "The Size Distribution of Particles Released by Garments During Helmke Drum Tests", Journal of IEST 44, no 4.

[17] Hoborn, J. (1981) "Humans as Dispersers of Micro-organisms – Dispersion Pattern and Prevention", Ph.D.-thesis, Dept of Clinical Bacteriology inst of Medical Microbiology, University of Göteborg, Sweden

[18] Whyte, W. And Bailey, P. (1985) "Reduction of Microbial Dispersion by Clothing", Journal of Parenteral Science and Technology, 39, pp51 – 50

[19] Ytterman, P. (1998) "Qualification of modified body-box for evaluation of cleanroom garments", MSC-thesis, Report No 59, Building Services Engineering, KTH, Sweden (in Swedish)

[20] Reinmüller, B. And Ljungqvist, B (2000) "Evaluation of cleanroom Garments in a Dispersion Chamber – Some Observations", European Journal of Parenteral Sciences, 5 (3), pp55 – 58

[21] Ljunggren, A. (2006) Unpublished results, The Textile University of Borås

[22] Ramstorp, M. (2003)"Contamination Control in Practice", Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany

[23] Ramstorp, M. (2006) “Integrity Testing of Fabrics for Cleanroom Garments – A New Method for Analysing and Controlling the Filtration Efficiency of Cleanroom Clothing” Forum Proceedings 18th International Symposium on Contamination Control, International Confederation of Contamination Control Societies (ICCCS), Beijing

[24] ASTM D412 – 06ae2 (2006) “Standard test Methods for Vulcanised Rubber and Thermoplastic Elastomers – Tension”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[25] ASTM D573 – 04 (2010) “Standard Test Method for Rubber – Deterioration in an Air oven”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[26] ASTM D882 – 10 “Standard Test Method for Tensile Properties of Thin Plastic sheeting”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[27] ASTM D991 – 89 (2010) “Standard Test Method for Rubber Property Volume Resistivity of Electrically Conductive and Antistatic products”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[28] ASTM D3577 – 09e1 “Standard Specification for Rubber Surgical Gloves”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[29] ASTM D3578 – 05 (2010) “Standard Specification for Rubber Examination Gloves”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[30] ASTM D3772 – 01 (2010) “Standard Specification for Natural Rubber Finger Cots”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[31] ASTM F739 – 07 “Standard Test Method for Permeation of Liquids and Gases through Protective Clothing Material under conditions of Continuous Contact”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[32] ISO 12127-1:2007, “Clothing for protection against heat and flame – Determination of contact heat transmission through protective clothing or constituent materials, Part 1: Test method using contact heat produced by heating cylinder”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[33] ISO 12127-2:2007, “Clothing for protection against heat and flame – Determination of contact heat transmission through protective clothing or constituent materials, Part 2: Test method using contact heat produced by dropping small cylinders”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[34] ISO 10282:2002, “Single-use sterile rubber surgical gloves – Specification”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[35] ISO 12243:2003, “Medical gloves made from natural rubber latex – Determination of water-extractable protein using the modified Lowry method”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[36] ISO 21171:2006, “Medical gloves – Determination of removable surface powder”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[37] ISO 11193-1:2008, “Single-use medical examination gloves – Part 1: Specification for gloves made from rubber latex or rubber solution”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[38] ISO 11193-2:2006 , “Single-use medical examination gloves – Part 2: Specification for gloves made from poly(vinyl chloride)”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[39] ISO 25518:2009, “Single-use rubber gloves for general applications – Specification”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[40] AATCC Test Method 22 (2010) “Water Repellency: Spray Test”, American Association of Textile Chemists and Colorists (AATCC), North Carolina, USA

[41] AATCC Test Method 118 (revised 2007) “Oil Repellency: Hydrocarbon Resistance Test”, American Association of Textile Chemists and Colorists (AATCC), North Carolina, USA

[42] AATCC Test Method 127 (2008) “Water Resistance: Hydrostatic Pressure Test”, American Association of Textile Chemists and Colorists (AATCC), North Carolina, USA

[43] AATCC Test Method 76 (2005) “Electrical Surface Resistivity of Fabrics”, American Association of Textile Chemists and Colorists (AATCC), North Carolina, USA

[44] STANDARD TEST: WSP 80.8 (2005) “Standard Test Method for Alcohol Repellency of Nonwoven Fabrics”, Association of the Nonwoven Fabrics Industry (INDA), North Carolina, USA

[45] ASTM D257 – 07 “Standard Test Methods for DC Resistance or Conductance of Insulating Materials”, American Society for Testing and Materials, Philadelphia, Pennsylvania, US

[46] ASTM D737 – 04(2008)e2 “Standard Test Method for Air Permeability of Textile Fabrics”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[47] ASTM D1777 – 96(2011)e1 “Standard Test Method for Thickness of Textile Materials”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[48] ASTM D2261 – 07ae1 (2007) “Standard Test Method for Tearing Strength of Fabrics by the Tongue (Single Rip) Procedure (Constant-Rate-of-Extension Tensile Testing Machine)”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[49] ASTM D3776/D3776M – 09ae1 (2009) “Standard Test Methods for Mass Per Unit Area (Weight) of Fabric”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[50] ASTM D3786/D3786M – 09 (2009) “Standard Test Method for Bursting Strength of Textile Fabrics-Diaphragm Bursting Strength Tester Method”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[51] ASTM D3884 – 09 (2009) “Standard Guide for Abrasion Resistance of Textile Fabrics (Rotary Platform, Double-Head Method)”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[52] ASTM D3885 – 07a (2011) “Standard Test Method for Abrasion Resistance of Textile Fabrics (Flexing and Abrasion Method)”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[53] ASTM D5034 – 09 (2009) “Standard Test Method for Breaking Strength and Elongation of Textile Fabrics (Grab Test)”, American Society for Testing and Materials (ASTM), Philadelphia, Pennsylvania, USA

[54] ASTM D5035 – 11 (2011) “Standard Test Method for Breaking Force and Elongation of Textile Fabrics (Strip Method), American Society for Testing and Materials”, Philadelphia, Pennsylvania, USA

[55] ASTM D6193 – 11 (2011) “Standard Practice for Stitches and Seams”, American Society for Testing and Materials, Philadelphia, Pennsylvania, USA

[56] ASTM E96 / E96M – 10 (2010) “Standard Test Methods for Water Vapor Transmission of Materials”, American Society for Testing and Materials, Philadelphia, Pennsylvania, USA

[57] ASTM E284 – 09a (2009) “Standard Terminology of Appearance”, American Society for Testing and Materials, Philadelphia, Pennsylvania, USA

[58] ISO 13937-1:2000, “Textiles – Tear properties of fabrics – Part 1: Determination of tear force using ballistic pendulum method (Elmendorf)”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[59] ISO 13937-2:2000, ”Textiles – Tear properties of fabrics – Part 2: Determination of tear force of trouser-shaped test specimens (Single tear method)”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[60] ISO 13937-3:2000, “Textiles – Tear properties of fabrics – Part 3: Determination of tear force of wing-shaped test specimens (Single tear method)”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[61] ISO 13937-4:2000, “Textiles – Tear properties of fabrics – Part 4: Determination of tear force of tongue-shaped test specimens (Double tear test)”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[62] ISO 13934-1:1999, “Textiles – Tensile properties of fabrics – Part 1: Determination of maximum force and elongation at maximum force using the strip method”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[63] ISO 13934-2:1999, “Textiles – Tensile properties of fabrics – Part 2: Determination of maximum force using the grab method”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[64] ISO 12947-1:1998/Cor 1:2002, “Textiles – Determination of the abrasion resistance of fabrics by the Martindale method – Part 1: Martindale abrasion testing apparatus”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[65] ISO 12947-2:1998/Cor 1:2002, “Textiles – Determination of the abrasion resistance of fabrics by the Martindale method – Part 2: Determination of specimen breakdown”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[66] ISO 12947-3:1998/Cor 1:2002, “Textiles – Determination of the abrasion resistance of fabrics by the Martindale method – Part 3: Determination of mass loss”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[67] ISO 12947-4:1998/Cor 1:2002, “Textiles – Determination of the abrasion resistance of fabrics by the Martindale method – Part 4: Assessment of appearance change”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[68] ISO 13936-1:2004, “Textiles – Determination of the slippage resistance of yarns at a seam in woven fabrics – Part 1: Fixed seam opening method”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[69] 13936-2:2004, “Textiles – Determination of the slippage resistance of yarns at a seam in woven fabrics – Part 2: Fixed load method”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

[70] ISO 13936-3:2005, “Textiles – Determination of the slippage resistance of yarns at a seam in woven fabrics – Part 3: Needle clamp method”, International Organisation for Standardisation (ISO), Central Secretariat, Geneva, Switzerland

Quality assurance in hospital pharmacies

Richard Bateman, Regional Quality Assurance Service, Guy's Hospital, London, UK.

22.1 Introduction

Although there is much focus on the manufacture and supply of medicinal products by the industry, it is essential to realise that many compounding activities are still carried out in hospitals and healthcare establishments. Additionally in many countries such activities may be carried out by third party service providers rather than in the hospital itself[1].

The types of product being compounded in these facilities may be very broadly divided into two categories:

1. Those products that are manufactured from first principles using pharmaceutical APIs and excipients. They often comprise of products where there is a specific need for a relatively small group of patients that cannot be met by any licensed product. The overall level of usage and demand for these products may well be such that the product is not viable or attractive for a pharmaceutical company to develop and manufacture.
2. Licensed products manufactured and marketed by the pharmaceutical industry often require further manipulation prior to administration to patients. These can potentially be hazardous materials or involve a process requiring multiple or complex steps and/or calculations. In such cases it is often preferable to remove higher risk activities from clinical areas of hospitals and bring them into a unit under pharmacy control where there is a well-defined quality management system in place to manage and mitigate preparation risks.

Principles of Quality Assurance as applied to hospital pharmacy compounding

The sterile preparation of medicinal products in hospital pharmacies includes:

- The preparation of terminally sterilised products
- The aseptic preparation of products using closed procedure
- The aseptic preparation of products using open procedure

Sterile preparations represent a highly risk-sensitive class of products[3], for example due to:

- The increased rate of microbiological contamination for products prepared in uncontrolled environments
- The higher levels of microbial contaminants in uncontrolled environments
- The increased risk of systemic infection associated with products prepared in uncontrolled environments
- The increased risk of medication errors when preparing injections without pharmacy supervision

The preparation must take place in well-controlled environments using well- established, quality assurance driven procedures. This considerably reduces the risk linked with these products.

Further specific risk factors are associated with specific product types:

Cytotoxics and radiopharmaceuticals: High level of hazard to the operator preparing the product and high risk of preparation errors.

Total parenteral nutrition solutions: May be very complex depending on the formula and the number of additions. Also, there is a high risk of microbial contamination and high risk of preparation error.

Epidurals and cardioplegia solutions: High risk associated with microbial contamination.

Infusors and ambulatory devices (e.g. patient-controlled analgesia): Risk of microbial growth; some products may be administered over significant periods of time at temperatures at or near body temperature during administration; technical complexity is also a risk.

Infusions, syringes and minibags: Risk of preparation errors and microbial contamination. Some solutions may promote bacterial and/or fungal growth. Some solutions may be administered over significant lengths of time.

Irrigations (excluding ophthalmic): Duration of administration.

Eye Preparations – unpreserved or preserved: Risk of microbial growth; complexity; risk of preparation error.

In order to protect patients, all medicinal products must be of a suitable quality, safe and effective. They must be prepared so that they are fit for their intended purpose and that their quality consistently complies with the defined requirements. To reliably achieve this objective, there needs to be an effectively managed and resourced quality management system, incorporating the principles of Good Manufacturing Practices[2].

22.1.1 Quality assurance

Quality assurance may be defined as the sum total of the organised arrangements made with the object of ensuring that medicinal products are of the quality required for their intended purpose.

Quality assurance systems must be in place to ensure that:

a) Production and control operations are clearly specified and carried out according to the principles of Good Manufacturing Practice.
b) Medicinal products are only supplied if they have been correctly processed, checked and stored in accordance with the defined procedures and released by an appropriately competent pharmacist or designated releasing officer
c) Adequate measures are in place to ensure that medicinal products are prepared, stored and handled in such a way that the required quality can be assured throughout their shelf-life.
d) Documentation systems are in place and maintained[5].

22.1.2 Good Manufacturing Practice

Good Manufacturing Practice (GMP) is defined as that part of the quality assurance system which ensures that products are consistently prepared to quality standards[6].

In order to achieve this, the following basic requirements have to be met:

a) Personnel have to be qualified and trained in accordance with their function. Responsibilities and competencies have to be clearly defined.
b) Premises and equipment have to be suitable for their intended purpose.
c) All quality related processes have to be risk assessed for their suitability and described by appropriate procedures.
d) Records need to be kept to show that all the steps required were completed. The documentation should demonstrate the complete history of a medicinal product.
e) The quality of prepared products has to be assessed prior to release. The assessment should usually include:
    - A review of the preparation documentation

- A review of test results, environmental results and specifications, where appropriate
- An assessment of any deviations

f) Medicinal products are only released by appropriately qualified and competent persons.

g) Medicinal products, starting and packaging materials have to be handled and stored so that their quality is ensured throughout their shelf life.

h) The cause of quality defects should be investigated, appropriate corrective actions taken and further measures put in place in order to prevent a re-occurrence of the defects. Suitable records of this process must be kept.

22.1.3 Quality Control

Quality Control is that part of Good Manufacturing Practice concerned with sampling, specifications and testing and with the organisation, documentation and release procedures. These ensure that the necessary and relevant tests are actually carried out and that starting and packaging materials as well as intermediate and finished products are only released if their quality complies with the requirements.

22.1.4 Registration and inspection of services

Arrangements must be in place to ensure that all hospital preparation units are subject to a regular external assessment of compliance of activities with GMP and all relevant standards and are operating in such a way that the risk to patient safety from medicinal products prepared in the units is minimised.

A hierarchy of standards that such services may be assessed against can be defined:

a) International/multinational regulations and guidance
b) National regulation and guidance
c) Professional body policy and guidance
d) Healthcare system/provider standards and guidance
e) Regional level policy and guidance
f) Individual healthcare provider policy and guidance
g) Pharmacy preparation unit policy and SOPs

This must be seen as an additional requirement to that for internal audit and self inspection as described later in this chapter.

There may be several mechanisms by which this external governance function can be provided:

a) Licensing of facilities and activities by national regulatory authority
b) Inspection and registration of facilities and activities by national professional body
c) Inspection and audit of facilities by independent quality assurance function within a healthcare system
d) Inspection and audit of facilities by independent quality assurance specialist engaged by unit management to provide this function

It is possible that within one country/healthcare system more than one of the above options may be used to ensure there is an external quality assurance function provided to all pharmacy units preparing products.

22.2 Design, use and maintenance of cleanrooms, clean air devices and isolators

22.2.1 General requirements

Premises and equipment must be appropriately designed, built, used, maintained and upgraded, ensuring that they are suitable for the intended activities and minimise the risk of errors. The design

and size must allow logical workflow and appropriate segregation of activities[7]. This includes:

- The design must minimise the risk of entry of contamination from the environment and the chances of cross contamination between different products
- Adequate measures should be taken against the entry of insects and other animals (pest control)
- Cleaning activities must not be a source of contamination itself and must be demonstrated as being effective in removing and reducing contamination
- Production, storage and quality control areas should be accessible to authorised personnel only
- Production, storage (including cold storage) and quality control areas should have temperature and environmental control. Monitoring records should be kept
- Good standards of housekeeping and maintenance should be maintained

22.2.2 Production areas

Production areas should be designed in such a way as to allow adequate segregation of different products and activities. This will include:

1. Dedicated rooms or isolators/cabinets are desirable for hazardous products, e.g. cytostatics, penicillins, biologicals, radiopharmaceuticals, blood products. In exceptional cases the principle of campaign working (CHECK WITH AUTHOR) can be accepted, provided that specific precautions are taken and the necessary validation has been performed.
2. Materials and products have to be stored and handled so that the risk of mix-ups of different products or of their ingredients is minimal, that cross-contamination is avoided and that the risk of a processing step being missed or wrongly performed is reduced. In the case of the preparation of cytotoxics and radiopharmaceuticals, measures should also be taken to protect the operator from the materials being handled.
3. Sterile preparations should be carried out in clean dedicated areas that have airlocks to allow the entry of personnel, materials and equipment. Changing rooms should be designed as airlocks.
4. Location and use of sinks should be carefully considered in view of their potential to cause microbiological contamination. Sinks or hand-washing facilities should not be available inside preparation rooms or the final stage of the changing rooms. If present in adjacent areas, they must be regularly monitored and disinfected.

22.2.3 Storage areas

Storage areas should be of sufficient size to allow orderly storage and segregation of the various categories of materials and products.

Materials and products in quarantine, rejected, returned or recalled have to be stored in segregated areas and should be clearly marked as such.

The storage conditions (e.g. temperature, relative humidity) necessary to maintain the material or product quality, should be specified and monitored. Control should be adequate to maintain all parts of the relevant storage area within the specified conditions. For monitoring purposes, storage areas should be equipped with recorders or other devices that will indicate when the specified conditions have not been maintained, so that out of specification situations can be assessed and appropriate measures taken.

22.2.4 Quality Control areas

Normally, Quality Control activities should be performed in a dedicated area. If this cannot be achieved, steps must be taken to avoid errors and contamination.

22.2.5 Ancillary areas
Rest and refreshment rooms should be separate from other areas. Toilets should not be directly accessible from production or storage areas.

22.2.6 Equipment
Standard Operating Procedures (SOPs) should be written and implemented for all equipment used for processing. Where appropriate, equipment should be regularly serviced, calibrated and the accuracy of volume measuring devices checked.

22.2.7 Special requirements for preparing terminally sterilised products
(Author's note: In this section, EU GMP classifications[2] are used for reference, other relevant national standards may be applicable as appropriate.)

Preparation of components and most products should be done in at least a Grade D (ISO class 9) environment in order to reduce the risk of microbial and particulate contamination. Where there is an unusual microbiological risk to the product, for example, because the product actively supports microbial growth, or must be held for a long period before sterilisation or is not processed in closed vessels, preparation should be done in a Grade C (ISO class 8) environment.

Filling of products for terminal sterilisation should be done in at least a Grade C environment.

Where the product is at unusual risk of contamination from the environment, for example because the filling operation is slow or the containers are wide-necked or are necessarily exposed for more than a few seconds before sealing, the filling should be done in a Grade A (ISO class 5) zone with at least a Grade C background. Preparation and filling of ointments, creams, suspensions and emulsions should generally be done in a Grade C environment before terminal sterilisation.

22.2.8 Special requirements for aseptic preparation activities
Aseptic operations (open and closed procedures) should be performed in a Grade A environment in a unidirectional airflow cabinet (UDAF) or pharmaceutical isolator. The room should have a positive pressure (ideally 10-15Pa) and airflow relative to the surrounding areas of a lower Grade in order to protect the product from contamination.

Preparation under negative pressure, to protect operator and environment from contamination, should be considered for the preparation of hazardous pharmaceuticals (e.g. cytotoxic drugs, radiopharmaceuticals and radio-labelled blood products), together with appropriate precautions against contamination of the medicinal product (e.g. appropriate background room air quality, positive pressure airlock systems).

Horizontal flow UDAFs are not suitable for the preparation of hazardous drugs. Pharmaceutical isolators or biohazard safety cabinets (BSCs) should be used instead, with a vertical down-flow exhausting vertically from the cabinet and not towards the operator.

As there is no terminal sterilisation of aseptic products, the microbiological environment in which they are prepared is of the utmost importance.

22.3 Personnel and training
The establishment and maintenance of a robust quality management system and the correct preparation of medicinal products relies upon personnel. For this reason there must be sufficient and competent personnel to carry out all the tasks. Individual responsibilities have to be documented and clearly understood by the individuals. All personnel must be aware of the principles of GMP and the system for quality assurance. Personnel must receive initial and

continuing training, which must also include the necessary hygiene instructions. The following should be in place:

1. A pharmacist with overall responsibility for the quality of the prepared medicinal products and for compliance with guidelines and regulations should be nominated by management. Specific duties may be delegated to appropriately competent persons. A deputy must be nominated in the absence of this individual.
2. The organisation must have an adequate number of competent personnel, so that purchase, storage, production, control and release of pharmaceutical products are consistently guaranteed.
3. The duties and responsibilities of all personnel, including any deputies, have to be laid down in a function or job description. There should be an overall organisational chart for the unit.
4. Systems should be in place to ensure new personnel receive training in all areas that are necessary for the completion of their duties. Records of this must be kept.
5. The continuing education of existing personnel must be undertaken and suitable records kept.

22.4 Hygiene

With regard to hygiene:

1. Standard Operating Procedures (SOPs) must be available for hygienic behaviour and for appropriate clothing of personnel. Personnel should be trained accordingly. Clothing for preparation and support areas should be appropriate for the activities to be performed.
2. Personnel must notify a manager/supervisor about infectious diseases and open lesions on the exposed surface of the body. The manager/supervisor should then decide on the fitness of the relevant person to carry out activities in the area of preparation or the specific protective measures that should be taken to avoid contamination of the product. If no adequate protection is possible, these persons should not be allowed to be involved in preparation activities.
3. Eating, drinking or smoking in preparation areas must be prohibited.
4. Hands should be washed and dried with a suitable method before any preparation activity begins. They should be additionally disinfected before any preparation step is performed that represents an increased risk of microbiological contamination.
5. Cosmetics and jewellery should be removed before entering controlled areas.

22.5 Personnel

The pharmacist with overall responsibility should have relevant knowledge and current practical and theoretical experience in the manufacture of the types of product being prepared.

All preparation must be carried out by appropriately trained personnel. The competency of trained staff must be assessed and records kept. All staff undertaking preparation activities should be made fully aware of the potential consequences of any deviation from the validated procedures, both to the integrity of the product and to the patient. Regular reminders of the critical nature of the process should be provided.

All staff should receive training which will provide them with:

a) An appropriate knowledge of Good Manufacturing Practice
b) A knowledge of local practices including Health and Safety
c) Competence in the necessary preparation skills
d) A knowledge of pharmaceutical microbiology
e) A working knowledge of the department, products and services provided

Regular reassessment of the competency of each member of staff to undertake sterile manipulations should be undertaken, and revision or retraining provided where necessary.

22.5.1 Special requirements for aseptic preparation activities

Senior personnel within aseptic preparation units should have an understanding of clean area and clean air device technology together with a thorough knowledge of all the particular design features in their department, e.g. ventilation systems, position and Grade of HEPA filters, type of work station, isolator design, etc.

Personnel involved in aseptic processing, should have specific competency and skills in aseptic technique[8]. Their aseptic technique should be periodically assessed by performing media simulations.

22.6 Gowning and cleaning

22.6.1 Clothing

The clothing and its quality should be appropriate for the process and the Grade of the working area. It should be worn in such a way as to protect the product from contamination.

(Author's note: In this section EU GMP classifications are used for reference, other relevant national standards may be applicable as appropriate.)

The description of clothing required for each Grade is given below:

- *Grade D:* Hair, arms and, where relevant, beard should be covered. A general protective suit and appropriate shoes or overshoes should be worn. Appropriate measures should be taken to avoid any contamination coming from outside the clean area.
- *Grade C:* Hair, arms and, where relevant, beard and moustache should be covered. A single or two-piece trouser suit, gathered at the wrists and with high neck and appropriate shoes or overshoes should be worn. They should shed virtually no fibres or particulate matter.
- *Grade A/B:* Headgear should totally enclose hair and, where relevant, beard and moustache; it should be tucked into the neck of the suit; a face-mask should be worn to prevent the shedding of droplets. Appropriate sterilised, non-powdered rubber or plastic gloves and sterilised or disinfected footwear should be worn. Trouser-bottoms should be tucked inside the footwear and garment sleeves into the gloves. The protective clothing should shed virtually no fibres or particulate matter and retain particles shed by the body.

Outdoor clothing should not be brought into changing rooms leading to Grade B (ISO class 7) and C areas. For every worker in a Grade A/B area, clean sterile (sterilised or adequately sanitised) protective garments should be provided at each work session, or at least once a day if monitoring results justify this. Gloves should be regularly disinfected during operations. Masks and gloves should be changed at least at every working session.

It is important to visually check that gowns are in good condition and that the seams are sealed. Periodic monitoring for particles[4] and bioburden (contact plates) is required. The frequency of laundering should be appropriate to the activity undertaken and the use of biocidal washes or gamma irradiation should be used for Grade C and B areas respectively.

22.6.2 Cleaning

With regards to cleaning:

1. Clean areas must be regularly cleaned according to a validated and written approved procedure. Any staff performing cleaning duties should have received documented training including the relevant elements of GMP, and to have been assessed as competent before being allowed to work alone.

2. Dedicated equipment should be used, and stored to minimise microbiological contamination. Mopheads must be disposed of or re-sterilised after each cleaning session. Cleaning and disinfecting agents should be free from viable micro-organisms and those used in Grade A and B areas should be sterile and spore free.
3. The effectiveness of cleaning should be routinely demonstrated, by microbiological surface sampling, e.g. contact plates or swabs.
4. Periodic use of sporicidal cleaning agents should be considered to reduce contamination from spore forming micro-organisms.

22.7 Compounding of sterile products

22.7.1 Principles

Production operations have to guarantee the required quality and must be performed and supervised by competent people.

22.7.2 General requirements

1. Production must be performed by trained personnel.
1. Starting materials, intermediate and finished products should only be used for production if they have been approved for use.
1. All preparation has to be performed based on written SOPs, in which all relevant processes are laid down in detail.
1. The process steps which have been performed must be recorded.
1. Equipment and material used for all operations have to be suitable for the intended use.
1. Products and materials have to be protected against microbial and other contamination at any preparation step.
1. At all times during preparation, all products have to be identified. Labels or indications on containers and equipment have to be clear and unambiguous.
1. At all times during preparation, the operational status (e.g. cleaned, in use) of rooms and equipment must be clearly identified.

Adequate measures must be in place to minimise the risks of cross-contamination.

22.7.3 Product risk assessment

The risk potential for health damage in case of failures (e.g. quality defects) varies with different types of products and must therefore be assessed. An assessment would include:

1. The necessary precautions in terms of facilities, rooms, equipment and processes need to be taken in relation to the identified risk potential, in order to guarantee the required quality.
2. Qualification is necessary, where facilities, rooms and equipment play an essential part for encountering an increased risk potential. All preparation processes must be validated and records kept.
3. The influence of changes of qualified facilities, rooms and equipment, the influence of changes in the composition or in the quality of starting materials and the influence of changes of validated processes on quality have to be assessed by an appropriately competent person with regard to the necessity and the extent of a re-qualification or of a re-validation, before a change is made.
4. The current status of existing validations must be checked at appropriate intervals in accordance with a predetermined procedure. If a validation is no longer current – for example, time
5. Expired or due to a series of small changes, which individually would not be considered as relevant – the process must be re-validated.

22.7.4 Processing operations

Before any processing operation is started, it should be checked and documented that the work area and the equipment are clean and free from any materials not required for the current operation. In addition:

1. Intermediate products have to be stored under suitable conditions and labelled unambiguously.
2. Packaging and labelling materials that are incorrect or excess to requirement should be destroyed and this disposal recorded.

22.7.5 Labelling

Labels should comply with national legislation and normally include the following information:

a) Product name
b) Dosage form
c) Active pharmaceutical ingredient(s) and amounts(s)
d) Content (amount, e.g. grams, number of tablets etc.)
e) Batch number
f) Expiry date and, if necessary, application date
g) Manufacturer

22.7.6 Packaging operations

Containers should be clean before use. To exclude mix-ups or mislabelling, labelling should follow immediately on filling and closing. Otherwise, adequate security must be provided.

22.7.7 Rejected and returned materials and products

Rejected materials and products have to be marked as such and stored in separated areas.

The reprocessing of non-compliant products should be exceptional and has to be authorised by the pharmacist responsible for the unit. It must carried out in accordance with written operating procedures and be recorded. A risk assessment has to be performed, which includes possible consequences for quality and the expiry date of the product, as well as the requirement for additional tests.

Dispensed products, which are returned and which have left the control of the preparation establishment, should be destroyed unless without doubt their quality is satisfactory. They may exceptionally be considered for reprocessing only after they have been critically assessed by the pharmacist responsible for the unit in accordance with a written procedure. Where any doubt arises over the quality of the product, it should not be considered suitable for re-issue or re-use. Any action taken should be appropriately recorded.

22.7.8 Sterile processing

1. All stages in the sterile process should be controlled by comprehensive SOPs to ensure the output of the process is a sterile product of the requisite quality.
2. All sterilisation processes should be validated. The efficacy of any new procedure should be validated, and the validation repeated at scheduled intervals or when any significant change is made in the process or equipment.
3. The preparation of different products with different formulations, in the same workstation at the same time is not permitted. Before beginning the next activity, a line clearance must be performed. Where applicable, additional cleaning may be required during the same working session.

4. Where there is more than one workstation in a room, there should be a documented risk assessment performed and appropriate measures taken before different products are handled at the same time.

22.7.9 Preparation of terminally sterilised products

Precautions to minimise contamination should be taken during all processing stages. This would include:

1. Microbiological contamination of starting materials should be minimal.
2. Materials liable to generate fibres should be kept to a minimum in clean areas.
3. Where appropriate, measures should be taken to minimise the particulate contamination of the end product.
4. Components, containers and equipment should be handled after the final cleaning process in such a way that they are not re-contaminated.

22.7.10 Sterilisation by moist heat

For moist heat sterilisation devices like autoclaves:

a) Sterilisation records should be available for each sterilisation run. They should be approved as part of the batch release procedure.

b) For effective sterilisation, the whole of the material must be subjected to the required treatment and the process designed to ensure that this is achieved. The validity of the process should be verified at least annually and whenever significant modifications have been made to the equipment.

c) Validated loading patterns should be established. It is recommended that photographs or detailed drawings are used to ensure that loads are packed in a consistent way.

d) Temperature and pressure should be recorded during each sterilisation cycle. The independent temperature and pressure gauges on an autoclave should be monitored and logged during mid cycle and compared with the chart readings for similarity.

e) Air removal tests and leak tests on the chamber should also be frequently performed with porous load cycles.

f) Clean steam should be used where contact with critical surfaces is expected.

g) Thermal indicators should be used to indicate whether a load has been sterilised (in order to avoid a mix-up with non-sterile product).

22.7.11 Aseptic processing

Key aspects of the aseptic process that must be considered are:

1. Maintaining the integrity of the aseptic processing area, and care of the workstation and its environment.
2. Handling and preparation of starting materials, especially any disinfection processes.
3. Disinfection of materials on entry into the processing area.
4. Standard aseptic processing techniques, including `no-touch' of critical surfaces, correct positioning of materials within laminar flow, and the use of specific pieces of equipment and regular sanitisation of gloves.
5. Segregation and flow of materials to ensure no accidental cross-contamination or mix up of prescriptions or products.
6. Removal of product and waste materials from the processing area.
7. All aseptic processing must be carried out by competent staff who are formally authorised to perform their work by unit management.
8. The number of people present in the room should be kept to a minimum.

9. Only sterile materials should be taken into Grade A areas, e.g. settle plates, swabs, and cleaning materials.
10. Process validation of aseptic procedures should be performed by using broth or a similar nutrient media to simulate the aseptic procedure and should be performed initially as well as subsequently on a regular basis. The process simulation test should imitate as closely as possible routine aseptic procedures and include all the critical production steps.
11. Media fill vials should be incubated at an appropriate temperature (e.g. 30-35ºC) taking care to invert containers periodically to ensure contact with all surfaces. Any contamination should be fully investigated.
12. Any interventions or deviations occurring during the manufacturing process should be recorded on batch documents.
13. The shelf-life of any bulk solution used as an ingredient (e.g. a bag of parenteral infusion or a vial of cytotoxic agent) must be validated and containers of unpreserved products used as starting materials should not be used beyond 24 hours after first use. Any justification for re-use of these ingredients and for withdrawing volumes more than once must pay particular attention to the method of puncturing the container, withdrawing the solution and the manner in which it is stored during use.
14. Sterile disposable components such as filters, tubing, etc. must not be used beyond one working session and should be removed at the end of each session. Syringes and needles used for individually dispensed items should only be used for that single patient.
15. The transfer of materials into the Grade A workstation is usually done by disinfection or sanitisation rather than sterilisation and therefore it is important to have a written, validated SOP for this process. It is essential to validate this method by practical studies that demonstrate the effective removal of viable organisms from all surfaces. Spraying and wiping is considered more effective than only spraying to sanitise surfaces.
16. Where available, purchasing bulk gamma irradiated or sterile components in double/triple wrapped form is recommended rather than spraying many individual components into the Grade A zone.
17. The cleaning procedure should also effectively remove product residues from surfaces of the workstation.

22.8 Documentation and records

22.8.1 Principles

Good documentation systems underpin and constitute an essential part of the quality assurance system. Documents should be easily understandable and clearly written in order to prevents error from spoken communication and permit traceability with regards to all aspects of the preparation of a medicinal product

22.8.2 Principles of documentation control

1. All documentation has to be approved by authorised staff. There must be a periodic review of all authorised documents or after significant changes
2. Documentation control systems must ensure that only approved versions of documents are used. Superseded documents must be withdrawn from circulation and a copy archived.
3. Reproduction of documents must ensure approved copies are error free.
4. All written documents have to be legible, clear, unambiguous and up to date.
5. Electronic records have to be adequately protected against unauthorised changes and against data loss. The readability of electronically stored data needs to be guaranteed over the whole retention period.

6. Documentation systems in the preparation unit must ensure the complete traceability of the preparation process of a medicinal product.

Any alteration made to a document must be signed and dated. The alteration has to permit the reading of the original information. The reason for alterations has to be evident. Equivalent measures have to be applied to electronic records.

22.8.3 Types of documentation

a) **Specifications**
There should be appropriately authorised and dated specifications for starting materials, packaging materials, and finished products and where applicable, for intermediate products.

b) **Product specific instructions**
There should be processing, packaging, quality control and release instructions available to describe the composition, specifying all starting and other materials used and laying down all processing and packaging operations as well as quality control tests and release.

c) **Batch records/worksheets**
Processing, packaging and quality control documents, which record the quality relevant facts of the history of a medicinal product during preparation.
In some operations, it may be appropriate to combine the requirement of categories b and c.

d) **SOPs and additional documentation**
These consist of detailed written, approved documents describing operations to be carried out, precautions to be taken and the measures, controls and checks that are to be applied that are directly or indirectly related to the preparation and supply of the product They may give directions for the completion of certain tasks, e.g. cleaning, changing or environmental monitoring to ensure they are completed to a consistent standard. Also covered here are the associated logs and record sheets for recorded results and the documents used to record details of deviations, changes and exceptions together with follow up actions and investigations.

22.8.4 Specifications

Specifications for starting materials and, where applicable, of packaging materials should include:

a) Name (including reference to pharmacopoeia, where applicable)
b) Description
c) Procedures for sampling and testing with references
d) Qualitative and quantitative requirements with the acceptance limits
e) Where applicable, requirements concerning storage and precautions
f) Maximum period of storage before a re-test needs to take place to decide on a further release of the material

Specifications for intermediate or finished products should include:

a) Name
b) Description of dosage form and strength
c) Formula
d) Package details
e) Instruction for sampling and testing, or a reference to procedures
f) Qualitative and quantitative requirements with the acceptance limits
g) Storage conditions, microbiological requirements and any special handling precautions, where applicable
h) Shelf-life

22.8.5 Instructions

Processing instructions

Processing instructions should include:

a) Product name
b) Description of dosage form and strength
c) Batch size
d) Type and quantity of all starting materials to be used
e) Expected yield of intermediate or finished product
f) Detailed instructions for the processing steps
g) Instructions for in process controls with the acceptance limits
h) Where applicable storage conditions (also for intermediate products) and precautions

Packaging instructions

Packaging instructions should include:

a) Product name
b) Dosage form and strength
c) Package size
d) Labelling text or master label
e) List of all necessary packaging materials, including type, specification, size and quantity
f) Detailed instructions for the packaging steps
g) Instructions for in-process controls with the acceptance limits
h) Storage conditions (also for intermediate products) and precautions when applicable

22.8.6 Batch records/worksheets

Individual records should be reproduced from a suitably approved master format and approved prior to use. The worksheet should be sufficiently detailed to allow traceability of starting materials and components to establish an audit trail for the product.

Completed worksheets should be retained for a sufficient period to satisfy national legislative requirements.

Worksheets will vary for each unit and should be designed to minimise the possibility of transcription errors. For items made for individual patients they should include:

a) Name and/or formula of product
b) A unique number to identify the product
c) A method for preparation or reference to relevant SOPs
d) Name, supplier and batch number of ingredients and quantities used
e) Name and batch numbers of sterile components used to prepare the product, where used as a final container
f) Date of preparation and time
g) Expiry date and time of product, which should include the administration time beyond which the product should not be administered. Reference to the source of this data
h) Storage conditions
i) Signature or initials of the staff performing the initial checks on formulation and suitability
j) Signature or initials of staff carrying out preparation and checking procedures including any in process checks

k) A record of the label that has been put on the product and the reconciliation for all labels
l) The number or items prepared and a reconciliation of product and ingredients
m) The patients' names or identification number (where applicable)
n) A "Comments" section for recording any unusual occurrences or observations
o) Signature or initials of the authorised pharmacist who is releasing the product

In addition, for batch manufactured products the following also need to be recorded:

1. Qualitative and quantitative information of all materials used such as batch number of the material used or other references, enabling the traceability to further quality related documents (e.g. product, number of analysis, number of certificate)
2. Identification of the product (including batch number and preparation formula) and the date of preparation
3. Information on all operations and observations, such as documentation of cleaning, line clearance, weighing, yields of intermediate steps, readings and calculations, as well as sampling
4. Records on batch specific in process controls and on results obtained
5. Initials or signature of the responsible operators for significant processing steps and controls
6. Any deviations from the approved processing instruction
7. Yield of finished product

Quality control records

Quality control records have to include:

a) Product name
b) Dosage form and strength
c) Batch number
d) Manufacturer or supplier
e) Testing method; any deviations from the method must be justified
f) Test results; when applicable the certificate of analysis from manufacturer or supplier including the date of the test.
g) Retest date of starting material.
h) Date of the test.
i) Initials of the person performing the test.
j) Decision on release or rejection including the initials of the person authorising release.

22.8.7 General procedures and additional documentation

Written procedures have to be available for all processes. Critical SOPs include:

a) Receiving, sampling and releasing starting and packaging materials
b) Release and rejection of intermediate and finished products
c) Recalls of finished products
d) Calibration and qualification of equipment
e) Validation of processes
f) Cleaning, disinfecting and maintenance of equipment and facilities
g) Training of personnel

h) Operation of equipment, where applicable
i) Procedures for monitoring, including trending
j) Procedure for actions to be taken in the case of deviations and complaints.
k) Self-inspection

22.9 Starting materials, stability and storage

22.9.1 Starting materials

1. When preparing doses for administration to named patients, starting materials should ideally be licensed medicinal products[9]. If unlicensed products are to be used, there must be systems in place to ensure that these are of a suitable quality for their intended use. This may require audit, testing and manufacturer's documentation or a combination of these methods.
2. For batch manufactured products it is more common to find true active pharmaceutical ingredients used (APIs). As above, robust systems must be in place to approve both suppliers of APIs and individual batches of material that are received.
3. Starting materials used for the preparation of medicinal products should comply with the relevant specifications.
4. Starting materials should be stored in the original containers. If transferred into other containers, these have to be clean and labelled with all batch specific information. In this regard, quality has to be guaranteed during the whole period of use. Mixing of different batches is prohibited.
5. Components used in the preparation process and as final containers must also be of the required quality for their intended purpose. There should be systems in place to assess suppliers and individual batches of these as well and documentary evidence of compliance with quality standards should be available.
6. For aseptically prepared products components that have direct product contact must be sterile and supplied with documentary evidence of this.

22.9.2 Stability and shelf-life

1. Expiry periods assigned to prepared products must be derived according to an approved and documented SOP.
2. Data obtained from local testing, manufacturers or literature searches must be carefully assessed by suitably competent staff to ensure appropriateness for local conditions.
3. For aseptically prepared products in particular, the overall aim should be to minimise the time between preparation of the product and its administration so that the opportunity for any live micro-organisms in the product is limited.

22.9.3 Storage

1. All stages between product approval and product use must be assessed to ensure that the quality of the product is not compromised before its defined expiry date.
2. All storage areas for products and raw materials should be maintained at an appropriate temperature and records of this should be kept. Ideally, temperature mapping of store areas and refrigerators should be completed. Temperature conditions during transit of products and the duration of the transit time must also be considered and risk assessed.
3. For aseptically prepared products, where stability factors allow, they should be stored in a refrigerator to minimise the risk of microbial growth.[5, 8]
4. There should be defined SOPs outlining actions to be taken in the event of an excursion the storage temperature limits taking place.

22.10 Environmental monitoring and control

22.10.1 Monitoring the process

It is important that all staff, on commencing sterile preparation, assure themselves that all equipment is functioning satisfactorily. Potential problems should be reported to key personnel. Relevant records should be kept as defined in local procedures.

Environmental microbiological monitoring should be performed when the unit is in use. This may be achieved by the exposure of settle plates, performing finger dabs of gloves at the end of the work session, surface sampling with swabs or contact plates and active air sampling. Limits for different classifications of environment are defined in EU GMP standards[2] but other National standards may also be applicable. Suggested sample numbers and testing frequencies seen as best practice have been defined within some countries, for example the United Kingdom[5].

Processes must be fully validated. Revalidation must take place at regular, defined intervals.

22.10.2 Monitoring of the equipment and clean air devices

All areas associated with the sterile preparation process should be assessed by the pharmacist with responsibility for the unit for compliance with the appropriate standards:

a) On commissioning
b) Following maintenance procedures
c) Routinely at an agreed frequency

A written report of the test data indicating the significance of the results and recommended action must be brought to the attention of all relevant staff and full records kept on file for future reference.

22.10.3 Monitoring the operators

Regular reassessment of the competency of each member of staff should be undertaken, and revision or retraining provided where necessary.

A key element of operator competency is regular assessment of sterile technique using broth fills (media fills) for people performing aseptic operations. Media fills should be conducted frequently using an appropriate method that simulates as closely as possible the manipulations that are normally conducted. This should be complemented by regular observation of aseptic technique to ensure that the operator can prepare dosage units precisely and safely[8, 10].

For aseptic processing activities where the surface disinfection of materials used is a key control in reducing the entry of contamination into the critical zone, operator specific validations of the disinfection process should also be completed.

22.10.4 Environmental monitoring

Regular monitoring of the environment, process and finished product is an essential part of the quality assurance of all prepared products. Key personnel should refer to and have an understanding of these documents with particular emphasis on the sections relating to sterile processing.

Particular importance should be attached to monitoring trends and setting 'in-house' standards and action limits. In-house limits may not only reflect national GMP standards, but should be based on typical baseline contamination levels seen in a particular facility. Any trend away from this “average” baseline figure can be seen as the first stage in the loss of environmental control. This can allow investigations and corrective actions to be instigated before non-compliance with GMP standards is seen. Information should be actively and knowledgeably assessed and not merely filed for record purposes.

Each unit should have a programme of sessional, daily, weekly, monthly, quarterly and annual testing with all results documented and retained for inspection. The optimum frequency for testing will depend upon the individual unit and the activities undertaken together with relevant national legislation. The monitoring programme should confirm that the environment meets the appropriate standard. It is not a substitute for the continual vigilance of operators in ensuring the correct functioning of all equipment.

22.10.5 Microbiological monitoring

In the case of preparations for individual patients, it should be borne in mind that in the absence of end product testing, microbiological monitoring plays an extremely vital role in confirming that the product is unlikely to be contaminated. Many products are used before any microbiological results associated with its preparation, are known. The first indication that contamination has occurred in the workstation may well be a patient exhibiting pyrexia or septicaemia. Frequent monitoring and prompt reporting of results unit management should help reduce this possibility.

When undertaking microbiological testing, because of the imprecision of the methods compared to chemical and physical analysis and the expected low levels of contamination, the data requires most careful analysis. Warning levels should be established well within the overall action limits. Exceeding the warning levels on isolated occasions may not require more action than examination of control systems. However the frequency of exceeding the limit should be examined and should be low. If the frequency is high or shows an upward trend then action should be taken.

22.10.6 Physical environmental monitoring

Due to the imprecision and variability of microbiological test methods, it is also of great importance to demonstrate environmental control using physical testing data.

Defined schedules and SOPs for physical monitoring activities should be in place. Acceptance criteria must be set for all measurements.

Suitably detailed reports on physical monitoring activities must be available and out of specification results must be highlighted and investigated. Any such investigations should be fully documented.

Physical monitoring programmes should include:

a) Pressure differentials between rooms
b) Pressure differentials across HEPA filters
c) Particle counts in rooms, isolators and laminar flow cabinets
d) Room air change rates and supply volumes
e) Airflow velocities in clean air devices
f) HEPA filter leak integrity tests
g) Operator protection tests (where relevant)
h) Isolator glove leak testing
i) Isolator system leak testing
j) Isolator alarm function
k) HVAC alarm function

22.11 Product testing and approval (including sterility assurance)

22.11.1 Preparation for individual patients

1) All starting materials, components and packaging materials must be visually checked before use to ensure compliance with the required specification.

2) If starting materials are themselves licensed medicinal products, it is not usually necessary to test these before use[11].
3) If a product is prepared for a single patient, it is assumed that no end product testing will be required before release.
4) Chemical and microbiological quality control is not required for products that are prepared as specific doses for named patients provided that frequent process validation is performed using broth transfer tests and chemical and microbiological information is available to justify the allocated shelf life.
5) Microbiological analysis of these products is not necessary on each batch, although, a regular programme of microbiological analysis of the product or of process validation using broth may need to be in place[5, 8]
6) There are several ways of setting up this programme, e.g. broth simulations done on a daily basis at the end of the day or sending a sample of product each day for microbiological testing gives a useful indication that the process, operators and facility are still under control.
7) Any growth must be investigated and documented in a deviation report.
8) A formal, documented decision of the final product approval should be taken by a pharmacist authorised to do so before a product is released and after completion of all preparation, documentation and technical checking procedures.
9) Pharmacists undertaking this task should be suitably trained and independent from the actual preparation process.

There should be an SOP in place describing the approval and release process. This should include:

a) Visual inspection of the product
b) Product complies with prescription and specification if appropriate
c) Labelling details are correct
d) Products have been prepared in accordance with the relevant SOPs and any relevant quality exceptions taken into account
e) Recent monitoring results for the preparation facility
f) Relevant monitoring has been completed and the associated records produced
g) All relevant technical checks have been completed and recorded

22.11.2 Batch preparation

Quality control ensures that all requirements related to quality are met. In particular it ensures that the necessary tests are carried out and that products are only released if they comply with the quality requirements.

22.11.2.1 General requirements

Test equipment has to be suitable for its intended use and calibrated to relevant standards.

All operations have to be performed in accordance with defined procedures and documented.

22.11.2.2 Sampling

Systems must be in place to ensure that samples for analysis taken for testing are representative of the material tested.

For the testing of active pharmaceutical ingredients or excipients, a sample should be taken from each container.

There should be a planned programme of physical, chemical and microbiological analysis of the finished product. Samples may be obtained from:

a) Unused products
b) Additional samples that were specially prepared

Sampling of the final container after completion of preparation and prior to issue may be a threat to product integrity and is therefore not recommended. However, containers closed by fusion, e.g. glass or plastic ampoules, should be subject to 100% integrity testing

From final products, which are subject to an analytical control. an appropriate number of analytical reference samples should be retained for an appropriate period of time after the expiry date.

22.11.2.3 Testing
Testing should be carried out to meet the quality requirements and test schedules defined in the relevant specifications and/or relevant pharmacopoeial monographs.

All test methods should be fully validated and stability indicating and the level of end product testing for these batch prepared products for stock will depend on the associated risk which takes into account the following:

a) Scale of the operation
a) Shelf-life given to the product
a) Type of product prepared and route of administration
a) Frequency of preparation
a) Type of facility that it is prepared in

The testing laboratory must be fully conversant with the technical background and requirements of the product types being handled and have validated methods for analysing the products and samples. The pharmacist with overall responsibility for the unit should ensure that the testing laboratory has a comprehensive knowledge of pharmaceutical microbiology and the relevant analytical techniques, and that there is an effective quality management system in place which is regularly reviewed. Off-site testing facilities should be regularly audited.

22.11.2.4 Release
The pharmacist with overall responsibility for the service is responsible for the quality of the medicinal products prepared and released. The actual release can be delegated to another appropriately competent person. The person releasing the product should be independent from the preparation of the actual product.

The release consists of verification that the medicinal products comply with the valid specifications and that they have been prepared in accordance with valid procedures and with the principles of GMP.

22.12 Internal audit in the hospital pharmacy

The quality assurance system, including personnel and training, premises, equipment, documentation, production, quality control, distribution of the medicinal products, arrangements for dealing with complaints and work contracted out, has to be examined at regular intervals in order to verify their conformity with the principles of Good Manufacturing Practice.

In part, this may be completed by external regulation and inspection activities as described in section 2 of this chapter. In addition there should be a self inspection plan with records and

evidence that self inspections result in adequate corrective actions. Self-inspections should be conducted in an independent and detailed way by designated competent person(s).

22.13 Complaints and recalls

All errors, defects, complaints and other signals indicating non-compliance with quality standards should be reviewed carefully according to written SOPs. Systems must be in place to be able to recall finished products promptly and effectively when a severe quality deficiency has been identified.

Errors, defects, complaints and other signals indicating quality problems have to be investigated. In order to ensure that the reasons for occurred deficiencies are remedied, appropriate corrective and preventative measures have to be taken.

22.13.1 Recalls

When defects are potentially harmful to patients' health, a batch recall should be initiated immediately. In addition:

1) A written procedure for a recall must be in place.
2) Recalled products have to be marked as such and stored in segregated areas. It is essential that they cannot be supplied by mistake.
3) The progress of the recall process has to be recorded. A final report has to be issued, including reconciliation between the delivered and recovered quantities of the products.
4) The effectiveness of the recall SOP must be tested periodically even if no product recalls have actually occurred.

22.14 Summary

Many hospitals have compounding facilities whose function is to prepare sterile medicines. These medicines are usually, but not exclusively, administered by injection. Patients can be harmed by medicines that are incorrectly formulated, prepared or non-sterile, and pharmacists are responsible for ensuring that these criteria are met.

Medicines prepared in a compounding unit may be stored for several days before administration. As few of these medicines contain antibacterial preservatives, the consequences of low-level contamination, even with non-pathogenic organisms, can be profound.

However, these facilities provide a vital service in delivering products to patients that are ready to use or ready to administer. This removes complex and high-risk preparation activities from clinical areas and brings them under the control of the pharmacy department.

In order for pharmacy compounding facilities to provide these products to patients safely, a well-designed and effective quality management system needs to be in place.

This chapter has outlined the key elements that must be considered and the controls that must be implemented when developing a pharmacy compounding service.

22.15 References

[1] PIC/S Guide PE 009: Guide to Good Manufacturing Practice for Medicinal Products http://www.picscheme.org

[2] Eudralex Volume 4 – Medicinal Products for Human and Veterinary Use: EU Guidelines to Good Manufacturing Practice: http://ec.europa.eu/enterprise/pharmaceuticals/eudralex (accessed October 2011).

[3] ICH Guideline Q9: Quality Risk Management. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Geneva, Switzerland. http://www.ich.org

[4] EN/ISO 14644: Cleanrooms and associated controlled environments. European Committee for Standardization (CEN), Brussels, Belgium. http://www.cen.eu

[5] Beaney AM (ed). Quality Assurance of Aseptic Preparation Services, 4th Edition, London, UK; Pharmaceutical Press, 2006. http://www.pharmpress.com

[6] Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2009, MHRA. London, UK. Pharmaceutical Press. ISBN 978-0-85369-719-0

[7] Midcalf B, Phillips WM, Neiger JS, Coles T J. (eds). Pharmaceutical Isolators. London, UK Pharmaceutical Press, 2004, ISBN 0-85369-573-3

[8] Aseptic Dispensing for NHS Patients, Department of Health, January 1996

[9] Guidance to the NHS on the Licensing Requirements of the Medicines Act 1968, Medicines Control Agency, September 1992.

[10] National Guidance on the Safe Administration of Intrathecal Chemotherapy, HSC 2001/022, Department of Health, 5th November 2001.

[11] The Unlicensed Medicinal Products for Human Use TSE (Safety) Regulations 2003 [SI 2003/1580].

Building management systems for cleanroom process parameters monitoring and control

Sunil Chand Singhai, Vice President, Validations & Technology Transfer
Rajesh Thempadiyil, Manager, Validations, Dr Reddy's Laboratories Ltd, India

23.1 Introduction

Building Management System (BMS) is a computerised control system that controls and monitors mechanical and electrical equipment such as heating and ventilation, lighting, power systems, safety systems and security systems, etc. BMS mainly consists of Graphic User Interface (GUI) software, components to be controlled, controllers, hardware and instrumentations. The core function in most BMS systems is to control heating and cooling, manage the systems that distribute this air throughout the building (for example by operating fans or opening/closing dampers), and then locally control the mixture of heating and cooling to achieve the desired room temperature. In general, the three basic functions of a modern day Building Management Systems are: controlling, monitoring and optimising of the process parameters of a facility or cleanroom.

History

Building Management Systems have been employed for as long as commercial buildings have existed, whether this is through the manual loading of coal into coal-fired boilers or opening water pipe valves manually with the use of a handle to enable heated water to flow through a radiator circuit. However, the term "BMS" is a relatively new concept, being introduced in the early 1970s (the terms BAS-building automation system, and EMS-energy management system are also used); the term has only really existed since the introduction of complex electronic devices that are capable of retaining data for the purposes of managing services such as power, lighting, heating, etc. It was the advent of the "modem", or "modulator-demodulator" that allowed analogue signals to be digitised so they could be communicated over long distances with a high degree of accuracy that spurred the development and deployment of the modern BMS[13].

Benefits of BMS

The implementation of a modern day BMS brings several benefits to organisations, including, but not limited to[12]:

- Enabling automatic control of the process parameters, resulting in protection of the process and product
- Enables the user to control, record, monitor and alarm a variety of process parameters of varying risk to the attributes of the manufactured product (e.g. purity, safety, quality and efficacy)
- Facilitates effective control of building/cleanroom environmental process parameters resulting in better control over related processes and equipment.
- Centralised and/or remote monitoring of the facilities and equipment. e.g. HVAC, Chilled Water System, Hot Water System, etc.
- Ease of information availability to resolve a problem
- Computerised and predictive maintenance planning
- Early detection of problems and early warning of process deviations
- Implementation of standardised building management strategies, which will lead to effective system management and support

- Possibility of extending/increasing the control levels (centralised/localised controls)
- Effective monitoring and targeting of energy consumption resulting in optimisation of operational and utility cost

This chapter intends to present an overview of the approach to be followed for design, validation and operation of Building Management System and the corresponding regulatory requirements, with emphasis on the cleanroom-specific BMS aspects. The objectives of this chapter are as follows:

- Introduce the pharmaceutical professional to the basics of BMS technology from the cleanroom process parameter point of view
- To define an approach to evaluate the need to validate BMS
- Provides an approach and the points to be considered when performing risk assessment for design of the system and to understand BMS risk and process/product risk
- To understand the parameters to be considered for design, qualification and operation of a BMS in a pharmaceutical regulated environment

23.2 Review of existing regulatory requirements

The Building Management System is not directly mentioned in the regulations; however, compliance with several regulatory requirements on manufacturing environment monitoring can be obtained through a Building Management System. The requirements from various regulations are given below.

a) EU GMP Annex 1[2]

Point 16: Other characteristics such as temperature and relative humidity depend on the product and nature of the operations carried out. These parameters should not interfere with the defined cleanliness standard.

Point 73: Activities in clean areas and especially when aseptic operations are in progress should be kept to a minimum and movement of personnel should be controlled and methodical, to avoid excessive shedding of particles and organisms due to over-vigorous activity. The ambient temperature and humidity should not be uncomfortably high because of the nature of the garments worn.

b) EU GMP Chapter 3: Premises and Equipment[3]

Point 3.3: Lighting, temperature, humidity and ventilation should be appropriate and such that they do not adversely affect, directly or indirectly, either the medicinal products during their manufacture and storage, or the accurate functioning of equipment.

Point 3.12: Production areas should be effectively ventilated, with air control facilities (including temperature and, where necessary, humidity and filtration) appropriate both to the products handled, to the operations undertaken within them and to the external environment.

Point 3.19: Storage areas should be designed or adapted to ensure good storage conditions. In particular, they should be clean and dry and maintained within acceptable temperature limits. Where special storage conditions are required (e.g. temperature, humidity) these should be provided, checked and monitored.

c) US FDA 21 CFR Part 211[6]**, US FDA Aseptic Processing Guide**[1]

21 CFR 211.42(c) states, in part, that "Operations shall be performed within specifically defined areas of adequate size. There shall be separate or defined areas or such other control systems for the firm's operations as are necessary to prevent contamination or mix-ups during the course of the following procedures: Aseptic processing, which includes as appropriate: (i) Floors, walls, and ceilings of smooth, hard surfaces that are easily cleanable; (ii) Temperature and humidity controls;

(iii) An air supply filtered through high-efficiency particulate air filters under positive pressure, regardless of whether flow is laminar or non-laminar; (iv) A system for monitoring environmental conditions; (v) A system for cleaning and disinfecting the room and equipment to produce aseptic conditions; (vi) A system for maintaining any equipment used to control the aseptic conditions."

21 CFR 211.46(b) states that "Equipment for adequate control over air pressure, micro-organisms, dust, humidity, and temperature shall be provided when appropriate for the manufacture, processing, packing, or holding of a drug product."

The regulatory requirements of PIC are provided in **Table 1**.

Table 1: Requirements from PIC/S PI 009-3, Inspection of Utilities[4]

1.	Area of operation/ items HVAC for medicinal products	Notes	Crucial questions	Supporting documents
1.1	Key design parameters	• Need for separate systems • Level of filtration (Filter specifications) • Recirculation or makeup air • Location of filters • Position of inlet and air return dust extractors • Temperature • Humidity • Air changes • Pressure differentials • Design of ducting • Easy and effective cleaning • Alarm system • Air flow direction- LAF and/or turbulent	How do you prevent cross-contamination by air?	PIC/S GMP Guide 3.10, 3.14, 5.10, 5.11, 5.18, 5.20. Annex 1- 29-31, Annex 2- 9,10,14,15, Annex 15-9, 10 ISO 14644-4: Cleanrooms and associated controlled environments – Part 4: Design and construction. International Organisation for 2001) EN 1822: High efficiency particulate air filters (HEPA and ULPA): Part 1 – Requirements, testing, marking; Part 2 – Aerosol production, measuring equipment, particle counting statistics; Part 3 – Testing the planar filter medium; Part 4 – Testing the filter element for leaks (scan method); Part 5 – Testing the efficiency of the filter element. European Committee for Standardisation, Brussels (parts 1-3 were ratified in March 1998, parts 4-5 in August 2000). EN 779: Particle air filters for general ventilation – Requirements, testing, marking. European Committee for Standardisation, Brussels (July 1993).
1.2	Monitoring of HVAC Systems	Environmental monitoring (particles, micro-organisms, humidity and temperature).		Guide 4.15, Annex 1 4-6,

c) IPSE Baseline Guides

Various ISPE Baseline Guides also provide a number of references about BMS related issues. The following sections should be referred to:

a) ISPE Baseline Guide Volume 1: Bulk Pharmaceutical Chemicals, Section 6.8 HVAC[8]
b) ISPE Baseline Guide Volume 2: Oral Solid Dosage Forms, Section 2.4 Extent of Validation[9]
c) ISPE Baseline Guide Volume 2: Oral Solid Dosage Forms, Section 6.6 HVAC.
d) ISPE Baseline Guide Volume 3: Sterile Manufacturing Facilities, Chapter 5 HVAC[10]
e) ISPE Baseline Guide Volume 3: Sterile Manufacturing Facilities, Chapter 8 Control and Instrumentation

23.3 Review of relevant regulatory citations

A review of citations given by various regulatory agencies are reviewed and summarised in **Table 2**. It is to be noted that not all of the observations listed directly indicate observations in BMS; the process and equipment referred to are typical of those associated with BMS.

Based on this review, it can be understood that most of the citations reveal the significance of monitoring of key environmental parameters against predetermined limits such as temperature, humidity and differential pressures in the cleanrooms.

Table 2: Compilation of observations from US FDA Warning Letters, relevant to pharmaceutical cleanroom monitoring

Clause (Where stated)	Citation	Citation date
The quality control unit did not assure that adequate systems and controls were in place to monitor the functioning and to detect malfunctions of the air handling systems used to control and assure aseptic conditions in aseptic manufacturing areas.		
21 CFR 820.70(c)	Firm failed to establish and maintain procedures to adequately control environmental conditions which could reasonably be expected to have an adverse effect on product quality, as required by 21 CFR 820.70(c). Failed to establish formal parameters for temperature, humidity, and pressure. A review of Filling Room temperature records revealed several instances where the temperature range limits (on the form) were exceeded	11 August 2005
	1. Failure to establish and maintain procedures to adequately control environmental conditions, as required by 21 CFR 820.70(c). For example: d. Appropriate procedures were not followed for controlling environmental conditions. Specifically, our Investigator observed loss of power to the 'white area' during the establishment inspection. During the power outage, your firm did not monitor the partial pressure differential and/or airflow between the 'white area' and the uncontrolled areas as described in procedure. Further, your firm did not have an established procedure to control environmental conditions of the white area during power outages.	25 February 2005
21 CFR 211.46(b)	Failure to provide equipment for adequate control over air pressure, micro-organisms, dust, humidity, and temperature when appropriate for the manufacture, processing, packing or holding of a drug product. For example, the manufacturing and filling rooms are not equipped with differential pressure monitors and not equipped with temperature and humidity control monitors.	08 November 2001
	Differential pressures should be monitored continuously (not twice daily) in the aseptic fill controlled environment areas.	June 2001 April 2001
	Differential air pressures should be monitored between aseptic filling areas and surrounding support areas during dynamic aseptic filling operations.	December 2000
21 CFR 211.42(c)	Failure to establish separate or defined areas or other control systems for manufacturing and processing operations to prevent contamination or mix-ups in that data is not available to demonstrate that adequate pressure differential is maintained during filling operations.	21 October 1999
21 CFR 820.70	Appropriate procedures have not been defined for controlling environmental conditions. Specifically your devices are labelled for room temperature or refrigerated storage. Your current manufacturing and warehousing facilities do not have temperature controlling equipment and you have no assurance that devices are maintained below the labelled 86ºF.	02 February 2007
	Building Monitoring SCADA Systems used to monitor temperature, pressure; humidity and alarm conditions for environmentally classified areas, used for aseptic manufacturing should be able to produce alarm reports for critical and sub-critical areas. Alarms should be adequately documented to assure that evaluation of the impact on product quality and trending can be conducted.	March 2007
21 CFR 820.70(c)	Failure to establish and maintain procedures to adequately control environmental conditions, as required by. Specifically, temperature conditions within the aseptic processing area are not being documented to ensure such conditions are consistently within established specifications of degrees Celsius.	31 October 2006

Even though access to specific European regulatory observations is restricted, a summary of regulatory citations from the UK Medicines and Healthcare products Regulatory Agency (MHRA) was published February 2003 (GMP News 13.11.2003: Current Inspection Findings of the British Supervisory Authority MHRA (Formerly MCA))[14]. It reveals that about 30 observations are made on environmental monitoring between the years 2002 and 2003, out of which four were critical deficiencies.

The inspection findings reiterate the significance of monitoring of critical physical environmental parameters. The observations also demonstrate the importance of sterility assurance and cross-contamination risks. By reviewing the issues relating to differential pressure, containment, sterility, temperature and humidity and a BMS provides an effective control of all these critical parameters. However, the criticality of the BMS should be determined only through a risk assessment that considers the consequence on product attributes of failure of a parameter.

As with the review of regulatory citations, at least the following key inferences can be made:

- Monitoring and logging of critical environmental parameters should be done on a routine basis
- Aseptic processes should be considered as direct impact systems
- Validation of direct impact systems should be performed
- The selected monitoring method should be based on criticality of monitored parameters with respect to product attributes

23.4 Scope of BMS in pharmaceutical manufacturing and its criticality[12]

BMS may be used as a collective noun for a range of computerised systems including Programmable Logic Controllers (PLC), Supervisory Control and Data Acquisition Systems (SCADA), Distributed Control Systems (DCS), outstations/controllers and instrumentation. BMS may be deployed and managed centrally as a large network of systems that may comprise different vendor products, or as a low complexity standalone system. The type of the processes controlled and monitored and their impact on the manufactured product will define the criticality of the system. Typically, BMS system can control or monitor the processes as shown below; however, these processes vary in their risk to the product:

- Production facility Heating, Ventilation and Air Conditioning (HVAC)
- Office HVAC
- Laboratory HVAC
- Cold storage facility control and monitoring
- Fire and security alarm systems
- Energy management

The criticality of the BMS should be evaluated based on the impact assessment of the process parameters being controlled by the system on product purity, safety, quality, and efficacy. The necessity and extent of validation of a BMS system should be defined based on the criteria mentioned above.

23.5 Components of a typical BMS

Instrumentation and devices

Instrumentation and devices or controllers, collect and communicate measurements and status information to the control and monitoring system usually in the form of digital and analogue inputs. The digital or analogue information is converted wherever required and the data analysed by the control system in order to provide control actions used to refine the control of the process. The installation, calibration and tuning or routine verification of such instrumentation is critical to the process control and monitoring.

Control recipes

Control recipes enables users to modify the control parameters in order to achieve the desired characteristics of the process, e.g. temperature and humidity set points, tolerances, time spans, alarm limits, dynamics. The parameters can be modified, based on the variation in the characteristics of input utilities and major environment changes. These parameters can be changed via graphical user interfaces or local devices/controllers. The range of the control parameters should be tested during initial system implementation and are modified based on the requirements. A configuration specification should be maintained throughout the life cycle of the system, with changes in the recipe.

Adjustment of calibration parameters

Calibration adjustment parameters are established and configured while initial calibration and should be maintained during periodic calibration of instruments. The tolerance range for the calibration adjustment parameters must be specified.

Control of process

Control is typically provided by assembling standard control functions, e.g. control loops (PandID or Ratio), written logics and Start/Stop functions, into the required control scheme. The control parameters from the field devices constitute the inputs to the control scheme that establish process, characteristics, process timings, and responsiveness of the control scheme. Control and calibration adjustment parameters also contribute to the accuracy of the control logics. Feedback from instrumentation and field devices influences the control scheme that will respond, in order to maintain process parameters within configured limits.

Monitoring of process

Integrated and/or independent monitoring functions scale and check inputs against pre-configured statuses and limits, setting alarm conditions when deviations are detected.

For the monitoring and control of the system following typical tools are used:

a) Tool for monitoring the systems
b) Scheduler for scheduling the activities for energy management
c) Alarm viewing and monitoring tools
d) Alarm escalation route with workflow and signature
e) Trending tool for data analysis
f) Reporting tool and generation of the report in the required format

Data logging and trending

An integrated trend data processing system allows easy evaluation and analysis of real-time (online) data and (offline) historical data. The trend monitoring and evaluation facilitates fine-tuning of the process/systems. The data logging and trending enables the capture and recording of process events and data in order to enable a process of optimisation, investigation or monitoring. Critical data from such a system can often form part of the regulated records such as batch records.

Alarm and event reporting

One of the most important functions of a building automation and control system are the automatic alarms for faults in the building services plants. The management of alarms (generation, display and handling) must be simple, efficient and consistent at all levels of the system. In general, alarms are categorised as follows:

- Basic alarm (for alarms not requiring user interaction)

- Simple alarms (for alarms requiring acknowledgment)
- Extended alarms (alarms requiring acknowledgement and reset)

When an alarm occurs, it is automatically detected, registered and can be transferred by the local controller into the system. Informative alarm messages are also can be transmitted to remote devices such as mobile phones, fax machines, printers or computers and web browsers, via SMS and e-mail. Alarm lists provide a view of all pending and time-stamped alarms at a glance and permit proper analysis.

Alarms typically warn of pending, actual and continued deviations from process limits. Event reporting typically provides an indication that a process step or condition has been achieved (e.g. start-up complete). Alarms and events may be used to indicate the need for maintenance and/or to report process deviations. Alarms and events may be logged in addition to display and/or printing.

23.6 Design and validation of BMS

The BMS must be validated before it is used for control or monitoring in any of the manufacturing processes. The type of computerised system deployed and the scope, size, and complexity of the system will determine the level of difficulty in demonstrating that the system is fit for purpose[5,3].

The life cycle approach for any system consists of four major phases:

a) Concept
b) Project
c) Operation
d) Retirement

At the concept phase a decision is made on whether to proceed to the project phase by considering various factors such as cost of the system, benefits of the system, etc. During this phase, validation documents are not usually prepared; however, a project risk assessment can be carried out, that will reveal several facts to be considered.

The first activity during the projects phase is to prepare a User Requirement Specification (URS). Based on this specification, supplier selection and evaluation is performed where the specification may be subject to revision during the course of the project. This phase includes design, construction and commissioning and qualification steps required until released for operation. The validation documents should be prepared at each step of activities such as:

- Risk assessment[11]
- Project Validation Plan
- URS (User Requirement Specification)
- DQ (Design Qualification)
- SAT (Site Acceptance Test)
- IQ (Installation Qualification)
- OQ (Operational Qualification)
- PQ (Performance Qualification)

At each stage it is also necessary to perform risk assessment[5] for the following reasons:

1. URS: By considering the product knowledge, process knowledge, regulatory requirements and company quality requirements, risk assessment should be prepared.
2. DQ: In addition to the above risk assessment, a risk assessment at this stage will enable to build more control parameters into the equipment/system/process.

3. Further validation steps: the assessment of equipment risks classification determines the requirements for verification and at what step in the validation process the tests should be done, i.e. SAT versus commissioning versus qualification, depending on the risk category assigned.

Unlike other systems performance qualification of the BMS can be a long term activity as during this stage the system is operated as per the defined operational procedures by appropriately trained personnel. Monitoring of the system should be done during this phase for its continuous operation during actual production environment, where activities such as alarm trending and fine tuning the operational parameter limits. All the changes and revalidation activities during the operational phase should also be monitored as part of the continuous validation strategy.

A decommissioning procedure should be in place to handle the retirement of the system from operation. This procedure should have details about review of the data available, data retention, migration or destruction and management of these processes.

23.6.1 Risk assessment

During the concept and requirements phase of the project, a risk assessment should be performed to formulate a proper user requirements specification. The key to the risk assessment is understanding the critical process parameters to be monitored or controlled. Critical process parameters are those that have a high probability of affecting product attributes if they deviate from stated limits for a defined period of time. During the risk assessment, evaluation can be done on the consequence of functional failure, probability of impact, and ability to detect product impact[11,12]. When conducting the risk assessment, the following must be evaluated:

- Criticality and stage of the product to be manufactured in the area
- Product characteristics (e.g., is the product hygroscopic)
- Impact on the product attributes that effects the deviation of process parameter (e.g. temperature, humidity, airflow)
- Probability of a critical parameter deviation being detected before it could reasonably affect product attributes
- Usage of data to demonstrate the state of compliance with a registered process
- Data from the system recorded as part of the batch record, lot release, or other GMP record/documentation
- Review of the designed or existing utility capacity versus the heat load calculation for the plant
- Review of boundary of the proposed BMS system
- Probability of failure of control logic based on the criticality (e.g. control of room temperature based on the return air temperature, control of room temperature based on the hot point temperature, control of room temperature based on the average temperature from room sensors, etc.)
- Probability of cross-contamination due to failure of airflow or pressure differential
- Review of probability of the failure of field devices/controllers
- Adequacy of computerised system hardware design specification
- Requirement of procedural controls required to run the system (such as operational procedures/alarm handling procedures/IT system procedures/business continuity plan, etc.)

In order to define a strategy for qualification, a risk assessment should be conducted to determine whether or not to qualify particular aspects/components of the BMS and any associated monitoring system. It is important that each component of the overall computerised system be considered, including automated and non-automated components and that criticality is not assumed simply by

association (e.g, aspects of the process are critical therefore the equipment and automated controls must likewise be considered critical). Some examples of the relationship between product, process, equipment and BMS are illustrated below:

- Consequential impact (e.g. a raise in temperature may not directly impact product; however, the resultant heat profile may give rise to changes in humidity or particulates)
- Parameter relationships (e.g. room differential pressure may not be an accurate reflection of air change rates), hence airflow must be monitored
- Monitoring strategies (e.g. not all critical parameters may be covered by the monitoring system, such as environmental recovery rates, air change rates, airflow patterns)
- Criticality of controlled equipment (e.g. HVAC) may not infer criticality of a BMS (e.g. the HEPA filter may be critical, but is not necessarily controlled or monitored by the BMS)
- Relationship between control parameters and product (e.g. deviation from control parameter may not impact product quality within a reasonable time frame)

The validation activities shall focus on the process end points as defined in the relevant User Requirement Specifications, based on the requirements of ISO 14644, EU GMP Guidelines and FDA regulatory guidelines.

23.6.2 Design of a Building Management System

Physical architecture of a BMS

The Building Management System carries out the monitoring and control for various parameters. These are:

- Chilled water supply system
- Hot water supply system
- Heating Ventilation and Air Conditioning (HVAC) unit
- Fire dampers
- Modulation of chilled water valve to HVAC
- Modulation of hot water valve to HVAC
- Pressure or flow of chilled water to HVAC
- Pressure or flow of hot water to HVAC
- Inlet and outlet temperatures of chilled water
- Inlet and outlet temperatures of hot water
- Room temperature/relative humidity sensors
- Differential pressure sensors
- Airflow sensors
- Non-viable particle count monitoring

During normal operation, control stations carry out these respective functions. The system should allow for operation scheduling, sequencing of various systems, apart from allowing recording of the operational history for the various systems.

The components or instrumentation of BMS are shown in **Table 3** overleaf.

23.6.3 Development of the User Requirements Specification

Of the validation documents from the outset of any project, the first to be developed should be the User Requirement Specification (URS). The URS is significant irrespective of the GMP criticality of any system. For a complex system like BMS, there may be several "users" including

Table 3. Components and functions of BMS

Sr. No.	Component	Function
1	Server with database and client	To facilitate BMS information, viewing, controlling and data storage to client workstation
2	User interface	Interface between BMS and user
3	System interface	Interface between controller and BMS workstation/server
4	Controllers (programmable logic controller, distributed control system, direct digital controller)	To monitor and control the Air Handling System (AHU) operation in stand-alone mode and networkable mode
5	Room-mounted differential pressure transmitter	For measuring room differential pressure with specified range and accuracy
6	Temperature and RH sensors	For measuring either common return air duct temperature and relative humidity (RH) or room temperature and RH with specified range and accuracy
7	Smoke detectors	For measuring the smoke in return duct and supply duct
8	Differential pressure switch	To monitor filter (fine filter) dirty conditions
9	On /off type smoke damper	Isolation of AHU from conditioned area when accident happens
10	Velocity sensor with transmitter	To measure and control air supply volume hot water

engineering, system owners, data owners, Safety and Quality Assurance. The requirements from all stakeholders shall be captured in the URS. The URS must clearly define the relationships between the BMS and the processes being controlled and monitored. Preparation of the URS should be a combined responsibility of subject matter experts from cross-functional areas such as manufacturing, projects/engineering, product development, validation, quality assurance, safety, etc.

Each requirement shall be categorised to safety critical, process critical (direct impact), GMP critical (direct impact), business critical or otherwise. The categorisation of the requirements will facilitate determination of the most appropriate approach to the verification each requirement, i.e. Good Engineering Practice or qualification.

A typical User Requirement Specification for a BMS would be as follows (but not limited to):

a) The design of the environmental systems should take into account the following:
   - i Product quality and product safety requirements
   - ii Regulatory requirements
   - iii Needs and constraints imposed by equipment and processes
   - iv The contamination control concept adopted in the facility
   - v Manual vs. automated – capital and operating costs (life cycle costing) and energy conservation
   - vi Safety requirements in the facility
   - vii Health and comfort of personnel

b) Control of Temperature and humidity[15]

- i The set point and variation limits of temperature and RH should be specified in the URS for the performance of the BMS

c) Consideration should be given to control the temperature and humidity for:

- i Manufacturing processes
- ii Equipment and materials
- iii Comfort and stable conditions for personnel wearing cleanroom garments
- iv RH control for reduction of electrostatic charges;

d) Consideration should be given to control the humidity in case of external influences (such as weather changes)

e) For each room the temperature and relative humidity monitoring/control locations should be mentioned.

f) Normal outside environmental conditions should be recorded and assessed

g) Control logic required for the HVAC system may become part of the URS; an example is as follows:

- i Chilled water valve control: the air temperature and RH shall be monitored by the temperature sensor, located at a defined location and shall modulate the chilled water valve to maintain the temperature and RH to its target set point.
- ii Hot water valve control: the temperature shall be monitored by the temperature sensor and shall modulate the hot water valve to maintain the air temperature to its target set point.
- iii A constant air supply (Cubic Feet per Minute, CFM) can be achieved by modulating the variable frequency drive control based on the measurement of the velocity monitored by the velocity sensor.

h) Alarm requirement from the system shall be mentioned in the URS:

- i Temperature upper and lower alert/action limits
- ii RH upper alert/action limits
- iii Pressure differential between rooms
- iv Pressure differential across filter
- v Trip status of any of the components
- vi Supply air flow alarm
- vii Safety alarm such as fire alarms, etc.

i) Process monitoring records for at least the following:

- i Pressure differentials
- ii Air velocity
- iii Airflow volume
- iv Recycled flow rate/exhaust flow rate (wherever required)
- v Temperatures and relative humidity
- vi Particle counts (wherever required and integrated)
- vii Alarms and events recorded during the operation
- viii Audit trail

23.6.4 Qualification of Building Management System

Design Qualification:

The DQ functions to verify that the system has been designed as specified in the URS (User Requirements Specification), FDS (Functional Design Specification) and relevant equipment

specifications satisfying all GMP requirements. For hardware and software of the control system, verification data should be collected to prove that the system has been designed in accordance with the URS and FDS including the requirements of EU GMP, FDA GMP, 21 CFR Part 11, and EU Annex 11[5,3,7].

Functional Design Specification

The Design Specification (DS) should be prepared and the system objective clearly defined in the DS. The list of products to be manufactured in the manufacturing facility shall also be identified. When designing, the following drawing should be considered and included in the DS:

a) With respect to facility and room
   - i Facility layout
   - ii Facility pressure differential layout
   - iii Facility air classification layout
   - iv Facility material man movement layout
   - v Air Handling Unit scope layout
   - vi Filter cut-out layout for room (where pressure differential across the filter to be controlled)
   - vii Equipment layout
   - viii Utility system, including chilling unit, hot water unit, steam unit, etc.

b) With respect to HVAC
   - i General Arrangement (GA)/isometric drawing
   - ii Process and instrumentation diagram of HVAC
   - iii Layout with utility connection
   - iv Heat/cooling load calculations for HVAC system with resulting airflow rates/air change rates

c) The following lists/specifications should be prepared;
   - i List of instruments/components to be installed
   - ii List of interlocks required in the system
   - iii List of PLC inputs/outputs
   - iv Electrical specification
   - v Utility specifications
   - vi List of alarms required in the system along with strategy
   - vii List of parameters to be included into the pharmaceutical monitoring concept including monitoring location and number of monitoring location for each parameter
   - viii Functional specification of the automatic control system, highlighting features for ensuring GMP compliance
   - ix Calibration plans for measuring instruments.

Installation Qualification (IQ) tests

The following tests are encompassed during the IQ phase of a Building Management System (but not limited to):

1. Engineering documentation verification
2. Major component verification
3. Electrical utility verification
4. Non-electrical utility verification

5. Instrumentation verification
6. System hardware verification
7. Network communication verification (e.g. LAN)
8. System security verification
9. Input and output verification
10. System backup verification
11. Software and application programme verification
12. Configurable parameters/set points verification

During installation qualification the following should also be ensured:

- Controlling and monitoring system: statement regarding completeness, correct installation and functionality
- Change control logbook and change control forms correctly filled in, complete and up-to-date till IQ
- Changes requiring qualification correctly executed and qualified up to IQ status
- Training and corresponding certificates complete for the personnel involved in commissioning and qualification activities
- IQ report, prepared after successful termination of IQ, with confirmation that all errors and omissions identified during the IQ stage have been rectified

Operational Qualification (OQ) tests

The Operational Qualification (OQ) is intended to verify in a formal documentation process that the installed system operates according to the design specification and manufacturer's requirements. The following tests should be performed to verify the system operation (but not limited to):

1. Control loop testing
2. System interlocks testing
3. Alarms testing
4. Power loss and loss of communication testing
5. Radio Frequency Interference (RFI) testing
6. Electro-Magnetic Interference (EMI) testing
7. Operational interface (Graphical User Interface) testing
8. Verification of reports
9. Backup verification and restoration verification
10. Verification of compliance with EU Annex 11 and 21 CFR Part 11

Verification of compliance with EU Annex 11 and 21 CFR Part 11

The installed system along with its software shall be verified for compliance with EU Annex 11 and 21 CFR Part 11[3,7] for computerised system compliance. Compliance can be verified using detailed checklists. The following requirements shall be verified wherever applicable:

- Electronic signature and records
- An audit trail at the database level for GxP critical data like log, trend, alarms/events, etc.
- Backup strategy based on the criticality of the data
- It is to be verified that the user actions must be justified with a reason or comment
- Data integrity shall be verified with suitable means
- User administration and access protection

- Reporting requirements such as the generation of an "Out Of Specification Report". The routine report may have only the deviations from the specified limits and critical alarms.
- Calculations ranging from summation, averaging to calculating the Mean Kinetic Temperature (MKT)
- Business continuity plan
- Disaster recovery plan

Upon completion of the Operational Qualification, the following should be prepared:

a) Test certificate confirming the correct functioning of the control system and the pharmaceutical monitoring system, including alarm and safety functions.

b) Change control logbook and change control forms correctly filled in, complete and up-to-date up to OQ status. Changes requiring qualification correctly executed and qualified up to OQ status.

c) OQ report stating that OQ of the isolator system is complete, with confirmation that all errors and omissions identified during OQ have been rectified.

23.6.5 Approach for handling of alarms

BMS alarms should be carefully designed and appropriately configured in the system. There should be a segregation strategy for handling alarms based on the alarm type such as GMP alarm, operational alarm, etc. BMS alarms are used to provide information for a variety of purposes including[12]:

a) Notification of failures or status events in the facility/system.

b) Pending and actual equipment or control system failures.

c) Upper/lower control limit excursions.

d) Failure of controlled conditions.

It is important to understand the purpose of all alarms in order that processing and response to such alarms is commensurate with the information being conveyed, For example, an alarm indicating upper/lower control limit excursions of critical process parameter will warrant a different response to an alarm-notifying engineer of the need for equipment maintenance.

The approach for handling alarms should consider:

a) Purpose and criticality of each alarm.

b) Priority and response plan of each alarm derived from the purpose.

c) Escalation matrix of the alarm based on priority and criticality.

d) Segregation of alarms to GMP critical vs. non-critical, high priority versus low priority.

e) Segregation alarms requiring acknowledgement and or reset.

f) Requirements for retention of alarm history.

g) Routine reporting requirement of alarms.

h) Delay time to be set for each alarm.

The priority and criticality of alarms also shall determine who responds to alarms and its escalation workflow for review of the same. For example, where alarms indicate possible product attribute issues, an incident or deviation may need to be raised or it may be necessary to instigate an investigation by production and Quality Assurance departments. Investigation of such alarms is enhanced when product attributes related to alarms are clearly differentiated from other alarms such as operational and maintenance alarms.

While defining the control limits for alarms (alert and action) several factors shall be considered:

a) Exposure of product in the area, if the product is handled in the closed condition such as isolators/negative pressure systems/in closed contained systems. The alarms limits for pressure differential shall be defined by reviewing the above criteria as its primary objective is to reduce contamination to the personnel and to the environment.
b) Scenarios such as the isolators and negative pressure systems may have their own control/alarm system for control of pressure differential.
c) Pressure differential alarms, may depend upon the activities performed such as door opening for a long period (process may demand), practice of the personnel, etc.
d) When the pressure differential is only a monitoring parameter and not a controlled parameter. In such cases, manual control may require some time to take responsive actions.
e) Alarms for the process parameters where the HVAC system caters for areas such as air locks (temperature and RH limit as material is not handled in such rooms), area where water (RH limit) is handled and areas where material is not handled.
f) Delay time settings to record as an alarm where automatic controls/responsive actions are in place, such as alarms for temperature or for Relative Humidity i.e. 'Alert' and 'High'. The response action is based on the Response time of modulating the valves for chilled water and hot water, and to open and close for maintaining the temperature conditions and automatically taken care by the system. Hence by design the response system in built into the system. In this case, delay time may be increased for such excursions.

23.6.6 Performance Qualification

Performance Qualification (PQ) of a Building Management System should be a prolonged phase as it requires stabilisation of the whole system such as:

a) Utility generation and supply system
b) Functioning of HVAC
c) Operations and its impact in the area
d) Man/material movement and its impact in the area
e) Finalisation operational procedures,
f) System controls and functions

The duration for PQ Monitoring shall be decided based on the complexity involved in the system, operations and component status. During monitoring, the system generated data shall be evaluated and fine-tuned based on assessing the impact. The following steps shall be followed for PQ activity:

a) Preparation of the SOP for operation of the system and verification of the SOP.
b) Monitoring of the system for sufficient duration.
c) Review and analysis of the results obtained for this duration versus control requirements for the process parameter.
d) Consideration for resetting of set parameters and alarm parameters (set points, delay time, etc.).
e) Monitoring for some more duration after changing the parameters to check the system behaviour and repeat the steps, if considered necessary.
f) Assessment of criticality of alarms generated and preparation of procedure for handling of alarms.
g) Release the system for routine operation once the system observed to be functioning as intended,

h) Environmental changes/seasonal variation can also be considered during performance qualification of the system.

23.7 Operation and maintenance

As in every GMP system, the operation should start with training the staff at each section of the organisation. It is important that the Building Management System controls and requirements are understood by the staff operating and monitoring it. It is important for the operational staff to actively participate throughout the qualification activities, so that routine operation and trouble-shooting becomes much easier.

During the process, SOPs should be developed and various activities such as, operation and maintenance of the BMS. The process parameters, which should be part of Batch Record or verified during routine manufacturing operations, should be finalised. In addition to routine functions, the actions to be taken in the event of any failure should also be described in the SOPs, such as the SOP for handling alarms.

The following tests and programs should be considered in the operation and routine maintenance activity:

- Operational procedure for BMS
- Roles and responsibilities for the personnel involved in monitoring, taking field responsive actions, supervising, and production and QA functions.
- Procedure to handle alarms
- Procedure for examination of control systems
- Calibration procedures and frequency of calibration adjustment procedures
- Procedure for back-up and data storage of the system
- Procedure for administration of the system
- Procedure for accessing control of the system
- Procedure for configuration management/set value change management
- Procedure to manage and document external support activities and for handling remote sessions
- Business continuity plan and disaster recovery system with risk assessment for acceptable down time and recovery rates

23.8 Summary

In writing this chapter, the authors' intention was to address the specific peculiarities of pharmaceutical Building Management Systems. The specific aspects in relation to a BMS can be summarised as:

- A risk assessment needs to be performed in order to decide the extent of validation and qualification of the BMS along with the appropriate quality management controls.
- The risk assessment is essential for determining the criticality of the BMS and any associated controlling and monitoring systems.
- For the design of the BMS, and to generate a User Requirement Specification, a risk assessment needs to be conducted that must focus on those process parameters controlled by the BMS and understand how these will impact upon product attributes.
- Major components and control system functions, with examples, are discussed in this chapter.
- Critical monitoring/controlling parameters for pharmaceutical cleanrooms and the facility monitoring and safety requirements must be delineated.

- Consideration must be given to validation and qualification of potentially high criticality aspects of the BMS controls (e.g., aseptic environmental controls).
- The alarms should be categorised based on criticality and priority. A practical approach towards handling and configuring the alarms should be adopted.

23.9 References

[1] Guidance for Industry: Sterile drug products produced by aseptic processing – current Good Manufacturing Practice. US Department of Health and Human Services, Food and Drug Administration, Bethesda, MD, USA, September 2004.

[2] EudraLex, the rules governing medicinal products in the European Union – Vol. 4: EU guidelines to Good Manufacturing Practice for medicinal products for human and veterinary use. Annex 1: Manufacture of sterile medicinal products. European Commission, Brussels, Belgium (frequently updated).

[3] EudraLex, the rules governing medicinal products in the European Union – Vol. 4: EU guidelines to Good Manufacturing Practice for medicinal products for human and veterinary use, European Commission, Brussels, Belgium.

[4] PIC/S PI 009-3: Aide memoire – Inspection of utilities. Pharmaceutical Inspection Co-operation Scheme PIC/S, Pharmaceutical Inspection Convention. PIC, Geneva, Switzerland, 25 September 2007.

[5] GAMP 5: A risk-based approach to compliant GxP computerized systems. ISPE (in collaboration with FDA and other regulatory authorities), Tampa, FL, USA, 2008.

[6] 21 CFR 211: Current Good Manufacturing Practice for finished pharmaceuticals. Code of Federal Regulations, Food and Drugs Administration. US Government Printing Office, Washington DC, USA.

[7] 21 CFR 11: Guidance for industry – Electronic records; electronic signatures – Scope and application. US Department of Health and Human Services, Food and Drugs Administration, Rockville MD, USA, April 2007.

[8] ISPE Baseline Guide Volume 1: Bulk Pharmaceutical Chemicals, Section 6.8 HVAC. IPSE, Tampa, FL, USA.

[9] ISPE Baseline Guide Volume 2: Oral Solid Dosage Forms, Section 2.4 Extent of Validation. IPSE, Tampa FL, USA.

[10] ISPE Baseline Guide Volume 3: Sterile Product Manufacturing Facilities; Second Edition. IPSE, Tampa, FL, USA, September 2011.

[11] ICH Q9: Quality risk management. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH, Geneva, Switzerland, January 2006.

[12] Position Paper: Use of Building Management Systems and Environmental Monitoring Systems in Regulated environments. ISPE GAMP Forum, Special Interest Group, Vol. 25(5), IPSE, Tampa, FL, USA, September/October 2005.

[13] GMP News 13.11.2003: Current Inspection Findings of the British Supervisory Authority MHRA (Formerly MCA).

[14] ISPE Good Practice Guide: Heating, Ventilation, and Air Conditioning (HVAC). IPSE, Tampa FL, USA.

Energy management and sustainable cleanrooms

Nigel Lenegan, Energy & Carbon Reduction Solutions Ltd, UK
Ulla Thomsen, Novo Nordisk A/S, Denmark

24.1 Introduction

From their inception over fifty years ago, cleanrooms have provided safe, compliant and effective manufacturing environments across many industrial sectors, without due consideration to their operating energy costs or associated carbon emissions. However in the new age of energy conservation, with both internal and external pressure on running costs and carbon emissions, it has been shown that cleanrooms are heavy energy users and hence very costly to run. Of this cost, the HVAC (heating, ventilation and air conditioning) is the major drain on energy, yet over the years very little has changed regarding HVAC design principles or operating strategies, whereas significant improvements in gowning, operator competence, cleaning systems and procedures have enabled many cleanrooms to operate well beyond their required levels of cleanliness and wasting valuable energy.

There is a growing belief that pharmaceutical cleanroom HVAC in particular over-performance, is wasteful and can, using a risk-based and scientific approach, be optimised to reduce energy use, whilst maintaining critical product, process and people controls and ensure both compliance and product quality are maintained.

The aim of this chapter is to identify and develop these ideas and promote HVAC optimisation. In doing so, the authors aim to challenge the traditional mindset on cleanroom HVAC design and operation and to promote a reduction in energy use whilst maintaining compliance and product quality

24.2 HVAC in the pharmaceutical industry

HVAC is one of the means of establishing and maintaining a classified cleanroom environment by minimising the residual contamination from the main sources such as people and raw materials. As people are the predominant source of viable and non-viable contamination, physical barriers surrounding the critical areas are provided to prevent or minimise uncontrolled access. Good quality gowning is another key control measure to contain personnel emission alongside good training of occupants on how to act and function, including deliberate and slow movement within the cleanroom. Regular and robust cleaning strategies ensure that particulates settled via sedimentation (viable & non-viable) are minimised. Thus, the actual emissions from people and the risk to product is minimised and hence the HVAC provides additional protection to ensure cleanliness levels are achieved.

Cleanroom clothing, cleaning and disinfection of facility and material, aseptic techniques, etc. are therefore critical in ensuring that the required cleanliness levels in the pharmaceutical production facility are provided, together with product quality. Because of traditionally high airflows, HVAC is usually the most energy costly part of running a pharmaceutical facility with cleanrooms to ensure that “operational” EU GMP Grades A (ISO 5), B (ISO 7), C (ISO 8) and D (ISO 9 or higher) are achieved. As a result, HVAC naturally takes centre stage when there is an effort to reduce the energy consumption in pharmaceutical production. Theoretically therefore, the higher the cleanliness grades in production, the higher the airflow and hence the higher the HVAC energy consumption per m^2 production space, although this is very much dependent on the idiosyncrasies of the product, process and people critical requirements.

Moving from the cleanroom to other parts of the production facility, such as packaging, laboratories and offices, the HVAC part of the total energy consumption is smaller but still significant. For most facilities, including cleanroom production, HVAC will take up more than 50% – 75% of the overall energy consumption of the plant.

The total HVAC energy consumption is comprised of:

1. Electricity to run the fans – governed by flow volume, system pressure, fan efficiency and full load operational hours
2. Use of hot water or other means to heat the air – governed by the volumes of fresh air used, HVAC design, temperature requirements in the cleanrooms, local climate and insulation of the building
3. Use of cooling media to reduce the temperature of the air and usually also to dehumidify the air (but this can be done by other means) – governed by the volumes of fresh air used, the temperature and humidity requirements in the cleanroom, the fan electrical energy consumption, design and the insulation of the building
4. Means of humidifying the air, usually steam or clean steam – governed by the volumes of fresh air used and the degree of humidification needed in the cleanrooms
5. Use of staged filtration up to HEPA efficiency grades increases the resistance of the system, which fan energy is required to overcome.

HVAC provides and maintains the required cleanliness levels by:

- supply of sufficient filtered air to protect the product from contaminants (displacement/UDAF) and to dilute the residual contamination emissions to maintain required levels of non-viable and viable particulate (dilution/turbulence)
- Supply of filtered air to maintain an overpressure/cascade in cleaner areas towards less clean areas, or other airflow protection strategies such as bubble or sink airlocks.

Further to this, the HVAC system also takes care of the climatic conditions in the cleanroom – ie. temperature and humidity – to ensure product quality, ease of process and personnel comfort.

24.3 Primary sources of contamination

In general, the primary sources of contamination within pharmaceutical manufacturing cleanrooms are from people who by and large account for the majority of airborne viable and non-viable particulate emissions. Other sources include raw materials and consumables, machinery and moving parts and the ventilation system due to faulty filtration. According to CK Moorthy in his book "Principles and practices of contamination control and cleanrooms"[1], a large number of reports of contamination in well-designed and well-run cleanrooms revealed that 5% of cases were due to contaminated raw materials; 10% through utilities and defective or soiled equipment, tools and implements; 5% due to faulty air filtration; and 80% due to breaches in the product – person interface.

Therefore, contrary to popular belief, more focus appears to be required on people, raw materials, utilities and equipment, not just ventilation systems. Key contamination control measures must include reliable and effective gowning, competent operatives, effective cleaning and sterilisation procedures and raw materials and equipment controls. With these in place, ventilation would become a secondary control measure, not the only control measure.

24.3.1 Viable (microbial) particulate contamination

Microbial contamination is of greatest concern within aseptic manufacture, although even in non-sterile applications control is vital. In a well designed facility with effective HEPA filtration, people are the primary if not the only source of microbial contamination. For many years, isolating people from the product has been a stalwart for aseptic manufacturing, using RABS (restricted access

barrier systems), and isolators where the product itself required containment. This physical barrier, supported by unidirectional airflow (UDAF), helps to ensure that no airborne contamination can reach the product under normal operating circumstances.

As micro-organisms cannot exist without some form of carrier and food source, viable particles tend to be of a size and of sufficient mass to cause them to settle out of the airstream relatively quickly. These are known as Newtonian particles and are in most cases. due to their mass, unaffected by usual turbulent ventilation processes. Studies from operating theatre microbial contamination control and ventilation design[2] suggests that; *sedimentation is unaffected by the ventilation process, therefore increases in the amount of ventilation produce a less-than-proportionate increase in the rate of clearance. To take a simple example, if the dispersed particles have a sedimentation rate of 1 foot per minute – a common average value for naturally dispersed airborne particles carrying bacteria, the time for the initial numbers to fall to 1/10, will be 23 minutes within an unventilated room. If six air changes per hour (ac/hr) of ventilation are provided, this reduces to 12 minutes, and at 20ac/hr this is reduced to 5 minutes.*

Aside from physical barriers, the only way to reliably control and influence Newtonian particles is by using unidirectional airflow with velocities of between 50-100ft/min, ubiquitous in aseptic manufacture.

24.3.2 The usefulness of ventilation as a contamination control measure

It is written in many books and papers that clean airflow into cleanrooms is proportional to cleanliness and particulate control. This is true in relation to Stokes-Cunningham particles, those belonging to the drag regime and not Newtonian, subject to sedimentation. The only truly reliable method of preventing contamination of product is by physical isolation of the product from the people or by removing the people from the process by automation.

Where automation or barrier solutions are inappropriate, those larger particles must in the first stage of contamination control be prevented from becoming airborne, using effective gowning, masks, gloves and restricted and deliberate movement (people controls). These people controls should also prevent smaller particulate release, without being too restrictive and uncomfortable. The next step is to ensure cleaning materials, regimes and resources are robust. Once these primary measures are in place, alongside raw materials and equipment aspects, the role of the ventilation system and HEPA filtrations becomes somewhat secondary regarding dilution of particulates, which has already been minimised. The room pressure regime then becomes the priority for ventilation and to ensure that temperature and relative humidity are controlled.

24.3.3 Attitudes to cleanliness

Historically and in many cases currently, the attitude to cleanliness in a pharmaceutical manufacturing environment was “the cleaner the better” which resulted in facilities being design, validated and run to provide levels of cleanliness many orders of magnitude greater than the required classification, as well as associated alert and alarm level requirements for compliance and product quality.

However, these facilities were designed and validated in the days when energy use, cost and associated carbon emissions were not of widespread concern. As times, attitudes, pipeline, profitability and corporate social responsibilities have changed, the existing attitudes to cleanliness must be reconsidered. However, due to quality assurance controls and regulatory persistence, the paperwork and risk assessments necessary to verify and approve any changes are laborious, time consuming and potentially costly which for those unwilling to adopt this change see as justification for the status quo. It is also true that facility use has changed without due consideration of the supporting infrastructure such that many less strict operations are being undertaken within an environment suitable for much cleaner demands.

Hence over the past 50-60 years, gowning, cleaning, operator competence and product risk and susceptibility to contamination has developed, whereas HVAC has remained rooted in traditional belt-and-braces energy intensive solutions.

There is, however, a win-win scenario, where energy cost and emissions can be reduced without conflicting with existing quality assurance and control values. This is because the systems are so much oversized; optimisation and energy reduction can be achieved without compromising minimum defined requirements and within existing internal and regulatory constraints to deliver significant energy and carbon reductions whilst maintaining compliance. Of course, further savings are only possible by de-classification or by breaching minimum defined levels of airflow to maintain original classification demands.

24.4 Classification and HVAC techniques

The higher the cleanroom grades, the higher the HVAC energy consumption because the air changes increase with increasing demands. A typical European pharmaceutical facility will have 10 – 20 air changes in Grade D, 20 – 30 air changes in grade C and 30 – 40 air changes in grade B. In Grades C and B (and often D, but it is not a requirement) the air is delivered into the cleanroom through individual terminal filter housings supplying the air in a way so it is constantly mixed within the air already in the cleanroom. This method of ventilation is called “diffuse”, “mixing” or “turbulent” airflow. Although a key metric known as “ventilation effectiveness” is an important consideration for turbulently ventilated cleanrooms, some terminal HEPA devices have perforated plate diffusers which results in columns of poorly mixed clean air, and dead-spots in between these columns.

Moving into Grade A, the air change rate goes up to several hundred an hour and the air supplied is no longer mixing with the air in the room (as **Figure 1** opposite illustrates). In Grade A the air is delivered by a fully filter covered ceiling or wall and the air acts as a ‘piston’ pushing out contaminants lingering in the air. This ventilation method is called “unidirectional” or “laminar” airflow. The term ‘laminar’ is not a scientifically correct terminology for the actual flow, but it sounds good and it is often used – still – but only a very small proportion of the flow in Grade A is technically “laminar”. Again, this is subject to a 1:3 rule in terms of sidewall extract, which if too wide or far away from the UDAF, the airflow can shear to create conical shaped internal dead-spots.

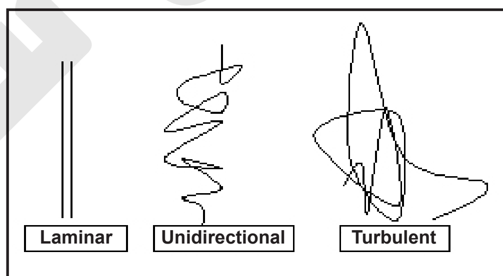


Figure 1: *Commonly used terms in relation to flow. Please note that “laminar” does not necessarily describe flow in straight lines. Laminar flow around a ball forms curves.*

24.5 HVAC compliance demands in the pharmaceutical industry

There are many slightly different GMP guidelines. Some countries have their own, others refer to WHO, FDA or EU. It is important to understand that while, for example, an FDA guideline is not prescriptive, a company needs to have a good reason for not following the recommendations in this guide if sale to the to the US market is in question, and some GMPs are more prescriptive.

From an industry perspective, different types of companies will have different interests in how GMPs should develop. Smaller companies will tend to require precise instructions for operating pharmaceutical facilities while larger companies that have the intellectual muscle to develop good practices themselves will be in favour of less prescriptive GMPs leaving room balancing quality/productivity/energy consumption, as they see fit. From an energy consumption point of

view, it should be obvious that if there are adequate resources, the optimal balance can be achieved by detailed analysis of the specific process instead of blindly following general rules.

Some of the most cited GMPs are the US FDA "Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice, Sept 2004"[3] and the "EU Guidelines to Good Manufacturing Practice: Medicinal Products for Human and Veterinary Use: Annex 1, Manufacture of Sterile Medicinal Products, 2009"[4]. In these, the requirements for room cleanliness for different kinds of pharmaceutical operations are spelled out and guidelines on how to achieve the cleanliness are given. Below, in **Table 1**, a brief overview of the requirements affecting the energy consumption as treated in the FDA and EU GMP guidance documents is given.

Strict adherence to specific rules does not guarantee either optimal air quality or optimal energy use. An example is blindly following the 20 air changes per hour for Grade C. In some instances with a lot of personnel activity – for example, for a changing room during change of clothes – 20ac/h is not

Table 1: The impact of HVAC energy requirements in relation to pharmaceutical GMPs

Impact of HVAC requirements on energy consumption in relation to FDA and EU GMPs			
Aspect	FDA	EU GMP	Notes
Air cleanliness Grade A – D	Max limits for particle and viable contamination + For Grade C (ISO 8 – in operation) supporting rooms, airflow sufficient to achieve at least 20 air changes per hour is typically acceptable. Significantly higher air change rates are normally needed for Grade A & B	Max limits for particle and viable contamination + maximum clean-up period of 15-20 minutes (recovery)	(a)
Air supply functionality specific grade A	HEPA-filtered air should be supplied in critical areas at a velocity sufficient to sweep particles away from the filling/closing area and maintain uni-directional airflow during operations	Laminar air flow systems should provide a homogeneous air speed in a range of 0.36 – 0.54m/s (guidance value) + "It should be demonstrated that should be demonstrated that air-flow patterns do not present a contamination risk"	(b) (c)
Room pressure	A positive pressure differential of at least 10-15 Pascals (Pa) should be maintained between adjacent rooms of differing classification (with doors closed)	Adjacent rooms of different grades should have a pressure differential of 10-15 Pascals	(d)

Comments

(a) The FDAs specific per hour requirement for air changes is "old fashioned". In terms of quality the importance is ventilation effectiveness, number of personnel and how they act and are gowned. Grade C conditions can be achieved with smaller air change rates in a large room with limited activity whereas 20/h is not sufficient in a smaller room with lots of personnel activity. EU GMP is more to the point because the aspect of ventilation efficiency is captured by the concept of 'recovery', however often the industry validate recovery going from the operational classification to the at-rest classification. If a room is never contaminated anywhere near the operational classification level during operation, this approach will call for larger quantities of air than needed to maintain the required air cleanliness.
However, EU GMP recovery calculations are often misunderstood, as the guidance states, "operational to at-rest"; this is not necessarily class limit values, but actual values upon completion of operations, which is often significantly less than the operational class limit.

(b) The focus is gradually shifting from the arbitrary 0.36 – 0.54m/s (originally 90 feet per minute ±20%) towards validating the actual performance through smoke studies which capture interaction of the HVAC system, the process equipment and the operations. FDA guidelines are most outspoken on this point.

(c) EU GMP actually suggests 0.36 to 0.54m/s "at the working position". Pharmaceutical companies usually interpret this to be 0.36 to 0.54 m/s 100 – 300 mm below the filter or diffuser where the ISO 14644 - which EU GMP refers to for validation testing methods – says it should be measured. Going by the exact wording ("at work height") would drive the air-change and hence energy consumption up dramatically. The filters used in common grade A supply air devices are not capable of such high airflows.

(d) Magnitude of room pressure is not interesting in itself. It is the presence or absence of adverse airflows which matter from a quality standpoint[5].

sufficient to clean the air whereas this number of air changes will very often result in air quality much higher than specified for grade C in rooms with medium or low level of activity. Heat gains must also be considered if product, process or people require critical temperature control.

24.6 Demand-side energy use

24.6.1 Metering and sub-metering

In order to understand where and how much energy is being used, good metering and sub-metering is a fundamental energy demand management tool. Most buildings have meters on the energy supplies for billing purposes but these do not inform where the energy is being used or identify areas for energy reduction. Nor do they identify areas where the building is performing badly.

Effective sub-metering provides the means to identify and respond to these challenges and quantify and focus on energy reduction opportunities.

1. Benefits of effective sub-metering are:
   - Establishes the breakdown of energy use within a building
   - Provides a better perspective on building operation
   - Identifies where energy use is greatest
   - Identifies what the minor loads are
   - Reveals usage patterns and trends – week-day/summer, week-day/winter, weekends, etc.
   - Enables benchmarking – kWhr/m^2/year, etc.
   - Enables carbon emissions to be assessed for specific energy users
   - Facilitates compliance with legislation, e.g. European (EPBD) Energy Performance of Buildings Directive/Display Energy Certification/Part L2B (2006)
   - Sustains improvements
   - The ability to provide:
     - Activity-based costing
     - Determine load imbalance
     - Monitor and record voltage levels to identify tap switching faults
     - Identify power quality issues – harmonics, etc.
2. Elements to be metered/sub-metered

   It is broadly accepted that as good practice, a reasonable metering provision at building level would be as follows:

   Meters at building level
   - Electricity meter
   - Gas/oil/fossil fuel meter,
   - Heat and cool meters (kW/BTU) on heating and cooling source
   - Water – see separate guidance

   Sub-meters within the building (heat kW or BTU and power kW)
   - Different area types within a common facility:
     - Production/offices/labs/catering
   - Lighting – floor x floor
   - Small power floor x floor
   - IT systems and server rooms
   - Central domestic hot water storage or local point of use
   - Energy consumed by HVAC plant (refer to Chartered Institution of Building Services Engineers (CISBE) document Technical Memoranda number 39 "Building Energy Metering" – Table 1, for example)[6]:
     - Air handling units, pumps, extract fans, cooling towers and chillers

- Fume and process extract systems
- Large process equipment/systems:
  - Compressed air, vacuum and nitrogen generation
  - Purified Water and Water-for-Injections
  - Steam and condensate

The broad objective of sub-metering is to ensure clear energy paths are understood which will enable focus prioritisation for improvement.

24.6.2 How HVAC design has an impact on energy consumption

There is a multitude of different designs for cleanroom HVAC. Here we will look into the energy consumption of three to four different HVAC setups and explore the energy consumption relating to each system in a given climate.

Some of the dividing lines between the different set-ups are:

1. Is the room ventilated with "full fresh air", also called "once through" or is recirculation of air applied?
2. Do air handling units serve many rooms or are they dedicated to single rooms/smaller areas?
3. Is all air treated in AHUs with the full air treatment package – filtration, heating, cooling and (de)humidification?
4. For recirculating systems: is the necessary fresh air pre-treated before entering the recirculating system or is all air conditioning taking place in the recirculating units?
5. For recirculating systems with pre-treated fresh air: is the pre-conditioning unit active with its own fan or passive using the fan power of the recirculating system?
6. Is it a "single line system" or is air conditioning handled in a sub-system with a secondary fan?
7. Does the facility have to run a full load 100% of the time, or can the flows be reduced out of hours?

To demonstrate the design impact on energy consumption, three different systems are compared below (**Figure 2**). While the fan electrical energy consumption will be the same in whichever climate the facility is located, the cost of electricity for cooling, the cost of heating and humidification will vary very much with the location. The three different systems are here compared in a north-eastern Chinese location and calculations are based on real climatic measurements for 8,760 hours per year for three years.

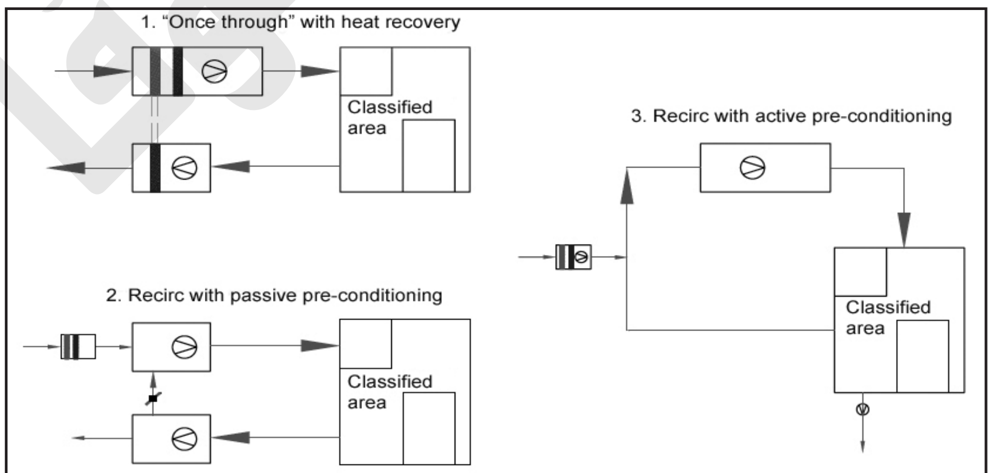


Figure 2: *Comparison of three different systems in terms of energy consumption.*

24.6.3 System descriptions

1. A "once through" system with 'run around' heat and/or cooling recovery.
2. A recirculation system where the main recirculation fans both treat the recirculation flow, the intake of fresh air and the exhaust to the outside. In order to handle the relatively small fresh air and exhaust volumes the main recirculation flow is "taxed" with an extra pressure drop ~450Pa.
3. A recirculation system with 'active preconditioning' and dedicated exhaust ensuring that the recirculation flow has no unnecessary pressure drops.

It is obvious that the "once through system" is much more costly to operate than the two systems recirculating the air in the cleanrooms, even when heat recovery is used.

Detailed comparison of the two recirculation systems shows that:

- the use of active precondition is less costly in terms of energy
- that the active preconditioning systems has a lower relative share of electrical energy in comparison with the passive preconditioning system.

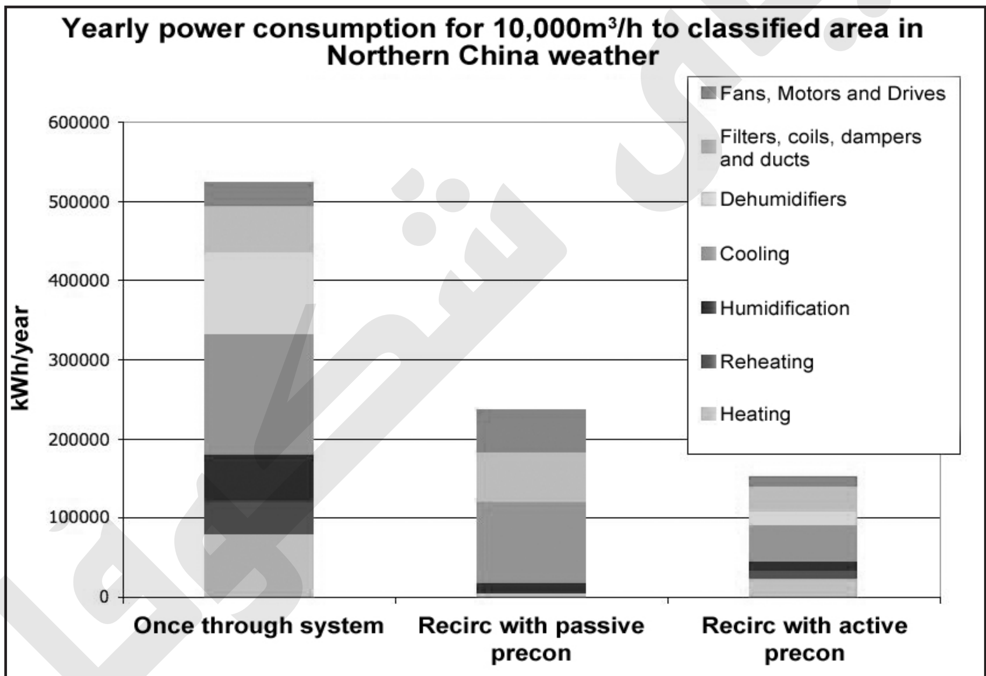


Figure 3: *Illustration of comparative energy costs.*

Comparing the energy costs of the three systems based on local electricity and steam prices (see **Figure 3**) for 2008, the relation between the costs comes out at 3:2:1 because electricity is more expensive per kWh than steam for heating. The local price reflects the carbon footprint fairly precisely in this case so the relation between the carbon footprint of the three systems is also 3:2:1.

24.6.4 Component efficiencies

Typical component efficiencies are:

- Fans = 65-80% (higher end of direct drive, avoiding belt losses)

- Heating and cooling coils = 50% from point of generation to air
- Humidification = 50-90% central steam generation is lower than local electric generation.

24.6.5 Air conditioning parameters affecting energy consumption

The choice of temperature and humidity limits and control range has a great impact on the energy consumption. The worst set-ups have a fixed set-point for temperature and humidity. There should **always** be separate set-points for humidification and dehumidification and likewise with cooling and heating to avoid conflicting simultaneous operations. The following graphs (**Figures 4** and **5**) demonstrate the energy impact.

24.6.6 The energy consumption impact of humidity requirements

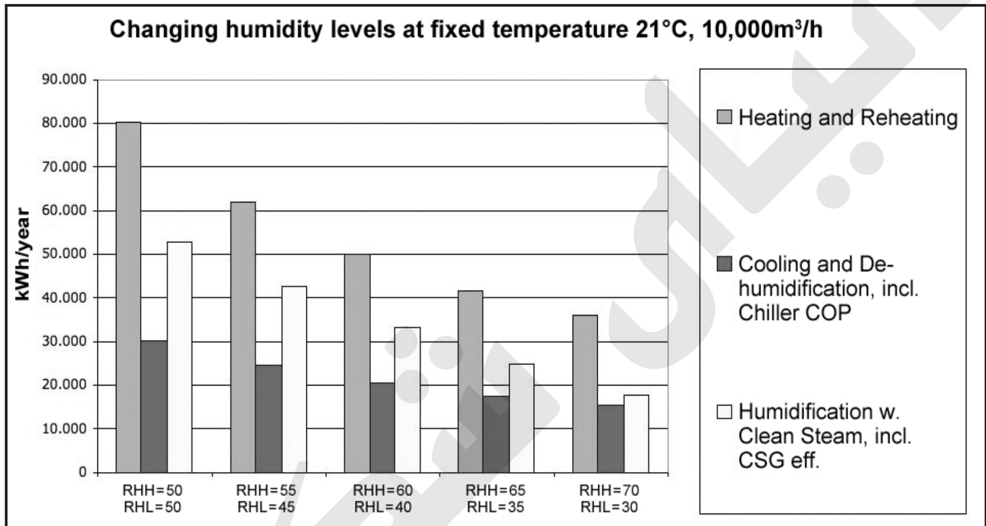


Figure 4: *Graph showing energy consumption in relation to humidity.*

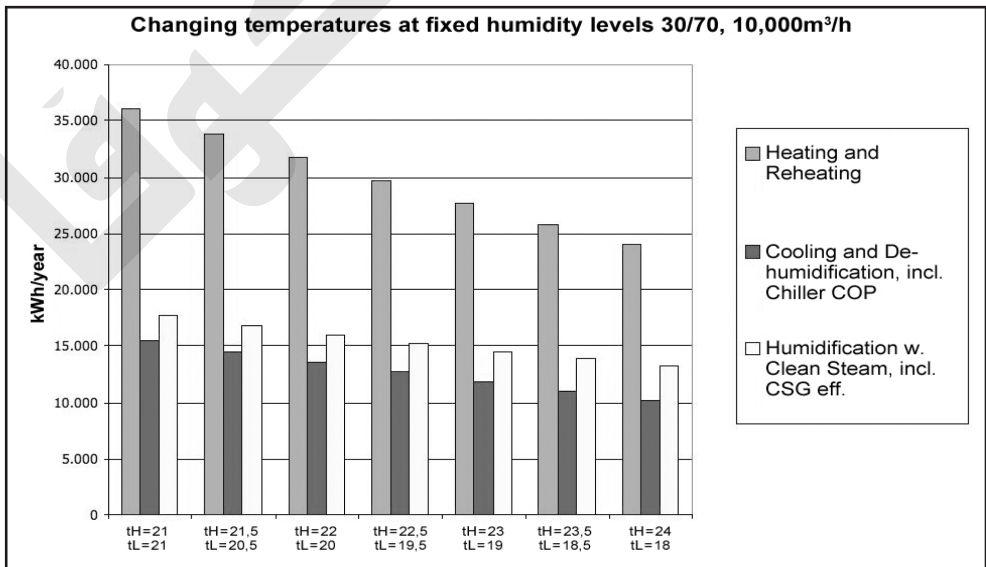


Figure 5: *Graph showing changing temperatures at fixed humidity levels.*

This comparison of energy consumption (**Figure 4**) does not take into account the (nearly) concurrent heating and cooling which often takes place when there is only one set-point for both heating and cooling, ie. a fixed temperature. In most systems there will be this extra cost when there is no temperature interval, but the magnitude varies. Often when cooling is used for de-humidification, air is sub-cooled to the dew point of the air and is then re-heated to avoid over-cooled areas.

24.6.7 Separating the flow for different purposes

The air supplied to a pharmaceutical cleanroom serves three purposes:

1. Establishing a pressure cascade
2. Air conditioning
3. Cleaning the air

Of the above, points 1 and 2 can be achieved with five air-changes an hour – this being a much lower air-change rate than what is used in the cleaner parts of pharmaceutical production. To meet the purposes in points 1 and 2, the air must pass prefilters for outside air, heating and cooling coils and rather long duct runs. All in all, the pressure drop for this part of the air is seldom below 1,000Pa total, even in a well-designed system. On the contrary, it is possible to clean the air in a local dedicated unit without coils and with only F9 and HEPA filters. Such an “air handling unit” installed locally can perform at a maximum of 500Pa total pressure drop. Therefore the immediate benefit of separating the flow is to roughly halve the energy consumption for more than half the flow. The next benefit in terms of energy consumption is that such a system is very favourable to “night set-back” because setting back the local recirculation flow does not impact pressure and air conditioning.

24.6.8 Energy savings and installation cost reduction hand-in-hand

We have often heard that it is more costly to install “energy correct” HVAC systems. It is true that the most energy efficient motors, the best fans, ducts with a larger cross-section, larger filter media areas, larger Air Handling Units and energy optimised coils will be a little more costly. However, the ensuing installation cost savings are often forgotten. When reducing the electrical consumption of the fans then the transformers, the motors, the cables and the variable speed drives can all be reduced in size and cost, but most importantly, the fan electrical savings has a huge impact on the cooling compressor installation. Each installed KWh cooling compressor capacity comes at a cost of 500 – 1000 USD and therefore energy savings initiatives have a very immediate and very positive impact on installation cost.

24.6.9 Theoretical “ideal” airflow requirements

Ideal airflow requirements can be illustrated with a simplified example:

Operational – 100m^3 cleanroom/5 competent people (fully gowned); no other contamination; HEPA quality air with no 0.5µm particles.

(Ideal mix = perfect ventilation effectiveness)

$$\text{ideal airflow required per minute} = \frac{\text{number of particles generated per minute}}{\text{required airborne concentration per minute}}$$

Assume 5 people @ 1,720,000 particles/min = 8,600,000 particles/minute (Whyte W *et al* 2007 – particle emissions from people)

<u>**Grade D**</u> = ac/hr = 8,600,000/not defined (say ISO9 = 35,200,000) = **0.15 ac/hr**

<u>**Grade C**</u> = ac/hr = 8,600,000/3,520,000 = **1.5 ac/hr**

<u>**Grade B**</u> = ac/hr = 8,600,000/352,000 = **15 ac/hr**

Hence the primary consideration is ventilation effectiveness to ensure optimised airflow. **Table 2** shows opportunities and ideas for HVAC optimisation.

Table 2: Opportunities and ideas for HVAC optimisation

Type	Opportunities & ideas for optimisation (subject to risk review)		
	Excellent	***Good***	***Ok***
Laboratories	-Switch off – out of hrs -Or Set-back if cannot switch off - Reduce fume extract - Set-back temp & %RH	- Recover heat from extracts - Use VSD on fans & pumps - Avoid humidification - Employ low-energy filter strategy	-Retro commission - Connect oversized motors in permanent STAR
Asset Facilities	-Design airflow to match stock density -Avoid over-conditioning / control of temp & %RH	-Heat recovery from extract -Use IVC's -Use VSD on fans & pumps -Employ low-energy filter strategy	- Ensure refrigerant charge is monitored
Primary API	-set-back airflow rates permanent of out-of hours / VSD or two speed fans	-Recover heat from process rejection systems -Allow temp & %RH to drift -- Use VSD on fans & pumps	-Reduce pressure regimes to minimise leakage -Employ low-energy filter strategy
Secondary Production	-Set-back airflow rates permanent or out-of hours -Recirculate air where possible -Improve ventilation effectiveness	-Use free-coolers to support chilled water - reduce pressure regimes to minimise leakage -Use UV sterilisation of purified water -Employ low-energy filter strategy	-Check controls set-points to prevent hunting
Packaging & Labelling	-Recirculate air where possible -Avoid over-conditioning the space -Set-back airflows out of hours	-Recover heat from other processes -Allow temp & %RH to drift about a broader control range	- Employ low-energy filter strategy
Warehousing "controlled"	-Localise materials which require a controlled environment - Choose appropriate temp % RH range	Use VSD on fans and pumps Switch off when external ambient allows	- Employ low-energy filter strategy
Data Centres / Hubs	-Use low energy server technology -Enable free-cooling using outside air -Use free cooling on chillers	-Ensure effective airflow / ventilation -- Employ low-energy filter strategy	

24.7 Conclusion

When the people, product and process controls are in place and regularly monitored, and the ventilation system is effective, then lower airflows, hand-in-hand with other control measures, can deliver significantly reduced energy costs. As this chapter has demonstrated, this can be achieved whilst still controlling the residual contamination emissions to maintain the airborne cleanliness levels required for compliant and quality-driven pharmaceutical production.

24.8 References

[1] Moorthy C K. *Principles and practices of contamination control and cleanrooms*. Ansham Ltd: Kent, 2004.

[2] Lidwell OM and Towers AG. “Protection from microbial contamination in a room ventilated by a uni-directional airflow", *Journal of Hygiene* 1969; **67**(1):95-106.

[3] Food and Drug Administration. Guideline on Sterile Drug Products Produced by Aseptic Processing, *Food and Drug Administration*, Rockville, MD: 2004.

[4] EU Guidelines to Good Manufacturing Practice Medicinal Product for Human and Veterinary Use, Annex 1 Manufacture of Sterile Medicinal Products. The Rules Governing Medicinal Products in the European Union, Brussels: Commission of the European Communities, 2009, Vol. 4.

[5] Farquharson, G and Whyte, W. "The design of Cleanrooms for the Pharmaceutical Industry", in Whyte W. *Cleanroom Design,* 2nd edition, Wiley: London. 1999, pp 79-114.

[6] CIBSE TM39. *Building Energy Metering*, Chartered Institution of Building Services Engineers: London, 2009.

Auditing cleanroom operations

Tim Sandle, Head of Microbiology, Bio Products Laboratory, St Albans, UK
Madhu Raju Saghee, Assistant Manager – QA, Novartis India Ltd, Thane, India

25.1 Introduction

This book has detailed all aspects of cleanroom operations and management. In order to maintain the quality of cleanroom operations, cleanrooms should be covered by the Quality Management System (QMS) of the organisation. Quality, in this context, can be defined as "freedom from defects or deficiencies"[1]. A good QMS will not function or improve without adequate audits and reviews. Audits are carried out to ensure that actual methods are adhering to the documented procedures.

Quality audits of cleanrooms are undertaken as independent assessments, using regulatory guidelines. The objective of an audit is to look for operational inconsistencies and examples of regulatory non-compliance. The former European Standard EN 30011-1[i] described an audit as:

> *"A systematic and independent examination to determine whether quality activities and related results comply with planned arrangements and whether these arrangements are implemented effectively and are suitable to achieve objectives."*

According to Martin[2], the key elements of an audit are:

- That it is systematic
- That it is conducted by someone independent of the operations
- That the audit be documented
- That the audit findings be evidenced based
- That the auditor evaluates the findings
- That the auditor makes a decision regarding the extent that the audit criteria have been fulfilled

Undertaking audits is a regulatory expectation and the process of carrying out periodic audits is in itself an essential part of preparing for regulatory inspections. The general requirement for audits is outlined in the ISO 9000 guideline that deals with the fundamentals of quality management systems. The ISO standard 9001 deals with the requirements that organisations wishing to meet the ISO 9000 standard have to meet[3]. It is generally regarded as best practice if a whole quality system (and thus all facilities) are audited at least once per year[4].

Quality audits are additionally an integral part of compliance or regulatory requirements. Examples include the US Food and Drug Administration, which requires quality auditing to be performed as part of its Quality System Regulation (QSR) (Title 21 of the US Code of Federal Regulations) and the European Good Manufacturing Practice regulations, overseen by the European Medicines Agency and national level inspectorates like the UK MHRA (Medicines and Healthcare products Regulatory Agency).

All staff employed at a facility need to be fully committed to operating an effective audit programme or with the receipt of audits. Quality is the responsibility of those being audited. The audit system must be planned to be effective and achieve its objectives in an uncomplicated way. It should also not be static, but be flexible, to enable constant seeking of improvements.

This chapter is about audits of cleanroom operations. The chapter begins with some general guidance and then proceeds to discuss some of the specific areas for cleanroom auditing.

25.2 Part A: GMP auditing

25.2.1 Types of audit

Quality auditing is an important task and one which should be undertaken by trained personnel. Audits can be divided into internal audits (undertaken by personnel employed by the organisation) and external audits (undertaken by personnel from another organisation, often a customer of the services supplied by the organisation being audited). Internal system audits and reviews should be positive and conducted as part of the preventative strategy, and not as a matter of expediency resulting from problems.

The assessment of a quality system against a standard or set of requirements by internal audit and review is known as a first-party assessment or approval scheme. If an external customer makes the assessment of a supplier, against either its own, or a national or international, standard, a second-party scheme is in operation. The assessment by an independent organisation, not connected with any contract between the customer and supplier, but acceptable to both of them, is an independent third-party assessment scheme.

Furthermore, audits can be vertical or horizontal. A horizontal audit requires the auditor to look at a number of reports produced over a period of time. For example, HEPA filter test reports issued over a two-year period. A vertical audit follows a series of steps through the organisation. An example might be environmental monitoring samples: how they are prepared, transported, sampled, returned to the laboratory, incubated and then read and reported.

The purpose of audits is for establishing the facts rather than finding faults. Audits do indicate necessary improvement and corrective actions, but must also determine if processes are effective and that responsibilities have been correctly assigned. The key emphasis is on process improvement.

Audits are rarely single events and an audit is often repeated at a future date. The requirement for an audit will vary according to what is being audited, with aspects considered to be a higher risk audited more frequently. Thus there should be a schedule for carrying out audits, with different cleanroom activities subject to different audit frequencies, with such frequencies determined by a risk assessment (for example an audit of an aseptic filling cleanroom would be carried out more often than an audit of an EU GMP Grade D/ISO 14644 class 9 equipment preparation area). An audit should not be conducted just with the aim of revealing defects or irregularities.

25.2.2 Auditors

Auditors should be knowledgeable of cleanroom operations and be trained specifically as auditors. Auditors should have complete independence of the functions they are auditing. In addition to understanding the processes, strong interpersonal skills are critical to the success of an auditor and the audit he or she is performing. There is a natural defensiveness which occurs on the part of the auditee and an audit can be an emotional experience. Good auditors are persistent without being relentless; ensure they have their questions answered and thoroughly understand a situation before they evaluate what they have seen and heard.

The auditor can obtain a lot of information through good audit technique. Information can be gathered through interviewing staff, checking documents, observing processes, checking equipment status and by observing the overall layout and organisation of the cleanroom or laboratory.

Some the interpersonal skills required by auditors include:

- Objectivity
- Tact
- Fairness
- Not having any preconceptions
- Thoroughness
- Persistence
- Technical knowledge
- Strong questioning and interviewing skills
- Detailed understanding of Good Manufacturing Practices and clauses
- Confidentiality

As well as looking for negative points, auditors should highlight positive aspects of the area visited.

25.2.3 Undertaking audits

There are various different approaches which can be taken for conducting audits. An example of the generic steps involved in an audit are:

- ***Initiation***

  That the audit happens at all must be initiated by one party and the two parties (auditor and auditee) must agree to the audit taking place. This defines the audit schedule.

- ***Scope***

  The activities that are to be included in the audit must be decided in advance of the audit by the auditor. This information must be shared with the area to be audited. This should normally take the form of a statement from the auditor outlining the audit purpose and the area(s) which will be subject to the audit.

- ***Frequency***

  The frequency of the audit (how often an area is to be audited) must be determined. In one sense this will help determine the scope, for if an area is subject to many audits (such as internal monthly audits), this may lead to mini-audits focusing on defined parts of the larger area taking place rather than one large audit of an entire facility.

- ***Preparation***

  Before an audit is undertaken, the auditor must prepare for the audit and understand the function of the area to be audited and its key operations. This includes familiarity with the facility, understanding the type of product produced and how it is organised by personnel and function. This is sometimes referred to as the audit task plan.

  The preparation of the audit task plan will include a review of past audits, noting indications of possible problem areas and items that were identified for corrective action in a previous audit.

  The manager of the area to be audited will also prepare for the audit.

- ***Review of documentation***

  Often the auditor will read documents pertaining to the area to be audited in advance. This may include, depending upon the scope of the audit, the Quality Policy or Site Master File.

- ***The programme***

  The auditor should map out the programme for the audit in advance. This may include considering questions like: What will be covered? What needs to be looked at? What should be witnessed? Which key documents will be reviewed?

  In doing so, the auditor will decide upon how the audit will be executed.

- ***Opening meeting***

  Audits begin with a meeting. The meeting will consist of the auditor and representatives of the area to be audited (normally the area manager and, if it is an external audit, someone from the company's quality department). At the meeting the auditor will outline the scope of the audit.

- ***The visit***

  The 'audit' is divided into two key steps: visiting the area(s) subject to the audit and documentation review. The visit consists of the auditor being escorted around the area. During this phase the auditor will examine the area (noting things like fabric), watch activities being carried out, ask questions of staff working in the area and check records. The auditor may ask for other documents relating to the activities to be reviewed later.

  The auditor will take note of things which do not seem correct. These will later be classified into observations and non-compliances.

- ***Documentation review***

  Either during the facility tour or at the end of the tour, the auditor will review any requested records. These may include Standard Operating Procedures and training records. Documentation represents an important part of the audit, as indicated by FDA CFR 211.113, which states:

  "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilisation processes."

- ***Close the meeting with the auditee***

  At the end of the audit, the auditor will prepare the findings. These must be presented to the auditee in a closing or wrap-up meeting. The auditor will convey the importance of the findings and make reference to regulatory documentation where necessary. It is important that anything to be covered in the final report is included in the closing meeting, for the report should not contain non-compliances not raised with the facility staff at the end of the audit.

- ***Report***

  Shortly after the completion of the audit, the auditor must write a report. The report should be held by the auditor's company. Either the full report or, more normal for an external audit, a summary report, is sent to the manager of the area which has been audited.

  Where non-conformities have been noted, the area which has been audited should respond to the audit findings within a short time period (typically within thirty days). As appropriate, corrective and preventative actions should be set.

  There are a number of ways that an auditor can approach the audit. Some 'best practice' tips are outlined below:

  1) It is a good idea for the auditor to use a checklist. This can serve as a valuable tool. A checklist will contain some of the key issues to be considered in advance. For example, if an aseptic filling line is to be looked at, the auditor may wish to verify that regular weight checks on bottles or vials are undertaken. This would be written onto the auditor's checklist. The checklist is to be used with a notebook into which detailed entries can be made during the audit. However, although a checklist is a useful guide for the auditor, it should not be intended to be a substitute for knowledge of the GMP regulations.
  2) Although a single question may be included about any requirement, the answer will usually be a multi-part one since the auditor should determine the audit trail for several products that may use many different components. Enter details in your notebook and cross reference your comments with the questions.

3) When looking at processing, at least three production batches should be selected for thorough analysis to include: (a) traceability of all components or materials used in the subject batches, (b) documentation of raw material or component, in-process, and finished goods testing for the subject product batches, (c) warehousing and distribution records as they would relate to a possible recall.
4) The references to sections in the GMP regulation should be known by the auditor (that is, a thorough understanding of to what standard the audit is being undertaken). In some instances, two or more sections within the GMP regulation may have a bearing on a specific subject. The headings in the GMP regulation will usually offer some guidance on the areas covered in each section.
5) In terms of general advice, the auditor should focus most of the audit upon major issues and, consequently, a smaller portion of the time on minor issues.

25.2.4 Review of audits

The results of audits should be reviewed periodically. This will be undertaken by those tasked with undertaking audits and those subject to receiving audits. This process is part of most Quality Management Systems. Once a year, as a minimum, a review meeting should consider the results of audits, non-conformities, the status of preventative and corrective actions, follow up actions from previous management reviews, identification of resources and recommendations for improvements.

In addition, the procedures for conducting audits and reviews and the results from them should be documented, and also be subject to review.

25.2.5 Regulatory trends

Reviewing regulatory trends such as reviewing the 483 notifications raised by the FDA, can provide a useful overview of the kind of topics that regulators are interested in and that auditors are considering. FDA warning letters can be examined to determine which section of Good Manufacturing Practices the citation relates to[5].

25.3 Part B: Auditing cleanrooms

There are a number of different aspects of cleanroom operations which are important to audit. This section of the chapter takes the form of check-list, with the aim of serving as a practical tool for those less experienced to the auditing field or as a an aide-mémoire to more experienced auditors.

25.3.1 General controls and facility operations

The audit finding on control systems and the facility operations is, in many ways, the overall finding of the audit and one which often rests on first impressions. The auditor will need to establish if the facility and its departments (organisational units) operate in a state of control as defined by the GMP regulations.

The auditor should request a map(s) of the facility which details cleanroom grades, process and equipments flows and pressure differentials. The auditor can gain an impression of the contamination controls in place. The cleanroom design should take into consideration the flow of personnel, product, equipment and components. The most effective facility designs will take into account the flow of all of these from the receipt of raw materials at the warehouse through the facility to the packaging of the product (although the latter take place outside of a cleanroom, this may fall within the scope of the audit).

25.3.2 Quality systems

A cleanroom audit will, depending upon the scope, require a consideration of the quality systems of the

organisation. This will include a review of Quality Assurance operations. Lines of inquiry may include:

- Is the quality manual up-to-date?
- Does the Quality Assurance unit alone have both the authority and responsibility to approve or reject all components, drug product containers and closures; and approve important validation activities like cleanroom certification reports?
- Does the QA department or unit routinely review production records to ensure that procedures were followed and properly documented?
- Does the QA unit have a person or department specifically charged with the responsibility of designing, revising, and obtaining approval for production and cleanroom procedures, forms, and records?

25.3.3 Personnel and staff training

Another important area to be examined is personnel and training. Here the auditor will wish to be assured that suitably qualified staff have been recruited and that these staff have been trained correctly in cleanroom disciplines. Given that personnel are absolutely necessary to work in cleanrooms but are, at the same time, the greatest microbial risk for a product or process contamination, then such training should include an understanding of hygiene and of basic microbiology. Another important aspect which an auditor should take note of is aseptic technique as all personnel working within the cleanroom should be undertaking this in a competent manner. Aseptic technique is a set of specific practices and procedures performed under carefully controlled conditions with the goal of minimising contamination. This relates to sampling, undertaking connections, making interventions and so forth.

Questions which an auditor may consider would include:

- Are all the personnel working within the cleanrooms suitably qualified to do so?
- Does each staff member have a training record and is that training record up-to-date?
- Does each staff member have a job description?
- Does each employee working within the cleanroom receive retraining on an SOP (procedures) if critical changes have been made in the procedure?
- Is all training documented in writing that indicates the date of the training, the type of training, and the signature of both the employee and the trainer?
- Are training records readily retrievable in a manner that enables one to determine what training an employee has received, which employees have been trained on a particular procedure, or have attended a particular training program? For cleanrooms, topics like general hygiene are essential.
- Gowning procedures:
  - What is the gowning procedure in place?
  - How are employees trained against the gowning procedure?
  - What is the acceptance criteria for passing the gowning test?
  - How often do employees need to perform the gowning test?
  - What is the procedure for qualification and disqualification for working within a cleanroom?

Another area regarding to the suitability of staff to work in cleanrooms relates to medical health. Here the auditor should ascertain if supervisory personnel are instructed to prohibit any employee who, because of any physical condition (as determined by medical examination or supervisory observation) that may adversely affect the safety or quality of drug products, from coming into direct contact with any drug component or immediate containers for finished product? The auditor should also establish if employees are required to report to supervisory personnel any health or physical condition that may have an adverse effect on drug product safety and purity. Strict controls

should be in place to stop employees who are ill (with illnesses like colds) or who have conditions like eczema from entering cleanrooms. Such controls should also be in place for temporary employees and contractors. For contractors, written records should be maintained stating the name, address, qualification, and dates of service.

25.3.4 Auditing cleanrooms

Whilst each type of cleanroom will vary in its function, there are some common aspects of cleanrooms for an auditor to examine. The auditor should first consider if all parts of the cleanroom suite are constructed in a way that makes them suitable for the manufacture, testing and holding of drug products.

The auditor should assess the numbers and types of cleanrooms, the grades or classes of the cleanrooms, and how the cleanrooms interrelate with each other (are they, for example, part of a suite?). For the interrelationship between cleanrooms, the auditor may wish to assess the use of different changing facilities and airlocks as a means of separating different areas.
When examining specific parts of cleanroom operations, the auditor should consider:

25.3.5 Space and design

With space and design, one of the key concerns is whether there is sufficient space for carrying out operations and if such spaces are correctly segregated in order to prevent mix-ups and to contain contamination.

Points that an auditor should consider include:

- Is there sufficient space in the facility for the type of work and typical volume of production?
- Does the layout and organisation of the facility prevent contamination? Is this facility maintained in a clean and sanitary condition?
- Is control of air pressure, dust, humidity and temperature adequate for the manufacture, processing, storage or testing of drug products?
- Are all parts of the facility maintained in a good state of repair?
- Has any recent maintenance taken place? Was this controlled through a change control?

The auditor should also consider if the types of cleanrooms, and their grades or classes, are suitable for the activities taking place within the areas. **Table 1** below provides some examples of room classifications.

Table 1: Table displaying some examples of uses of cleanrooms for different grades and classes

EU GMP Grade	ISO Class (dynamic state)	Example of cleanroom use
A	4.8[(ii)]	Aseptic preparation and filling (critical zones under unidirectional flow)
B	7	A room containing a Grade A/ISO class 4.8 zone (that is, the background environment for filling) and the area demarcated as the ‘Aseptic Filling Suite’ (including final stage changing rooms)
C	8	Preparation of solutions to be filtered and production processing
D	9	Handling of components after washing; plasma stripping
U*	N/A	Freezers, computer conduits, store rooms, electrical cupboards, other rooms not in use, etc.

25.3.6 Process flow

The auditor should examine the material flow throughout the building with a particular focus on how items are transported in and out of cleanrooms, and how product is moved around the facility. The auditor should assess what control measures are in place to protect the process and product from contamination.

The auditor should also request information relating to room and building specifications.

25.3.7 Certification

- When was the cleanroom last certified for HVAC operations? Is the frequency of certification adequate? (ie. is a minimum of six-monthly for aseptic filling areas and twelve-monthly for other Grades of cleanrooms).
- Are HEPA filter certificates available?

25.3.8 Physical operations

The physical operation of cleanrooms is centred upon the Heating Ventilation and Air Condition (HVAC) system. The primary concern is with contamination control and ensuring that the cleanrooms have a suitable air supply, through High Efficiency Particulate Air (HEPA) filters, in order to maintain the correct pressure differentials and to provide air of a low enough particulate level appropriate to the class or grade of cleanroom. Perhaps the first issue that the auditor should check is the cleanroom certification, focusing on when this was performed and if it was undertaken to an approved standard (such as ISO 14644) and that all of the requisite tests have been completed.

These cleanroom certification tests fall within the following areas:

a) Control of air borne particulates
b) Sufficient airflow and air changes
c) Positive pressure differences
d) Testing of HEPA filters
e) Temperature, humidity and lighting

The auditor should check that the tests have been completed by competent engineers, that the tests have been conducted at a suitable frequency and that the test results meet the acceptance criteria required by an approved standard.

In relation to the above test criteria, and taking into account other aspects of the physical operation of cleanrooms and clean air devices, points that an auditor should consider are:

- Are laminar flow hoods/unidirectional airflow devices and the HVAC system which serve the aseptic operations areas validated and within date?
- Which department performs the validation?
- Was the validation approved by QC or QA?
- Was the report issued?
- If yes, was the report forwarded for QC or QA for review?
- Filters.

  The filters used in cleanrooms and in unidirectional airflow devices will be HEPA or ULPA grade. All filters have an efficiency rating and should be tested periodically for leaks. In addition, filters are studied for particulate penetration. The type of filter used in a cleanroom will be selected for and justified by the cleanroom manager. A common type of HEPA filter is one with an efficiency rating of 99.997%. The 99.997% efficiency is based

on particle sizes 0.3μm and larger (i.e. theoretically only 3 out of 10,000 particles at 0.3μm size can penetrate the filter). In addition to particulates penetration, filters which form part of unidirectional airflow devices need to be examined for air velocity. Here the auditor should question:

- What is the frequency for measuring air velocity through a filter?
- How often are air velocities measured?
- Are airflow velocities within operating parameters? (Here it should be noted that EU GMP defines an air velocity range at the working height:
  - 0.45 m/s +/-20% (unidirectional). However, the FDA do not and require air velocities to be justified and to be taken 6 inches from the filter face).
- Where, in relation to the filter face, are air velocities measured from? (that is, near the filter face or at the working height?)
- What is the equipment used to measure air velocities and is this equipment calibrated? For air filters, is there a written procedure specifying the frequency of inspection and replacement?

- Does the facility have separate air handling systems, if required, to prevent contamination? (for example, where antibiotics are produced).
- The auditor should review the airflow visualisation studies to determine that areas which require unidirectional airflow are protected.
- Positive pressure is an essential operational feature of cleanrooms. In order to maintain air quality in a cleanroom, the pressure of a given room must be greater relative to a room of a lower grade. This is to ensure that air does not pass from “dirtier” adjacent areas into the higher grade cleanroom. The facility will set appropriate limits for pressure differentials (as a guidance, 15-20Pa relative to lower grade rooms is commonly used).

The auditor should ask, for example, measurement data for pressure differentials between each room and all surrounding spaces, and between two rooms.

- Related to pressure differentials are airlocks. Airlocks are used to control the entry of personnel into cleanrooms and for dividing clean areas and where there is a risk of pressure loss between areas. The auditor should examine the facility map to ensure that airlocks are located in the correct locations and are functioning correctly. Where airlocks exist the door of the airlock should be interlocked to prevent both doors from being open at the same time and hence to avoid pressure loss.
- Cleanroom air changes should be checked. Each cleanroom grade has a set number of air changes per hour. Air changes should be defined by the facility and are calculated from supply air volume and room volume measurements.
- Cleanroom recovery rates ('clean-up times') stand as an optional test in ISO14644 part 2. Recovery rates are connected to air changes and is the time taken for a clean area to return to the static condition, appropriate to its grade, in terms of particulates. However, where these are performed they must relate to pre-set acceptance criteria (normally that the room returns to a satisfactory particulate level in 15 to 20 minutes).
- With temperature, humidity and lighting, it is common for EU GMP Grade B/ISO class 7 (dynamic state) rooms to have a temperature and humidity requirement. Commonly, and to meet EU GMP requirements, this is: Grade B/ISO class 7 (dynamic) – Temperature: 18±3°C, Humidity: 45±15%. Other clean areas have a temperature appropriate to the process step (e.g. if the process requires a cold room at 2-8°C).
- Lighting should be adequate, uniform and anti-glare, to allow operators to perform process tasks effectively. A range of 400 to 750 lux is recommended.

25.3.9 Equipment

Cleanrooms will contain a variety of items of equipment within the cleanroom (for weighing stations to centrifuges). The auditor should check that such items of equipment have been calibrated. In addition, items of equipment used to certify cleanrooms (such as anemometers) should also be inspected for their calibration status. The auditor should also enquire if the facility has approved written procedures for checking and calibration of each piece of measurement equipment.

The auditor may also enquire who is responsible for maintaining the equipment. Where the equipment is serviced and calibrated by external contractors, the auditor should verify that those carrying out the calibration and serving are competent to do so. The auditee should have detail of the work carried out (such as a method statement) and a copy of the contractors' curriculum vitae on file.

Other questions the auditor may ask include:

- Is there a maintenance schedule in place?
- What is included in planned preventative maintenance?
- What is the procedure for undertaking servicing and calibration?
- What are the calibration tolerances?
- Has equipment been verified as conforming to build requirements which is defined in the Installation Validation?
- Has the equipment been verified as having been correctly installed, and supplied with the required services and support equipment and facilities (often defined in the Operations Validation)?
- Has the equipment been verified as being able to operate as required in routine production under stressed (worst case within limits) conditions (often defined in the Performance Validation)?

Auditors will also focus on more specific aspects of cleanroom equipment. For example, a 'hot topic' with the FDA are vessel filters and whether these have been integrity tested.

25.3.10 Personnel

The personnel working within a cleanroom will be subject to the audit and it is important that personnel conform to procedures at all times. An auditor will focus on the way people behave and whether these aspects put the product, process or environment at risk. Auditors will often look at gowning and the frequency of glove spraying and the glove spray technique.

The auditor should also examine how personnel enter cleanrooms and the change procedures. This will also include an inspection of the changing rooms. Cleanliness levels relate to correct changing procedures. For example, Annex 1 of the EU Guide (paragraph 20) states that changing rooms in which outdoor clothing is worn should not be used to access Grade C/ISO class 8 (dynamic) and Grade D/ISO class 9 (dynamic) areas. For entering aseptic filling areas a two-change procedure should be in place.

Furthermore, the FDA aseptic filling guide requires that gowning is periodically reassessed. The auditor should review the gowning assessment procedure and, for aseptic filling area, ensure that both an observational and personnel sampling assessment is in place. The auditor may request a full audit trail of garments used in aseptic areas, including management reports and wearer analysis.

25.3.11 Aseptic processing

Aseptic processing takes place in the highest grade of cleanrooms within pharmaceutical and

healthcare environments and is often the subject to a self-contained audit. One of the most important aspects of aseptic processing is the validation of the process through media simulation (broth) trials and this area is likely to be subject to the greatest scrutiny by the auditor.

In relation to media trials, the auditor may check that:

- Media trials take place
- That media trials take place at a sufficient frequency
- That media trials are undertaken following significant changes to room or HVAC design
- That a representative number of vials are filled
- That simulated interventions take place
- That media fill containers are incubated for the correct time and at a dual incubation temperature regime
- That any micro-organisms are identified.
- That the culture media used is suitable. Media should meet the following criteria:
  - *Filterability:* If there is a filtration stage in the process, the liquid medium should be capable of being filtered through the same grade of microbial retentive filter as that through which the actual product is filtered.
  - *Selectivity:* The growth medium must have low selectivity so that it supports the widest possible range of micro-organisms growth.
  - *Clarity:* The growth medium needs to be as clear as possible to facilitate subsequent checking for any growth following incubation.

The second aspect likely to be studied is the environmental monitoring results. Environmental monitoring is examined below in general terms. In relation to aseptic filling, the auditor will seek assurance that the most critical parts of the filling area are part of the environmental monitoring sampling regime (such as stopper bowls and filling needles). The auditor may also consider:

- If environmental monitoring is taking place in the dynamic (operational) state?
- Are the environmental monitoring locations meaningful and selected and based on risk evaluation? Are justifications available to explain why each location was selected?
- Whether all critical steps are covered by the monitoring programme?
- That monitoring takes place at the point of fill?
- That settle plate exposure is adequate.
- That a sufficient volume of air is sampled for volumetric air-samplers
- That contact plates and plates used for sampling personnel fingers have an appropriate disinfectant neutraliser.
- That monitoring of aseptic filling is throughout the filling operation (and that particle counting is continuous)
- That operators are correctly identified
- That all operators present are sampled
- That exit suit gown plates are taken

25.3.12 Auditing cleaning and disinfection

An essential aspect of cleanroom management is the cleaning and disinfection programme. The auditor must consider if the facility hase written procedures that describe in sufficient detail the cleaning schedule, methods, equipment and material. The auditor should also check that:

- Cleaning and disinfection agents are suitable

- Cleaning and disinfection agents are compatible
- The auditor should examine the cleaning techniques used (for example, whether the two or three bucket cleaning technique is in use).
- Cleaning and disinfection agents have been validated
- If disinfectants, of different modes of operation, are rotated.
- The auditor may also wish to examine the environmental monitoring data in light of the disinfectants used.

Depending upon the scope of the audit, the auditor may also examine cleaning of equipment. Examples of areas to consider include:

- Are written procedures established for the cleaning and maintenance of equipment and utensils?
- Has a written schedule been established and is it followed for the maintenance and cleaning of equipment?
- Is equipment cleaned promptly after use?
- If appropriate, is the equipment sanitised using a procedure written for this task?
- Is idle equipment stored in a designated area?
- Is equipment cleaned promptly after use?
- Is clean equipment clearly identified as "clean" with a cleaning date shown on the equipment?
- Is clean equipment adequately protected against contamination prior to use?
- Has cleaning validation been performed on the representative equipment? Did the validation include swabs for total organic carbon and viable micro-organisms?
- Are written procedures available for each piece of equipment used in the manufacturing, processing or holding of components, in-process material or finished product?
- Are all pieces of equipment clearly identified with easily visible markings?

25.3.13 Auditing environmental monitoring

With environmental monitoring, the auditor must review whether a systematic and comprehensive environmental monitoring programme is in place (and that such a programme is documented)[6]. Listed below is a check list that could be used for auditing the environmental monitoring of cleanrooms.

- Is there an environmental monitoring program for the cleanrooms? Are Standard Operating Procedures (SOPs) in place and in date?
  - Are there separate programmes for aseptic batching area, sterile filling room, sterile filling line and ancillary areas?
  - What is the frequency of monitoring?
  - How are the monitoring locations selected? Are the located suits suitable? (Has reference been made to airflow visualisation studies for the selection of environmental monitoring locations?). The auditor should review the environmental monitoring sampling plan.
  - Does monitoring include all locations, including areas that are hard to clean such as walls, floors and ceilings?
  - Is the monitoring programme able to indicate if the cleaning and disinfection frequencies are appropriate?
  - Is monitoring undertaken in the operational or non-operational state? (Here, the operational state is deemed to be more representative of processing conditions).
- Viable monitoring
  - How is viable air sampling performed?
    - Are both settle plates and active air-samplers used?

- How have the models of active air-samplers been selected? (For example, why have centrifugal, filtration or impaction models been used?)
- Does the active air-sampler disrupt the unidirectional airflow?
- What volume of air is sampled? (one cubic metre is the norm, or a volume of air equivalent to one cubic metre.)
- Where active air-samplers are used, is a corrective factor applied when required?
  - How is surface sampling performed?
    - Are both contact plates and swabs used?
  - For aseptic filling areas, how are personnel sampled?
    - Are both finger plates and gown plates taken?
  - Which culture media is used?
  - Are growth support tests performed on each lot of nutrient media used for environmental monitoring?
  - How is sterility verified for each lot of nutrient media used for environmental monitoring?
  - How is nutrient media stored prior to use?
  - What are the post-test incubation conditions?
  - After incubation, how are test results documented?
  - Are the alert and action levels used appropriate for the cleanroom?
- Is non-viable particulate sampling performed?
  - What is the frequency of particle counting outside of formal re-certification?
  - Do the locations for monitoring relate to the formal certification data?
  - Have particle counters been calibrated to the required standard and are calibration records up-to-date?
  - Is environmental monitoring equipment on a maintenance/calibration program?
- Culture media
  - Has the culture media been subject to growth promotion testing?
  - For media manufactured in-house, are there standardised and validated autoclave loads for nutrient media used for environmental monitoring?
- Investigations
  - The auditor should examine the out of limits/out of specification procedures for environmental monitoring excursions. The auditor must be satisfied that any microbiological data deviation is examined and documented. An example record should be studied.
- Equipment
  - How is media and environmental monitoring sampling equipment transported into cleanrooms?
  - What is the decontamination procedure for equipment?
  - How is equipment calibrated? What standards are used?
  - For aseptic filling areas, is equipment captive to the filling area?
- Microbial identification
  - What is the frequency of microbial identification?
  - Is identification to genus or species level?
  - Are the methods used for identification appropriate?

An audit of environmental monitoring may lead to the microbiology laboratory. The auditor may wish to examine incubators and other items of laboratory equipment, particularly in relation to

calibration checks, service records and monitoring. The auditor should ascertain if incubators are operating at the correct temperature and humidity (that is within defined ranges), that incubators are tidy, that the incubator shelves and samples are clearly labelled and identified, and that the culture media used is within its expiry date.

The auditor should also examine some laboratory data to determine if the data are reliable and if documents are controlled (the control of documentation is discussed below).

25.3.14 Auditing cleanroom documentation

Cleanroom documentation, like all other GMP documentation, should fall under Good Documentation Practices. Listed below is a check list that could be used for auditing cleanroom documentation.

- Handwritten entries should be made in a clear, legible and indelible (permanent) way.
- Records should be made or completed at the time each action is taken. Have such records been completed contemporaneously?
- Records should be made in such a way that all significant activities concerning the manufacture of medicinal products are traceable.
- Any alteration made to the entry on a document should by signed and dated. The alteration should permit the reading of the original information. Where appropriate, the reason for the alteration should be recorded.
- Where an area of the document is not applicable, it must be struck through and a reason recorded.
- Are deviation reports available for non-compliances? Have these been completed satisfactorily with appropriate preventative and corrective actions?
- Have the correct date and time formats been used? To an extent this will depend upon company policies.
- However, should the documentation relate to international activities, the appropriate date formant is: dd/mmm/yyyy. Here the day must be two digits, the month at least three letters, and 2 or 4 digits for the year. For example 1st of April 2011 should be written: 01 Apr 2011
- Use the correct time format – normally the 24 hour clock. For example, ten past nine in the morning would be: 09:10 and ten past nine at night would be 21:10
- Documentation non-compliances must be noted. These can include:
  - Overwriting or scribbled out entries
  - The use of dittos, or bracketing multiple lines and signing just once
  - The use of unauthorised copies of controlled documents
  - The use of superseded or obsolete forms
  - The record signed by the person who did not complete the task
  - Discarded GMP records
- Use of pencil or correction fluid on forms
  As well as the product-related documents, there should be written policies, procedures, protocols and reports covering the support systems including:
  - Cleanroom validation
  - Equipment and facility maintenance, cleaning and calibration, including HVAC and cleanroom re-certification
  - Personnel signature lists
  - Training in GMP and technical subjects (including effectiveness of the training)
  - Clothing and hygiene

- Environmental monitoring
- Pest control
- Investigations
- Internal or supplier audits
- Change control and deviation management

In addition both the MHRA and FDA require log books to be kept for major or critical equipment used for analytical testing or production purposes as well as for areas where product has been processed. The log books should provide a chronological account of any use of the area, equipment/method, calibration, maintenance, cleaning or repair operations. The dates and names of people carrying out those operations should also be recorded.

25.4 Conclusion

This chapter has examined the activity of auditing in general and the application of good auditing practices to cleanrooms. A considerable amount of the content has taken the form of checklists, for the aim of the chapter was to assist auditors in carrying out their task of assessing cleanroom operations.

However, there is no universal checklist which can simply be picked up and used. Although the chapter has discussed some of the important aspects of cleanroom operations which an auditor should check it is noted that each cleanroom or cleanroom suite is different and the auditor will need to have a firm grasp of the activities and operations within the cleanroom. Nonetheless, most of the detail in the different cleanroom standards (and many of the topics discussed in the chapters in this book) are highly applicable to most cleanroom audits, and the auditor should have a thorough understanding of ISO 14644 and EU GMP and FDA regulations and guidelines in order to assess the operation of a cleanroom and its conformance to expected standards.

25.5 References

[1] Miller, MJ, Emerson R. Auditing the Pharmaceutical Microbiology Laboratory In: Prince R, ed. *Microbiology in Pharmaceutical Manufacturing*. Baltimore: Parenteral Drug Administration, 2001.

[2] Martin A. Auditing a QC Microbiology Laboratory. In: Saghee MR, Sandle T, Tidswell EC, eds. *Microbiology and Sterility Assurance in Pharmaceuticals and Medical Devices*. New Delhi: Business Horizons, 2011: 237-258.

[3] Tsim YC, Yeung VWS, Leung ETC. An adaptation to ISO 9001: 2000 for certified organisations. *Managerial Auditing Journal* 2002; **17**(5).

[4] Sharp IR. Quality Audit and Quality System Review in the Laboratory. In: Snell JJS, Brown DFJ, Roberts C, eds. *Quality Assurance: Principles and Practice in the Microbiology Laboratory*. Colindale: Public Health Laboratory Service, 1999.

[5] Moldenhauer J. Review of FDA Warning Letters. *Pharmaceutical Technology Europe,* April 2011, 44-51.

[6] Vincent D. Validating, Establishing and Maintaining a Routine Environmental Monitoring Program for Cleanroom Environments: Part 1. *Journal of Validation Technology*, August 2002; **8**(4).

Developments in cleanroom technology

Tim Sandle, Head of Microbiology, Bio Products Laboratory, St Albans, UK
Madhu Raju Saghee, Assistant Manager – QA, Novartis India Ltd, Thane, India

26.1 Introduction

Cleanroom technology is designed to provide either product or operator protection. The drivers for developing cleanroom technology are to increase the level of protection or to decrease operational costs. It is important that pursuing the latter does not impinge on the former objective.

Of all of the chapters in this book, one which attempts to capture the future direction of cleanroom technology is most likely to become outdated. The aim of this short chapter is to survey some of the recent developments in cleanroom technology which have occurred with the aim of allowing practitioners to remain aware of the contemporary technologies and latest trends.

The chapter also includes an overview of risk assessment. The use of risk-based approaches should be the basis of all changes to cleanroom design and consideration of new technologies. Our reason for including a brief overview of risk assessment is to introduce those unfamiliar with the concepts so that an appreciation can be gained as to the necessity of a risk-based approach to cleanroom management. Readers are advised to refer to Chapter 4 for more information about the modern approaches to cleanroom design and Chapter 9 for information on risk-based approaches to cleanroom commissioning and qualification.

26.2 Cleanroom design

26.2.1 Design principles

In recent years there have been considerable advancements in cleanroom design. These are aimed at ensuring that the cleanroom is designed in a way which ensures that it meets the requirements of the user and is designed in the optimal way to ensure contamination control. It is important to dedicate time to designing cleanrooms and the equipment located in cleanrooms for if there is a design fault in one part, this will affect the items of equipment and if there is a fault at the conception stage, this will be expensive and time consuming to rectify.

For cleanroom design, modern approaches utilise computer-aided engineering software for the design process, such as Building Information Modelling (BIM) software. BIM covers geometry, spatial relationships, light analysis, geographic information, quantities and properties of building components (for example manufacturers' details). BIM can be used to demonstrate the entire building life cycle, including the processes of construction and facility operation. Quantities and shared properties of materials can be extracted easily[1] . Systems, assemblies and sequences can be shown in a relative scale with the entire facility or group of facilities.

When designing modern cleanrooms, the following approach should be adopted:

- The type and function of the cleanroom should be established. This should include the required cleanroom grades or classes and how cleanrooms of different grades will interact (including requirements for air-locks and pressure cascades).
- The most important aspect is drawing up the process flow. Here the cleanroom management, together with engineers and quality assurance personnel, should map the path that equipment, product and operators will take in the cleanroom. Established quality risk management tools

like HACCP (Hazard Analysis and Critical Control Points) or FMEA (Failure Modes and Effects Analysis) can be used for this purpose. Areas that pose a contamination control risk should be noted and attempts made to design these risk areas out (the principles of quality by design). Other considerations can also be included at this stage, including whether there is adequate clearance under door frames for equipment to pass through.

- Engineering must be involved at the early design stage in order to ensure that the conceptual design for the cleanroom can be built as intended and within the required timeframe.
- In the design, there should be sufficient space for equipment and connections.
- The cleanroom should be constructed from a material compatible with different cleaning and disinfection solutions.
- A mock-up of the cleanroom should be constructed. This is particularly important for testing the process, product and personnel workflow. In terms of understanding contamination control it is essential to understand what objects are passed from one class of cleanroom to another.

There have been some recent advances in cleanroom construction to ensure that cleanrooms are built to a higher standard in order to further reduce contamination risks. For example, plasma welding can be used for potentially weaker areas, like ventilation ducting, to ensure improved leak tightness (a leak of air from a less clean area into the cleanroom is a major contamination risk). Another development is the use of 'double skin' constructions around air-handling units, as another means of minimising air leakage.

26.2.2 Conventional and modular cleanrooms

There have been a number of changes to the way in which cleanrooms are designed and constructed, with a wider use of modular cleanrooms. The traditional cleanroom design is sometimes described as "hard-walled" or "hard-lidded" to distinguish these cleanrooms from the softer walled modular designs[2].

The relative advantages of hard-walled and modular cleanrooms depends upon the required durability and the application. Hard-walled cleanrooms are often easier to clean and to sanitise because there are fewer joints and seams and this may be an important factor for contamination control. A second advantage is that, as conventional cleanrooms are well established, the materials for construction and the various coatings (such as epoxy, vinyl sheeting with heat welded seams) are well known within the industry and have an established history of reliability.

Nevertheless, modular cleanrooms are becoming more common and offer the advantage of a fast construction, normally at a lower cost, and allow users of cleanrooms to expand their clean area footprint relatively easily. Modular cleanrooms also offer flexibility and flexible designs are important for small-scale or emerging technologies, such as bio-technology. A modular cleanroom is sometimes described as a "soft-wall" cleanroom and tends to freestanding. A modular cleanroom is a series of pre-engineered and prefabricated components that are prefabricated at a factory. This allows for greater flexibility, and minimises on-site installation time. In general, modular cleanroom systems have lower costs than a traditional cleanroom build. Modular cleanrooms are often steel structures that require no external ceiling supports.

Companies that manufacture modular cleanrooms provide services whereby a cleanroom can be constructed including the changing room, transfer hatches, air showers and air locks. The main benefits of the modular cleanroom approach are that during construction, contamination of surrounding areas is minimised through the use of non-shedding materials and prefabrication. A second advantage is that modular cleanrooms can be easily modified, upgraded or expanded with minimal interruption to the existing cleanroom operation, or if the need arises, even relocated to a new facility. Furthermore, with appropriate cleanroom design, upgrading a cleanroom from one ISO class to another can be implemented with minimal interruption of the environment.

28.2.3 Isolators and RABS

As this book has clearly demonstrated, the key advancement in cleanroom technology is barrier technology through the use of barrier systems, like isolators and Rapid Access Barrier Systems (RABS). In many areas, such as aseptic filling of sterile drug products, such technology is being adopted to replace conventional cleanrooms. The adoption of RABS or isolators, leads to a reduced risk of operational failure and a lower risk of microbial contamination[3] .

Isolators and RABS are sometimes called Advanced Aseptic Processing (AAP) technologies. They have the same objective: to restrict operator access to the most critical areas of the machinery. These are physical barrier methods of product protection and containment that are used during manufacturing operations to separate (primarily) operators from the process. These methods are most often used during open processes or other critical process steps to ensure the product is not exposed to viable organisms and particulate contamination. Of the two, the isolator provides the most complete barrier and is the superior technology[4].

Arguably the most important contamination control step is the sterilisation of the isolator environment. Decontamination within the biopharmaceutical industry has evolved tremendously. Traditionally, formaldehyde was the gas of choice, but because of health and safety concerns it has been replaced by other gases, of which vaporised hydrogen peroxide (VHP) is the most common.

A related aspect to barrier technology, in terms of improving contamination control beyond that afforded from aseptic filling within a conventional cleanroom, is blow-fill-seal technology. For products filled in ampoules, blow-fill-seal technology allows the product to be filled and sealed into vials in a way that minimises the number of process steps involved (and fewer process steps provides fewer opportunities for contamination). Aseptic blow-fill- seal technology integrates blow moulding, aseptic filling and hermetic sealing in one continuous operation to produce aseptically manufactured pharmaceutical liquid products. The key advantage compared to traditional aseptic processing is that blow-fill-seal has the capability of rapid container closure and minimises aseptic interventions.

The use of barrier systems is well described in this book. The reason for highlighting the technology in this chapter is in due to its increased use.

26.2.4 Cleanroom robotics

Given that human manipulations and interventions into the critical zone represents the greatest risk, then reducing the need for human intervention to the lowest possible level is an important part of contamination control. To a degree this can be achieved with an increased use of automation and robotics within the filling zone. In addition, robotics provides a means of ensuring that activities are undertaken in a consistent manner and also a means of adapting the mechanical operation to suit the filling of different types of products. If designed correctly, robotics will not generate a high level of airborne particles and will be able to be sanitised using standard cleanroom disinfectants, without biocide residues impairing their function.

Although robotics remains in its infancy it provides the possibility of reducing human intervention and replacing human manipulations with a lower risk and better performing technology.

26.2.5 Energy conservation

As several chapters within this book have indicated, energy management and energy conservation for cleanroom operations are areas being given increasing attention (whether to reduce energy conservation as part of broader environmental protection for the planet or to reduce operational costs). There have been a number of global initiatives around using less energy and using energy more efficiently across industry. In the past year this has become an important issue for cleanroom

operators. Energy efficiency provides a means for cleanroom users to meet energy targets and save costs. In Europe, for example, the EN 16001 standard (as require by European Union directive: 206/32/EC) requires member states in the European Union to achieve an energy saving of 9% by 2016. The European standard is also connected to the International Standard ISO 14001, that describes environmental management and practices.

Whilst it is sometimes possible to scale down a process to a smaller area that requires clean air, the key way in which cleanroom energy is controlled is through the design and operation of the motorised fan units. As air supply is the main energy using operation, this has received the most attention. Cleanrooms require large numbers of relatively small horsepower motors, mounted directly in the air stream of the air handling system. Fans, powered by electric motors and variable-speed drives, are critical to moving air through cleanrooms according to user specifications.

Such fans are designed so that they require less energy to operate (low wattage), are less noisy (low sound through improved baffling technology), and are of smaller size (a low profile). Advances in microcomputing allow motors to be dynamically self-adjusting whereby the airflow adjusts in relation to changes in pressure or to the filter loading (particle challenge). This allows the motor to require less energy when the cleanroom is in the static state compared with the in-use state.

In relation to energy conservation, many discarded cleanroom products can be recycled. Cleanroom garments, including coveralls, hoods, boot covers, hair nets and masks can be collected, converted and resold as bulk plastics or changed into eco-friendly consumer products.

26.2.6 Antibacterial materials

An important preventative measure for contamination control is the use of antibacterial materials to coat cleanroom surfaces (this is sometimes referred to as "biotrunking"). Such surfaces include stainless steel where silver or copper can be introduced into the steel surface. An advantage of silver ions is that although they have antimicrobial properties they are rarely toxic to human cells.

There are different technologies which can be used to achieve this. One method is to creat an alloy using Active Screen Plasma (ASP), which was developed by the University of Birmingham, UK. ASP technology creates a composite or hybrid metal screen and the combined sputtering, back-deposition and diffusion allows the introduction of silver into a stainless steel surface, along with nitrogen and carbon. The silver acts as the bactemidal agent and the nitrogen and carbon make the stainless steel much harder and durable[5].

As well as cleanroom surfaces, silver has been used in implements like forceps. In addition to silver, other metals with antibacterial properties can be incorporated into surface materials, eg. copper and zinc (and alloys like brass)[6] .

26.2.7 Cleanroom decontamination

There are different techniques used to decontaminate cleanrooms. Traditionally, this has involved either manual cleaning or disinfection by operators using mops and buckets, or decontamination with fumigation units and chemicals like formaldehyde (which, in the gaseous form, creates significant health and safety concerns). More advanced means of cleanroom decontamination involve the use of vapourised hydrogen peroxide (VHP). VHP is created by liquid hydrogen peroxide being placed into special generators which, when operated, create a gas. VHP is an oxidising agent and readily kills most micro-organisms, including spore-forming bacteria like Bacillus. Gas generators can be placed into cleanrooms at known locations. This takes some time and effort to map the cleanroom (which additionally requires some form of validation using chemical and biological indicators)[7].

More advanced applications of VHP generators site the generator in the HVAC system so that the gas vapour can be evenly distributed into all areas of the cleanroom. Comprehensive validation studies are required to develop the appropriate cycles (which move from dehumidification, through conditioning, to decontamination and finally aeration), however once the validation studies have been completed, the time savings are considerable and the effectiveness of decontamination is greater than comparable manual cleaning methods.

26.3 Cleanroom equipment

26.3.1 Air showers

Personnel, once they have gowned, can harbour particles and bacteria on the outside of their cleanroom suit generated from the act of gowning[8]. In order to control personnel contamination, the use of air showers is becoming more widespread. Air showers are self-contained chambers installed at entrances to cleanrooms. The air shower consist of a cubicle, supplied with HEPA filtered air, where the air supply is provided at a high velocity in the form of an "air jet" designed to purge the person or object within the cubicle. The use of an air shower is an effective way of removing surface contaminates from gowned personnel, materials and equipment prior to entering the cleanroom. By providing a high-velocity stream of air through adjustable nozzles, the air shower ensures the efficient "scrubbing" action necessary to remove particulates from whatever might be introduced into the air shower. The time that the person or object remains within the air shower needs to be validated.

Once the particles become dislodged, they are held within the air and are quickly removed through extract air grilles and retained within the air shower's HEPA filter media. Air showers should be designed in a way that prevents both the entry and exit doors being opened at the same time. Where a large number of personnel enter an area air shower, tunnels are used. These are protected passageways that allow large volumes of personnel to pass into controlled areas.

26.3.2 Pass through chambers

In order to pass materials into and out of the cleanroom, pass through or transfer hatches are used more widely. The advantage with such systems is that the contamination risk can be controlled and the level of human intervention reduced. Control is maintained by the design of the chamber only allowing for one door at a time to be opened through interlocking mechanisms. A further control measure is through most chambers being fitted with a HEPA filter air shower purging system in order to maintain particle control. Objects are placed in the chamber and remain there for a validated period of time in order to remove any contamination deposited onto the surface of the object.

Some pass through chambers do not have filters and instead objects are sprayed with disinfectant and left within the chamber until the disinfectant contact time has elapsed. An alternative system is the use of ultra-violet light (UV-C). The ultra-violet lights are normally generated from low-pressure mercury-vapour lamps, which emit about 86% of their light at 254 nanometers (nm). The ultra-violet light is antimicrobial by dimerising microbial DNA through destroying thymine molecules. When sufficient destruction occurs, the micro-organisms are unable to replicate. However, neither manual disinfection nor ultra-violet light technologies achieves the cleanliness levels obtained from the sweeping action of the HEPA filtered air. Manual sanitisation is highly variable, and with ultra-violet light many micro-organisms are shielded from its effects by dust or surface cracks.

26.3.3 Cleanroom ovens

Other items of equipment found within cleanrooms have undergone development. One example of this is with cleanroom ovens. Ovens are used to dry, sterilise or to depyrogenate. They key control mechanism is the temperature and very high temperatures are required. These however, can

ligg.ir

create a problem for the other key aspect: the need for clean air and the temperature can damage re-circulating HEPA filters. In order to protect the HEPA filter, the best functioning ovens require good temperature control.

26.2.4 Cleanroom weighing stations

Following on from the earlier discussion about barrier technology, another type of separative device is the cleanroom weighing station. Cleanroom weighing stations are portable devices with controlled environments which prevent contamination of the sample or product but are also sufficiently stable to prevent the air from disrupting the calibrated balance. The construction of such devices, in stainless steel, makes them easy to clean.

26.3.5 Filling machines

The technology related to product filling machines in cleanrooms continues to advance with systems designed to increase throughput and to decrease the big risk of operator intervention. The most robust machines combine in-line filling and stoppering with continuous motion positive in-line transport systems, which are particularly suitable for filling liquid solutions into cylindrical vials and for rubber stopper insertion. The construction of modern filling machines means that they can be exposed to the VHP sterilisation gas agent required for isolators, whilst the flexible nature of contemporary design allows machines to be positioned under RABS or isolators.

Many of the current technologies utilise lasers to measure fill sizes, headspace gaps and closure seals. Furthermore, in keeping with the earlier discussion on single-use disposable technologies, many filling machines use peristaltic pumps with single-use tubing or tubing sets and disposable filling needles and manifolds. The advantage of peristaltic pumps is that the piston pumps associated with older models of filling machines have many moving parts which not only can break down but are also a common source of airborne particle counts. With these technologies, careful calibration is required to ensure accurate and consistent fill volumes. Nevertheless, provided that these difficulties of measurement are overcome, the many advantages of sterile disposable design can be successfully applied to filling lines.

26.3.6 Environmental monitoring

The methods for monitoring the cleanroom environment (microbial and particulate) have been established for a long time and the conventional methods for viable monitoring and airborne particulate counting are described in this book. With airborne particle counters – sophisticated instruments that measure airborne particles in a number of size ranges, technological developments have led to counters being able to take the required one cubic metre sample size in ever faster time periods, with the faster counters able to sample within ten minutes. The software used for analysis and classification has also advanced considerably. As the technology has advanced, the assessment criteria for particle counts has become more stringent (with the issuing of the ISO 21501 standard and the specification for counting efficiency)[9].

One more recent development with environmental monitoring methodologies is with optical instruments. These aim for the real-time counting of micro-organisms and non-viable particles from samples of air. Optical spectroscopy is an analytical tool that measures the interactions between light and the material being studied.

These instruments work by elastic light scattering. This measures two things:

- Particle counts: where the size of a scattering particle, as it passes through a light beam, is comparable to a certain wavelength of light. The intensity of the scattering is dependent upon the size of the particle. Such systems will detect and quantify particles within a 0.5 to 20µm range.

- Microbial counts: a 405nm laser that intersects the particle beam, so that as a particle passes through, its scattering measures the intrinsic fluorescence of the particle, from the metabolites (such as NADH, dipicolinic acid and riboflavin) inside micro-organisms. This distinguishes micro-organisms from inert particles[10].

The advantage of such instruments is that they produce a real-time result, thus overcoming the limitations of growth-based methods which require several days of incubation in order to produce a result. Furthermore, the instruments are capable of continuous monitoring.

26.4 Cleanroom apparel

26.4.1 Wipes

Cleanroom wipes have been used in cleanrooms for decades and are used to wipe up liquids and to clean or sanitise surfaces. When using wipes within cleanrooms, the primary concern is with the generation of particles from the shedding of fibres. Over time the manufacture of wipes has improved in order to make them low fibre shedding (and thus low particle generating).

The best designed wipes are made from material like polyester and have each edge sealed (the perimeter of the wiper can be a great source of contamination if it is not properly finished. For example, polyester wipers made from the same fabric can have drastically different levels of contamination based on the way the edges are cut and finished. The predominant ways that wipers are cut are heat-sealed, laser cut, hot wire cut, and knife cut). In order to aid "cleanability" and to avoid surface abrasion, wipes should be of a smooth knit construction (heavier wipers are of a double-knit design). A more recent development with the manufacture of wipes is with hydro-entangled polyester wipers which are created by spraying high-pressure water onto a bed of polyester to form a light-weight, non-woven fabric. These wipers have similar characteristics to knitted polyester wipers, but are much lighter and less costly.

For use in cleanrooms, wipes should be provided sterile or be able to be sterilised in the cleanroom.

Some wipes are supplied with the cleaning agent (detergent) or disinfectant saturated into the wipe material. These are useful in that the need to have a separate cleaning agent is avoided. However, the user must be able to demonstrate that the disinfectant is distributed evenly throughout the wipe, that it is at the required concentration and that the active ingredient remains activity throughout the manufacturer's expiry time.

26.4.2 Cleanroom mats and flooring

Cleanroom mats are located at the entrance to changing areas and at other important junctures, such as areas where equipment is passed into the cleanroom. Conventional mats are multi-layered mats designed to capture dirt, dust and debris from foot traffic and also from equipment wheels through particles adhering to the adhesive coated onto the mat. Mats are manufactured from acrylic-based materials which inhibit microbial growth. The main concern with using mats is the removal of each sticky sheet, which can lead to particle generation.

An alternative to the 'sticky mat' is polymeric flooring[11]. Polymeric flooring is manufactured from a non-toxic, plasticised material designed to retain particulate contamination (viable and non-viable) that comes into contact with its surface. A function of polymeric flooring is to attract particles to its surface and retain them for long periods of time (until such a time when they can be removed, totally, through cleaning and disinfection). Some types of flooring are also anti-microbial in that they contain silver ions[12] . The antimicrobial properties of silver stem from the chemical properties of its ionised form, Ag^+. This ion forms strong molecular bonds with other substances used by bacteria to respire. When the Ag^+ ion forms a complex with these molecules,

they are rendered unusable by the bacteria, thus depriving them of necessary compounds and eventually leading to their death[13].

26.4.3 Garments

Given that people are the primary source of contamination in any cleanroom; the selection of clothing and garment systems is critical to the quality of the products manufactured in the cleanrooms and the overall operation of the cleanroom. There is a range of different materials available of different qualities.

As with wipes and mats, cleanroom garments have improved in design to make them more comfortable for the user to wear and in improving the integrity of the gown to retain particles. High quality gowns are tested for material properties such as particle generation, particle filtration, and resistance to wear and tear-related damage. Although such improvements have taken place, it is important that the cleanroom facility has a time limit on how long a gown can be worn for before it needs to be changed, as gowns will have a limitation in terms of their ability to minimise the shedding of particulates[14].

There have also been improvements in the way that gowns are laundered, with particle counting being required in order to assess the cleanliness of the gown. It is also required that all gowns used in cleanrooms be irradiated by gamma radiation as a means of ensuring gown sterility before being worn by the operator.

26.4.4 Cleanroom consumables

The transfer of materials into and out of a cleanroom or clean air device presents one of the most significant contamination risks. Most items untreated by sterilisation or disinfection are contaminated with micro-organisms. This phenomenon creates a problem as many items, particularly plastics, cannot be sterilised. Although such items can be disinfected, where contamination originates from spore-bearing micro-organisms, the contamination cannot be removed by many of the standard cleanroom disinfectants. This risk has helped trigger the recent advance in disposable, plastic irradiated consumables for cleanrooms. These are typically presented in sterile multi-packs. These are bags, often constructed from polythene or other low particulate material, which contain consumables like connectors and syringes. The packs are normally double or triple bagged so that any contamination on the outside is removed prior to transfer into a critical area.

26.4.5 Furniture

In relation to cleanroom design and layout, more attention has been paid to the ergonomics of cleanroom design. The cleanroom as a work environment is a challenge to ergonomics because of the controlled and standardised conditions. However, attention to human work can lead to process efficiencies[15].

A second aspect in relation to cleanroom furniture is the construction material. Benches and tables are used in many areas within cleanrooms. Gowning benches are used to sit on while donning cleanroom attire. Tables are used for activities like inspection, assembly and packaging. Various materials and laminates are used, although for the highest levels of cleanliness, furniture of a stainless steel construction is used. For some applications, high quality plastics are used in place of stainless steel.

26.4.6 Documentation

Activities within cleanrooms require documenting as part of GMP. Paper is less commonly used in cleanrooms with the advent of electronic devices such as personal digital assistants (PDAs). Where paper is used, this needs to be of cleanroom design. Special types of paper have been

developed (containing low particulate filler and fibres coated with latex) that can be sterilised by steam or gamma irradiation.

26.5 Single-use technology

Arguably the most important development in the second decade of the 21st century for cleanroom operations has been with single-use systems. Single-use technologies are sterile, plastic disposable items used to replace traditional pharmaceutical processing items which require recycling, cleaning and in-house sterilisation. Single-use, sterile, disposable technologies (sometimes referred to as biodisposable technologies) are available in many different formats and confer different advantages for pharmaceutical manufacturers[16].

The main reasons for technological change are a mix of process efficiencies (including cost reduction) and sterility assurance. Single-use technologies include tubing, capsule filters, single-use ion exchange membrane chromatography devices, single-use mixers, bioreactors, product holding sterile bags in place of stainless steel vessels (sterile fluid containment bags), connection devices and sampling receptacles. Single-use disposable technologies are generally manufactured from plastic polymers involving processes of injection moulding, extruding and blow moulding. The assembly of components should be undertaken in an ISO 14644 class 5 cleanroom and sterilised by gamma irradiation.

Considerable research and investment has been directed into such technologies to reduce processing time, to reduce costs and seek improved sterility assurance. The primary time and cost savings arise through the removal of the need to clean and recycle equipment, like vessels. The investment into a higher level of sterility assurance is to improve controls whilst the product is being manufactured and filled, not least to overcome the concern that by the time a product is assessed for batch release using the final product sterility test, there is nothing that can be done to correct a sterility problem with the manufacturing of the batch should the sterility test fail.

Such technologies have reduced risks by allowing pharmaceutical organisations to move away from equipment which needs to be sterilised or consumables which are recycled or pose a risk with their transfer into cleanrooms, to disposable and single-use sterile items. Thus, the advantages of single-use technology can be listed as follows:

- eliminating the need for cleaning
- removing the requirements for the pharmaceutical company to perform in-house sterilisation of equipment like stainless steel processing vessels (typically by autoclaving) for all components
- reducing the use of cleaning chemicals
- assisting with storage requirements
- lowering process downtime and increasing process flexibility and reducing risks of cross-contamination[17].

Some types of single-use technology used in cleanrooms are described below.

26.5.1 Aseptic connections

A critical cleanroom step is the aseptic connection, especially for aseptically filled products. Types of aseptic connections include the connection of a vessel or filter to another item of equipment for the transfer of fluids. Conventional methods of connection involve steps such as clamping or heat-welding of tubing. The major risks arising from this step are from the external environment and from any microbial contamination which could be transferred from the operator’s hand. Although glove sanitisation is regularly undertaken for aseptic filling operations, a risk exists, when conventional connections are undertaken, that bacteria transient or

residential on human skin can be transferred from the hand of the operator onto the connector and potentially into the product.

Innovations in aseptic connection technology have led to the development of single-use connector systems to allow for a totally enclosed and automated process. These are based on the so-termed alpha-beta principle which allows the connection to be performed in an environment this does not require unidirectional airflow cabinets or other capital equipment to maintain sterility. This principle allows liquid sterile products to be transferred simply and safely, towards or from contained areas, via small-scale, rapid transfer ports. These devices shorten the time required for the connection and could, depending upon the risk-based position adopted by the pharmaceutical manufacturer, remove the requirement to undertake the connection under ISO class 5 air (that is, air provided by a unidirectional airflow cabinet). The design of the connectors also facilitate a fast and safe disconnection through the alpha-beta sections being disconnected through the pushing of an internal plunger which allows the two parts to become separated. The rapid disconnection means that any backflow of fluid is avoided.

26.5.2 Disposable product holding systems

In line with advances in aseptic connections, there is a drive towards the adoption of disposable bag technologies in biopharmaceutical production and away from fixed, stainless-steel equipment (which requires more complex engineering configuration and far more components in terms of separative valves and piping). This change has arisen because such technologies can reduce validation and clean-in-place requirements, lower the requirements for pure water (connected to clean-in-place systems), clean steam (produced from steam generators) and WFI, and cut costs (such as from reduced set-up times). For example, the cleaning and sterilisation down-times for stainless steel vessels, transfer lines, or filter housings might require 8–10 hours and copious amounts of cleaning solutions and Water-For-Injection (WFI), none of which is required with single-use approaches. The single-use technology takes the form of plastic bags or packs used to process or store product.

The common configuration of product holding bags is as single-use assemblies consisting of either two-or-three dimensional bags connected to a manifold of tubing, connectors and filters. The design is such that no part of the equipment will have direct contact with the product unless the component or part of the equipment is also sterile, single-use, and maintains the sterile liquid pathway of the closed system assembly. For the future, rapidly developing connectivity will enhance the development of connected, integral systems and potentially total disposable processes.

26.5.3 Biocontainer sampling bags

For sterile products, there is a regulatory requirement that a sample is taken of the bulk product prior to the final filtration (using a 0.2µm filter) of the bulk product. This filtration is either into a vessel which is then connected to a filling machine, or prior to the transfer of the product along a transfer line which directly supplies the filling machine. The sample is taken for bioburden testing using the Total Viable Aerobic Count (TVAC) technique. The conventional way to sample the bulk product for bioburden is to withdraw a quantity of the material from a holding vessel, using a valve or by syringe, and to transfer this to a sterile sampling container. This process, which is operator dependent and involves multiple steps, poses a risk of adventitious contamination and thus of a false-positive result being reported which, at a sufficiently high bioburden, could lead to product rejection.

The use of single-use, sterile biocontainer bags allows the sample to be taken in a way which eliminates the possibility of external or operator contamination triggering a false-positive result.

26.6 Risk management

Although "risk management" is itself not a 'technology', the risk management concept is now embedded in many of the design concepts and technological applications for cleanrooms. It is clear from many of the book chapters that quality risk management is a regulatory expectation, and indeed, risk management has always been an intrinsic part of the pharmaceutical operations and with healthcare. What is different is the implementation of a more standardised, formal processes of managing cleanroom operations.

An important application of risk management has been in building quality into a facility during the design, construction, commissioning, qualification as well as the validation stages of cleanrooms. Cleanroom technology and design can be divided into three areas, and to each area risk assessment should be applied:

1. The design and construction of the room. This involves an assessment of:
    - The design standards that should be used
    - The design layout and construction materials
    - The supply of services to the cleanroom
2. After the cleanroom has been installed and working, it must be tested to check that it conforms to the stipulated design. During the life of the cleanroom, the room must also be monitored to ensure that it continually achieves the standards required.
3. The cleanroom should be operated correctly so that the manufactured products are not contaminated. This requires that the entry of people and materials, the garment selection, cleanroom disciplines and the cleaning of the room are all carried out correctly.

Risk analysis and risk evaluation together represent the two fundamental parts of the risk assessment phase of a risk management process cycle. The assessment of risk involves either the quantitative or qualitative determination of one or more risks. Risks are generally recognised as being related to a situation, event or scenario in which a recognised hazard may result in harm. Quantitative risk assessment requires a type of calculation. This is often based upon the magnitude or severity of the risk and the probability that the risk will occur.

Risk assessment involves identifying risk scenarios either prospectively or retrospectively. With the former, this involves determining what can go wrong in the system and all the associated consequences and likelihoods; with the latter, this looks at what has gone wrong and using risk assessment to assess the process, product or environmental risk and to aid in formulating the appropriate actions to prevent the incident from re-occurring. Risk analysis is also highly beneficial in that it can also be used to identify and justify process improvements.

A significant advance in risk management practices took place when the International Convention for Harmonisation published a guideline Quality Risk Management (ICH Q9) which was a significant milestone in the development of quality risk management activities within the pharmaceutical industrial and its regulatory activities[18].

Risk assessment tools and techniques can be applied to every aspect of pharmaceutical processing. An important part of this application involves an understanding of the process.

Formal risk approaches normally share four basic concepts, which are listed below[19].

1. Risk assessment

Risk assessment is the assessment of effects of the incident or scenario (such as a microbiological

contamination event). It involves the use of risk analysis tools and the evaluation of risk. The object of using these tools is to identify the root cause.

2. Risk control

Risk control is centred on risk reduction or risk mitigation. This process consists of corrective actions taken to resolve the incident and preventative actions needed to avoid a recurrence of the incident in the future. At some point, risk control will also involve a consideration of risk acceptance as the measures put in place will ultimately need to be accepted or rejected.

3. Risk review

The risk review is the follow up of action items and the final summary and evaluation of the incident. This should be approved by senior management.

4. Risk communication

Risk communication discusses the important steps involved in reporting and discussing the incident. It should also ensure that the appropriate parties are included in resolving the incident.

Before commencing a risk assessment, it is important to define the magnitude and scope of the assessment (remaining focused on what is to be achieved); to select the appropriate team (often an interdisciplinary team is best); selecting and reviewing the appropriate risk management tool; deciding upon any numerical scale to be used (if any is applicable) and prioritising the different problems to be addressed. Most approaches begin by constructing a process map.

The various analytical tools used for conducting risk assessments are similar, in that they involve[20]:

- Constructing diagrams of work flows (these are pictorial representations of a process designed to break the process down into its constituent steps, this allows complex processes to be simplified)
- Identify hazards (which can be intrinsic, ie. specific or inherent to the process or equipment, or extrinsic, ie. factors which are external to the process or equipment but which might impact upon it). In doing so, this helps:
- Pin-point the areas of greatest risk
- Allow an examination of the potential sources of contamination
- Decide on the most appropriate sample methods
- Help to establish alert and action levels for on-going monitoring and to detect future risks
- They should be flexible enough to take into account changes to the work process and any seasonal activities

In addition, a standard series of questions should be asked. For example, when considering equipment breakdown[21]:

- What is the function of the equipment?
- What are its performance requirements?
- How can it fail to fulfil these functions?
- What can cause each failure?
- What happens when each failure occurs?
- How much does each failure matter? What are its consequences?
- What can be done to predict or prevent each failure?
- What should be done if a suitable proactive task cannot be found?
- If a risk cannot be eliminated then how can it be reduced?

- If the risk cannot be reduced then how can it be monitored?

Thus the general approach is to recognise a risk, rate the level of the risk and then set out a plan to minimise, control and monitor the risk. Subsequent monitoring of the risk will help to determine any follow up action.

26.7 Summary

This chapter, in closing the book, presents an overview of recent developments in cleanroom technology. It indicates that cleanrooms and associated technologies continue to evolve, driven either by regulatory guidance and the need to avoid contamination, or by the desire to reduce operating and energy costs.

26.8 References

1. Lee G, Sacks R, Eastman CM. Specifying parametric building object behavior (BOB) for a building information modeling system. *Automation in Construction* 2006; **15**(6):758–776.
2. Möllmann M. Modular cleanroom construction success. *Health Estate* 2007; **61**(8):74-6.
3. Agalloco J. Barriers, isolators and microbial control. *PDA Journal of Pharmaceutical Science and Technology* 1999; **53**(1): 48-53.
4. Sandle T, Saghee MR. Advances in cleanroom technologies. *Express Pharma*, 16th-30th September 2010. On-line Paper: Express Pharma (accessed 30th September 2010).
5. Weili Hu, Shiyan Chen, Xin Li, Shuaike Shi, Wei Shen, Xiang Zhang, Huaping Wang. *In situ* synthesis of silver chloride nanoparticles into bacterial cellulose membranes. *Materials Science and Engineering* 2009; **29**(4):1216-1219.
6. Noyce JO, Michels H, Keevil CW. Potential use of copper surfaces top reduce survival of epidemic methicillin resistant *Staphylococcus aureus* in the healthcare environment. *J Hosp Infect* 2006*;* **6**(3):289-297.
7. La Duc MT, Dekas A, Osman S, Moissl C, Newcombe D, Venkateswaran K. Isolation and characterisation of bacteria capable of tolerating the extreme conditions of clean room environments. *Appl Environ Microbiol* 2007; **73**(8):2600-2611.
8. Ljungqvist B, Reinmüller B. Evaluation of product protection efficiency for two clothing systems used in food manufacturing. *Renhets Teknik* 2008; **3**:14–17.
9. ISO 21501-4 Determination of particle size distribution – Single particle light interaction methods – Part 4: Light scattering airborne particle counter for clean spaces. Geneva, Switzerland: International Organization for Standardization, 2007.
10. Kunnil J, Sarasanandarajah S, Chacko E, Reinisch L. Fluorescence quantum efficiency of dry *Bacillus globigii* spores. *Optics Express* 2005; **13**(22):8969-8979.
11. Sandle T. The use of polymeric flooring to reduce contamination in a cleanroom changing area. *European Journal of Parenteral and Pharmaceutical Sciences* 2006; **11**(3):75-80.
12. Barrett GFC. Polymeric flooring demonstrates particle retention properties. *Cleanrooms,* November 1996.
13. Clibbon C. An evaluation of the effectiveness of polymeric flooring compared with peel?off mats to reduce wheel- and foot-borne contamination within cleanroom areas. *European Journal of Parenteral Sciences* 2002; **7**(1);13-15.
14. Ljungqvist B, Reinmüller B. *Cleanroom Clothing Systems; People as a contamination source*. River Grove, IL,USA: PDA/DHI Publishing, 2004.
15. Pheasant S. *Ergonomics, work and health.* Basingstoke, UK: Macmillan Press, 1996.
16. Goldstein A *et al*. Methods and considerations for disposable implementation – design, compliance, and validations. *BioProcess Int 2005;* **3**(6):S20–S35.
17. Sandle T, Saghee MR. Some considerations for the implementation of disposable technology and single-use systems in biopharmaceuticals, *Journal of Commercial Biotechnology* 2011; **17**(4):319–329.

[18] O'Donnell K, Greene A. A risk management solution designed to facilitate risk-based qualification, validation & change control activities within GMP and Pharmaceutical regulatory compliance environments in the EU, Parts I & II, *Journal of GXP Compliance* 2006; **10**:(4).

[19] Quality Risk Management (ICH Q9), International Conference of Harmonisation of Technical requirements for Registration of Pharmaceuticals for Human Use, November 9th , 2005, available at www.ich.org

[20] Saghee MR, Sandle T. Embracing quality risk management: The new paradigm, *Express Pharma,* 16th-30th September 2010, On-line paper: Express Pharma (accessed 30th September 2010).

[21] Sandle T. Risk Management in Microbiology. In: Saghee MR Sandle T, Tidswell E, eds. *Microbiology and Sterility Assurance in Pharmaceuticals and Medical Device*s. Hyderabad, India, Business Horizons, 2011.