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Introduction to Contamination Control and Cleanroom Technology

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Preface

Contamination control is becoming more and more important in industrial areas where there are ever-increasing requirements for cleanliness and hygiene in production. Most of all, contamination control is characterized by an holistic view, i.e. having a total overview of the entire production system whilst at the same time taking into account all the minor processes that are involved in creating the larger system. In practice this means that personnel working in this field should be aware of the interdependency of the component processes and the effect that this has on the outcome of the production process as a whole.

With this book, which is the first in a series of three books, I have tried to help the reader in developing this holistic view. The book deals with cleanliness and hygiene, how this may affect the outcome of a process and most of all how it can be controlled and in many cases also improved. The content of the book reflects the latest research and standardization work together with applications in both industry and research and development laboratories.

The book provides general and basic information for everyone that works with higher and advanced levels of cleanliness, in both controlled environments and cleanrooms as well as in clean zones. This book is most of all intended to be a rich and inspiring source of knowledge for anyone who works within the pharmaceutical, microelectronic, food and beverage and also the micromechanical and optical industries.

The content of the book is based on modern research and application and also on discussions I have had with people whom I met in my daily work as a lecturer and consultant. I would like to acknowledge the support of my family; Pia, Anna and Johan and thank them for their patience and inspiration. Without their never-ending support (especially on

late evenings and weekends) this book would never have been written. I also wish to express my gratitude to Mrs. Camilla Dahl for helping me to finish this manuscript, her friendly manner and humour helped to make the task an enjoyable one.

I hope that all of you who read this book will find it of interest and that you will make use of it in your work. Last but not least, I hope that you will find the area of contamination control and cleanroom technology as exciting and interesting as I do.

Malmö, August 2000 *Matts Ramstorp*

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1.1 Introduction

The overall purpose of contamination control is to prevent different types of contaminants, particularly those of a critical nature, from damaging products, production processes and also people. Contaminants, from a general point of view, are normally defined as substances (solids, gases or liquids) or physical risk factors that are found in the wrong place (and/or) at the wrong time.

When talking about contamination control, people in general tend to focus on that part of the technology concerned with the latest scientific and/or industrial approaches. Parallels are often drawn between the use of cleanrooms and contamination control, a comparison that is not completely appropriate. Within modern science, so-called interdisciplinary technologies, are used more and more frequently, as exemplified by the field of biotechnology. Contamination control is, like biotechnology, an interdisciplinary science.

Interdisciplinary sciences have become more popular than the traditional sciences, especially in industrial areas, mostly because of what the technology actually can accomplish from a practical point of view. However, there is a risk associated with this more popular approach, namely that one may totally or partially forget that new and interdisciplinary techniques do not stand alone. All interdisciplinary techniques usually have quite important and interesting historical as well as technical backgrounds.

Biotechnology for example, has for some considerable time, been utilizing a vast number of microorganisms in order to produce, among other things, food and beverage products. The area of biotechnology also includes the technology that has made a major contribution to the survival of mankind, namely in the field of agriculture.

The same applies to the technical field that in everyday

language is called contamination control. Many of the different parts that together create this technology have a long history. An example is filters and filtration, which is one of the most frequently used unit operations within contamination control. Filtration technology has, for example, been used routinely for many years to produce clear and ›particle-free‹ food products.

1.2 Contamination control

Contamination control, or C^2 for short, includes all the different elements that are used to protect products, process equipment and personnel from being negatively affected by contaminants. The use of cleanrooms is today incorporated as a modern part of contamination control. The first cleanrooms were built and used within hospital environments more than 100 years ago, but their development and improvement has accelerated over recent decades and especially during the Second World War. Today, the use of modern cleanrooms is often a ›must‹ in the production of many products used in everyday life.

Contamination control is used within many different industries and for the production of a large variety of different products. The following are among the various industries that are generally negatively influenced by contamination with inorganic matter, often in the shape of very small particles, e. g. solid material:

- Microelectronic industry
- Semiconductor manufacture
- Industries performing sensitive micromechanical work
- Optical industries

Among industrial branches that are mostly affected by material of organic origin, particularly viable as well as non-viable microorganisms together with other organic matter, are the following:

- Biotechnological industries
- Pharmaceutical industries
- Industries producing and packaging medical implants and medical devices

- Food and beverage industries
- Hospitals and other health care institutes

1.3 History

As stated previously, the first cleanrooms where built and used within hospitals. Historical, scientific work published by Pasteur, Lister, Kock and many others more than 100 years ago, focused on the fact that bacteria where responsible for many of the different infections that, at that time, posed major problems for patients. Other scientists later proposed that total elimination or even reduction of bacteria within the hospital environment would be of great benefit and would hinder the growth and dispersion of infectious diseases. This was actually the introduction to and start of hygiene, cleanliness, cleanrooms and clean zones.

Lister used a technique that was called antisepsis. He dispersed carbolic acid as a fine aerosol in the air surrounding the patient during a surgical operation, in order to reduce the possibility of infection caused by airborne microorganisms. The various experiments performed by Lister led to the development of different aseptic techniques for work in controlled environments and/or cleanrooms, as for example the use of clean instruments, sterile gloves, facial protection and specially designed garments for personnel to use. Many of the basic principles arising from the work of Lister are used today, although in different forms, for the provision and maintenance of a level of cleanliness suitable for the manufacture of sensitive products.

Although several of the different working techniques that were developed a long time ago are still in use today, the entire area of contamination control was revolutionized by the introduction of what is often called positive ventilation. Within the area of contamination control positive ventilation means in practice the addition of filtered, clean air.

Ventilation by the artificial addition of air was quite unknown approximately 60–70 years ago. Within the hospital sector this technique was applied more and more frequently, but the other main area that benefited from the use of positive ventilation was the area of manufacturing. Artificial ventilation and its use has promoted far greater possibilities for production under controlled conditions.

The industrial utilization of clean environments, i.e. the introduction of clean filtered air, had its breakthrough during the Second World War in the USA and England in connection with the production of guns, tanks and various critical parts of aeroplanes (gyroscopes etc.). The technique made it possible to manufacture higher quality products that were more accurate and maintained their high quality. The technique reduced the number of rejected products, for example when producing ball bearings and gyroscopes. There was also a very strong driving force to use this technique in the nuclear, biological and chemical fields of the war industry, in order to protect both the people working with the products and the products themselves. This forced the industry to develop more efficient and secure filter materials. This driving force led to the development of the HEPA-filter (HEPA = High Efficient Particulate Air). The HEPA-filter made it possible to supply critical production environments with cleaner air, which in turn led to lower levels of airborne contaminants.

During the period between 1955 and 1965 specially designed cleanrooms were produced in which large volumes of air were introduced through special equipment called ventilation distributors. This type of arrangement made it easier to supply air to the cleanroom in a more uniform manner; furthermore, it also provided a much better method of controlling the cleanliness of the room.

1961 was an extremely important year within the area of contamination control. During this year the technique of laminar flow or what is traditionally called LAF, (LAF = Laminar Airflow) was introduced. Today the term LAF has, to a greater extent, given way to the term UDF (UDF = Unidirectional Flow). UDF is, in comparison to LAF, the more correct term to use, since UDF, as its name implies, describes how the air moves on its way through a cleanroom (Figure 1).

Even if the two terms LAF and UDF describe two different types of air movements, they actually mean exactly the same flow pattern, and the two terms are thereby considered synonymous. The filtered and thereby cleaned air is supplied through filters mounted in the cleanroom, normally in the ceiling or in a wall. The introduced air is then allowed to pass through the room in a unidirectional flow pattern and, finally,

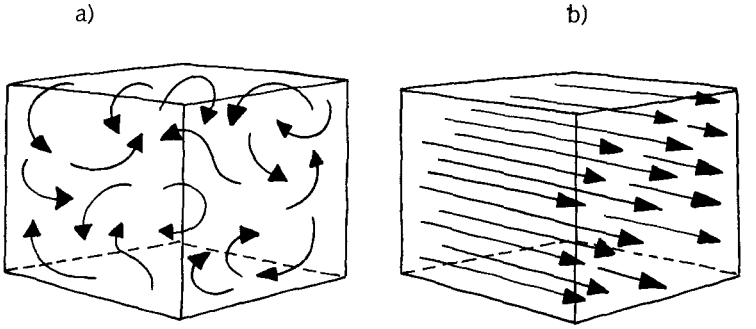

Figure 1. Conventional airflow versus unidirectional airflow. Conventional airflow in a room means that the airflow is more or less random (a), whereas a unidirectional flow (UDF-flow) means that the air is travelling along a unidirectional path from one end of the room, either from the ceiling or a wall, to the opposite end where it leaves either through a raised perforated floor or a perforated wall (b). A unidirectional flow room was previously called a LAF-room. Unidirectional airflow is the most effective way to eliminate contaminants from a room or a zone

leaves the room through a raised, perforated floor (for vertical systems) or in some cases, an opposite perforated wall (for horizontal systems). Due to the high velocity of the air when passing through the room, airborne contaminants are captured by the air stream and are by this action transported out of the room through the perforated floor or opposite wall. The airflow in a UDF cleanroom gives a much better and effective system as compared to the traditional, turbulent and, in fact, diluting airflow system (see Section 4.12.2).

Recent developments within contamination control and cleanroom technology have focused on the general air supply to the room as well as on the supply to smaller, specially designed, zones in certain local parts of the cleanroom where higher air quality is required in order to give products their desired quality. In other words the development of production systems tends to focus on a generally lower degree of cleanliness in the cleanroom overall, with certain areas in the room where specific steps in the manufacturing process are carried out, having a higher standard of air quality provided by point-of-use cleanliness systems or clean zones.

1.4 Standardization

The development of contamination control and cleanrooms has primarily been a result of research and development taking place from 1940 onwards. Some of these development and research projects are as follows:

- The atomic bomb (the Manhattan project) during the period 1942–1944
- The biological and chemical war industry
- Instrumentation for the air and space industry
- Industries working with miniaturization, i. e. the reduction in size of different components in equipment that is being miniaturized
- Work that is of a high-risk nature both to the personnel working on the project and to people living near industrial plants where this high-risk production process is carried out, e.g. industrial plants dealing with radioactivity or hazardous microorganisms
- The space program, for example the production of the Lunar module
- Surgical operations in humans requiring extreme cleanliness, e.g. within the field of orthopaedics
- Tumour therapy, both when producing, preparing and administering solutions containing cytostatic components
- Microelectronics, corresponding more or less to the area of miniaturization

The development of contamination control within different industrial branches in general fulfills the fundamental need of the present time and also those requirements that can be foreseen in the near future. In order to make full use of the development within these industries and also to try to systematize this development, efforts are being made on a global scale to employ a system of standardization. The quality standard ISO 9000 is an example of how, through discussions of quality, mutual agreement can be reached on how to describe and present the quality system used by a company for instance, or used in a production procedure.

Within the area of contamination control standardization is an ongoing area of work. When working with contamination control and cleanroom technology, different standards,

norms and other recommendations and practices are encountered. The most frequently used standards and norms are:

- General standards such as the US Federal Standard 209, British standard 5295 and ISO ›209‹
- Pharmaceutical standards such as for example, Good Manufacturing Practice (GMP)
- Laboratory standards exemplified by Good Laboratory Practice (GLP)
- Risk analysis such as Hazard Analysis Critical Control Point (HACCP)

Different standards and recommendations have different meanings as well as different target groups. US Federal Standard 209 for example, specifies the cleanliness of air in a cleanroom, e.g. it specifies the number of particles suspended in the air. This is measured as the number of particles of a given size or larger per unit volume. British standard 5295 and ISO ›209‹ generally have the same purpose as the US Federal Standard 209. Hazard Analysis Critical Control Point (HACCP) is a risk analysis that is basically intended for the food and beverage industry. HACCP however, cannot be used as a general quality system since it only deals with contaminants that can be dangerous to health, and in particular to the health of the consumer.

1.4.1 Good Manufacturing Practice (GMP)

Good Manufacturing Practice (GMP) was formulated by the FDA (Food and Drug Administration) in the United States during the 1960s. GMP consists of a series of rules that were formulated in order to avoid serious failures in the production process being caused by mistakes and defects occurring during production of pharmaceutical products. The following are examples of some of the outcomes of the failure to comply with GMP: blindness caused by non-sterile eye ointment, *Salmonella* infections due to infected raw materials and sepsis as a result of the use of non-sterile intravenous solutions.

Since the 1970s GMP has been developed further by different organizations: FDA-GMP (1962), WHO-GMP 1969, PIC-GMP (Pharmaceutical Inspections Convention) 1970 and EG-GMP 1997.

The general aim of Good Manufacturing Practice (GMP) is to standardize four vital areas *strength*, *cleanliness*, *effect* and *identity*. This ›standard‹ covers all parts of a production process including for example *personnel*, *production facilities*, *equipment*, *documentation* and *validation*.

With regard to the personnel, the following points are vital:

- Qualified personnel should be available in sufficient number
- All work should be performed according to an organizational scheme
- All areas of responsibility should be clearly defined
- A record of competence and education should be available for each individual employee

The facilities should be constructed so as to

- Prevent and avoid every kind of contamination
- Avoid the mixing of products
- Facilitate the flow of material
- Minimize unnecessary traffic of personnel

The process equipment should be designed and constructed in such a way that it shall not remove anything from the product nor add anything to the product. In short the equipment should be totally inert towards the product or process undertaken.

With regard to the documentation, the following should be considered:

- The title and type of document should be unequivocal
- It should be easy to identify the present version
- It should be easy to read as well as precise and clear
- It should be authorized

Validation is a very commonly used term, which in practice means that one should define and follow such activities that are demanded in order to secure and document a production process or a part thereof, so that it functions during a routinely performed task. Validation aims to approve the process and incorporate the following: *installation qualification*, *process qualification*, *product qualification* and *certification*. Certification

is the final judgement and also the formal approval after validation.

1.5 High levels of knowledge are of vital importance

In comparison with traditional and basic sciences, interdisciplinary sciences often require a broad but not too detailed range of knowledge. Contamination control is no exception in this respect. This situation is evident from the varying degree of competence that is required from personnel working with contamination control in order to perform their tasks.

The following list of knowledge criteria is often considered as a ›must‹ for persons working with contamination control. Personnel need a general knowledge of different types of contaminants. Furthermore, they need knowledge of the structure of the buildings where they work, often including different types of barrier systems, such as cleanrooms and other types of clean zones required for the protection and/or safety of the personnel as well as the product. Other examples include, being familiar with (1) the different equipment utilized for clean, hygienic and secure production, (2) the equipment, apparatus and methodology necessary for measuring different contaminants and (3) cleanroom garments and their maintenance, that is, the need for regular washing and decontamination. Finally, there should also be a general awareness among the personnel of the different standards and regulations that are used as holistic tools for production, maintenance, control and care.

1.5.1 Fifty years of contamination control

Even if the basic and primordial parts that are used within contamination control are not as recent as people in general tend to believe, the most important landmarks within this technical area have been developed during a relatively short period of time. The development has accelerated since the end of the Second World War, when equipment such as the HEPA filter was developed together with, for example, such analytical tools as the slit sampler and synthetic textiles for protective garments.

In total, the industrial development during the last four to five decades has resulted in the following:

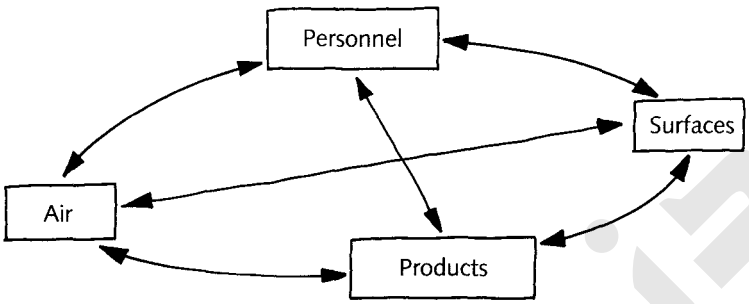

Figure 2. The interaction between the four major areas of contamination control. The figure shows how personnel can affect the cleanliness of the air, surfaces, products and/or the process. The same general pattern applies to how the cleanliness of personnel is affected by the cleanliness of the air, surfaces and/or the products

- The development and use of the LAF technique, LAF benches and LAF surgical cabinets
- Equipment such as the particle counter, that today can detect and count smaller and smaller particles and also with a higher degree of accuracy
- Computer-aided multi-channel particle counting instruments that can handle vast amounts of data from many different measuring points at the same time
- Different types of biohazard equipment to be used in order to gain total containment of various forms of dangerous material such as, for example, microorganisms
- The Ultra HEPA filter (so-called ULPA filter) with higher removal capabilities than the HEPA filter
- Non-woven textile materials for use in protective clothing (as an alternative to traditionally woven textiles)
- The Scanning Electron Microscope (SEM)

1.5.2 The source, dispersion and deposition of contamination

Contaminants are of central importance within the field of contamination control and cleanroom technology. Figure 2 shows a schematic representation of the source and dispersion as well as deposition of contaminants, in a general and holistic way. The figure shows the four general sources from where different contaminant may arise. Furthermore, this figure shows different ways that contaminants can be distributed from the source and finally where they normally settle.

The four general sources in this figure, personnel (i.e. the human being), air, surfaces and product, are all connected (shown in the figure with double arrows). All the four sources are equally important from a general point of view. From a practical point however, there is always a need to ask which of these four sources that has the highest significance with respect to the work being undertaken. The answer to this question is that naturally all four are of equal importance, but that their individual impact on the outcome of the production process depends on the nature of the product and also how sensitive the resulting product and/or the production system actually is.

As can be seen from Figure 2 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 source of contamination by human beings is through the natural process of regular shedding of epidermal cells. All cells on and in man, except for nervous tissue, are continuously being replaced by new ones. Old skin is in this way continuously replaced by new skin. It is generally assumed that the outer layer of human skin is replaced every fourth day. The old skin is not replaced in the same way as it is in reptiles for example, who cast their skin in one single operation. The skin of humans is renewed on a continuous basis and the released skin cells are dispersed as small particles away from the body. Even when sitting totally still the outer skin layer sheds cells which are then released into the surrounding air. When sitting still and quiet the human body is said to release approximately 100 000 particles per minute (see Table 1). When moving, even just slightly, the number of particles leaving the human being will rise dramatically.

Within the area of contamination control there is nearly always a never-ending discussion about particles. According to the US Federal Standard 209 E, a particle is defined as something that is either solid or liquid and with a size ranging from one thousandth of a micrometer (1/1000 μm) to one thousand micrometers (1000 μm). In some cases there is also a need to define the word fibre. A fiber is generally defined as

Table 1. Number of particles released from the human body during various activities

Activity	**Number of particles released** (greater than or equal to 0.5 μm) per minute
Sitting totally still	100000
Sitting with rotating arm movements	500000
Rising from sitting and sitting down	2500000
Walking up and down stairs, running	10000000

a particle, following the definition above, that has a relationship between one dimension, its length, and the other dimension, its width, that is equal to or larger than 10.

Particles released from the human body, so called skin scales, will be dispersed into the air of the surrounding room. If these skin scales are small enough and thereby not affected by the gravity forces of the earth they will be kept in a suspended state in the air, i.e. they will be floating around in the air in the room. Working within industrial fields where there is a need for and an interest in keeping particles, both dead particles and microorganisms away from products and/or production equipment, the human being will play a vital and important role as a generator as well as a distributor of contaminants. Since all the surfaces of the entire human body, both interior as well as exterior surfaces, are covered with microorganisms, this means that the skin scales which are continuously shed from the body, are covered with microorganisms. This fact means that the human worker is a vital as well as a critical contamination risk, nearly independent of the overall safety of the product.

From the figures given in Table 1 it is not easy to estimate the exact number of particles that are actually shed from the human body. The figure for sitting still can be considered very high, 100 000 particles per minute per individual. It is often better to look at other ways of estimating the amount of shedding from the human body. One way is to look at the number of particles that are shed from the body on average per day on a gram scale. The human body sheds between 6

and 13 g of skin scales per day. In a year this means that approximately 3.5 kg of skin scales will be released from the body. Men have a higher level of skin scale dispersion than women, this is mainly as a result of hormonal differences. Also, younger people have a larger production of skin scales than older people.

Humans do not only contaminate the surrounding air by the release of skin scales. Another way that air can be contaminated by human beings is through the mouth and nose. Talking, coughing and sneezing will create large numbers of particles that contain high levels of microorganisms. The generation of contaminants from the mouth and the nose is very critical, particularly as dispersion is directional. When talking, coughing, and sneezing the contaminants are spread over long distances in the direction of where individual is looking. In practice this means that contamination arising from the mouth and nose is spread in the direction of the product or the equipment that the personnel are working with.

A solution to all the contamination problems associated with personnel or other human beings is to keep the individuals totally separated from the production, for example by the use of robots. However, the introduction of robots is not always a solution to these problems. Robots also generate particles, mostly arising from movements in various joints between parts of the robot system. Furthermore, the flexibility of a robot system is not the same as that of a human being. Personnel can normally observe things that are happening and can take decisions on eventual corrections that might be necessary in the production process.

One very effective way to keep the grade of contamination to a low level is to see to that the number of persons in contact with the production process is kept to an absolute minimum. At the same time the personnel should be dressed in specially designed garments that will act as a barrier, so-called personnel filters. The use of specially designed cleanroom garments will dramatically reduce the number of particles that will be dispersed 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 as well as in some cases,

eye protection. Eye protection will reduce the likelihood of particles and droplets generated from the eye region entering the surrounding air.

Another activity that is associated with personnel and the creation and deposition of contaminants is 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 to a very high degree by, first of all, reducing the need to touch critical surfaces, and secondly by using different types of clean barrier gloves if there is an absolute need to touch an item or a surface.

The dispersion of contaminants from a surface to, for instance, the air within the production room is most of all dependent on the choice of surface material and the different pressures, mostly mechanical, that are put on the surface, either during production or during cleaning and decontamination. Construction materials used within contamination control and cleanroom technology should in general be stabile, inert and have a smooth surface. Another advantage of smooth surface material is that it will be much easier to clean.

The cleaning procedure is often a risk of contamination in itself. Particles that are released during a cleaning process can either originate from the surface itself, from the chemicals used for cleaning and/or from the different tools and equipment used for cleaning.

The nature of the production process also plays an important role in achieving and maintaining a low risk level of contamination. Parts in the process equipment that rotate have a major bearing on the outcome of the process from a contamination control aspect. Small particles can during operation be generated in such systems and these particles can be distributed widely in the surrounding air. Small droplets of liquid material, so-called aerosols, i.e. freely floating droplets in the air, are also critical in many cases. The overall cleanliness of the production process is of vital importance and the necessity of using correctly designed and correctly maintained processes cannot be stressed enough.

1.5.3 Air cleanliness

In many cases it is assumed that contamination control and the use of cleanroom technology are one and the same. This is not entirely correct, since one can actually work with contamination control but without performing the work in a cleanroom. Furthermore, there are certain parameters, other than the ones previously pointed out, that can heavily affect what is happening in a cleanroom. Despite all this, it is often of interest to study how different cleanliness classifications of cleanrooms are defined in order to understand the different standards and regulations that are being used to date. When studying cleanrooms and other controlled environments the most commonly used criteria for cleanliness is the number of, or concentration of, airborne particles in the air in the room.

The particle size distribution together with the concentration of atmospheric dust, measured either by number or by weight, (i.e. dust in the surrounding air can vary dramatically depending on where it is measured) is an important consideration in contamination control. The variations can for example be dependent on where the actual measurement is performed, what time of the year the measurement is done and also on what time of the day. In the colder, northern parts of the world one finds that both numbers and weight concentration of particles in the air is often higher during the colder part of the year as compared to the warmer. This is mostly due to the fact that many more particles are produced and released to the surrounding atmosphere when energy is produced through the combustion of different fossil fuels.

When planning a controlled production, either using a cleanroom or a clean zone or just a controlled production environment, it is of vital importance to study the overall external environment, i.e. the environment surrounding the production building. The internal environment, for example the cleanroom, that is intended to be controlled is dependent on everything that takes place in its surroundings. This means that the cleanliness level of the controlled environment is dependent on what happens in the environment where it is situated, i.e. the room next to it. This outer room is in turn dependent on what is happening in the room next to this one and so forth. In other words, one can say that there is a situation of dependence between the room of interest and the

Table 2. Variation of particulate content of air in relation to location of sampling

Measuring place	**Number of particles per cubic foot** (greater than or equal to 0.5 μm)
Industrial zone	10000000
Town zone	1000000
Mountain zone	100000
The moon	100

surrounding rooms, the outer environment and its total cleanliness.

When discussing different types of cleanliness classes, one has to take this into account – not only to decide if it is possible to achieve the desired cleanliness – but more importantly to decide from a technical point of view, how the cleanliness level of the controlled environment can be secured. It is of special importance to study how the outer environment varies and most of all to take into consideration what type of activities are taking place nearby.

Generally, air cleanliness is defined as the number of particles of 0.5 μm or larger per cubic foot. One cubic foot corresponds to approximately 28.3 litres. Table 2 shows, in a schematic way, how the particulate content of air can vary depending on where it is measured.

These figures can be compared to the different cleanroom classes found in US Federal Standard 209 D.

Table 3. Classes of cleanroom according to US Federal Standard 209 D

	Class limit, measured particle size (equal to or larger than stated size, measured in μm)				
Class	0.1 μm	0.2 μm	0.3 μm	0.5 μm	5 μm
1	35	7.5	3	1	–
10	350	75	30	10	–
100	–	750	300	100	–
1000	–	–	–	1000	7
10000	–	–	–	10000	70
100000	–	–	–	100000	700

Table 4. Aspects of the holistic view of contamination control

Contaminants	**Conditions within industrial premises**	**Equipment**	**Process cleanliness**
Dead particles	Air movement	Choice of material	Media cleanliness
Microorganisms	Choice of material	Design and layout	Surface cleanliness
Contamination source	Design and layout	Tables, chairs, shelves	Sanitation
Dispersion of contaminants	Filters for ventilation air	Lighting	Cleaning In Place (CIP)
	Temperature	Communication	Steaming In Place (SIP)
Deposition of contaminants	Humidity	Safety equipment	Sterilization In Place (SIP)
Measuring techniques	Vibrations		
	Clean zones		
Quality	**Working technique**	**Garments**	**Cleaning**
Measuring techniques	Education	Body garment	Premises
Control techniques	Training	Head gear	Equipment
GMP	Control	Facial cover	Cleaning material
HACCP		Changing facilities	Cleaning chemicals
Validation		Maintenance	Disinfection
Standards		Sterilization	Sterilization

All industrial branches and their different processes working with increased demands for cleanliness must actually be looked upon from an holistic point of view. The weakest part of the overall system will, in most cases, be responsible for the level of cleanliness of the entire system.

Contamination control is a very large and diverse technical area, which means that the different industrial branches that employ this technique will use many different and often unique methods to achieve optimum cleanliness. The holistic thinking within contamination control has a major impact on the total product quality. The different areas that are included in this holistic view are presented in Table 4.

1.6 Conclusions

Contamination control is an interdisciplinary science for the control of indoor environments. The major purpose of this technology is to reduce the possibility of critical contaminants negatively influencing the final product, the production process and the personnel present in the production facility, by using controlled and clean ventilation air.

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2 Contaminants

2.1 Introduction

Cleanliness, hygiene and quality are terms that are used more and more frequently in modern production, most often in the pharmaceutical and microelectronics industry. The reason for using cleanliness, hygiene and quality is to obtain as well as to maintain, a high level of cleanliness during operations so that the number of faulty products is minimal and the majority of the products are approved and usable.

Cleanliness, hygiene and quality are actually incorporated together. This is why it is important to consider all of them in relation to one other and also to the entire production process that is to be performed.

2.2 Cleanliness

The term cleanliness is used on many different occasions. In practice this means that just stating that something is clean is not sufficient. This is the underlying reason why it is always necessary to specify in greater detail the level of cleanliness. For example, one can use the term clean when discussing the environment in a home. A clean home is normally a home that has been set to order by putting all the different items in their right places followed by, for instance, vacuuming and wet mopping the floors, etc.

The level of cleanliness stated above is normally adequate in a domestic environment but it is not in general sufficient for industry, for example the pharmaceutical or microelectronic industry. The level of cleanliness that is needed in certain situations is dependent on what process is to be carried out in the different environments to be used. This is why it is extremely important to start every discussion concerning cleanliness by defining what is to be considered as a contaminant and in particular what is to be considered as a critical contaminant; the overall and desired level of cleanliness should also be defined.

2.3 Contaminants

The term contaminant has a very broad meaning. As a general definition one can state that a contaminant is something, either material (in solid, liquid or gaseous form) or a physical state, that is considered to be in the wrong place (and/or) at the wrong time. This general definition means that the different contaminants can be divided into groups consisting of solid materials, chemicals and physical conditions.

2.3.1 Particulate contaminants

Mostly within contamination control one tends to talk about contaminants that are of particulate matter. Particulate contaminants can be divided into two subgroups, namely ›dead particles‹ and ›live particles‹. Standards that primarily have an impact on most industries including the pharmaceutical and microelectronic industry, are US Federal Standard 209 E, British Standard 5295 and ISO ›209‹. According to the US Federal Standards 209 E a particle is defined as something solid or liquid (small droplets in the form of an aerosol) with a size ranging from 1/1000 µm to 1000 µm. ISO ›209‹ has a slightly different definition. A particle according to this standard is defined as something solid or liquid (in the form of an aerosol) with a size ranging from 0.1 µm to 5 µm. These definitions have been formulated in relation to how the different production areas are to be sampled for contamination and their respective air qualities.

In order to gain a better understanding of the different micrometer sizings used, one can make a comparison with a human hair. A human hair has a diameter of approximately 70–100 µm. The visibility limit to see and identify single particles with the naked human eye in normal daylight, i.e. a human eye without any technical aid, is normally stated to be 40 µm or larger. This means that a particle with a size of 40 µm or larger will be possible to distinguish, whereas particles smaller than 40 µm will not be detected without an optical aid or by illumination with strong light.

Particles can be more or less harmful, depending on the nature of what is being produced or handled. Dead or inert particles are particles that are not able to reproduce by themselves and form identical copies. Among inert particles one can list salt crystals formed on coastlines for instance, when the wind is blowing strongly at sea. Other inert particles can

be formed and dispersed into the air when using plain paper copying machines or laser writers. Fibres from textiles and from insulation material in connection with construction or re-construction of buildings, different types of aerosols from machinery having rotating parts, fans, etc., all fall into the category of inert particles. This category also includes dead microorganisms or subparts of dead microorganisms, which are of particular interest in relation to different disinfecting and sterilization methods that are used within the production process.

›Live particles‹ comprise the vast number of organisms that are included under the heading of ›microorganisms‹. These ›live particles‹ have an extraordinary ability to reproduce or multiply if the environmental conditions are suitable. Humid, warm environments that contain high levels of nutritional material provide suitable conditions in which microorganisms can reproduce.

2.3.2 The particle content of air

The outdoor air contains large numbers of particles. The number of particles as well as the type of particles present depend on the location where the measurement is performed. In general particle analysis, the concentration of particles and/or the size of the particles are measured.

Figure 3 shows a schematic presentation of the particle size distribution in a hypothetical air sample. As a rule of thumb one can state that up to 99.9% of all the particles in the air are less than 1 µm (1/1000 mm) in size. It is of vital importance to measure the percentage of particles of a given size on a defined and mutually agreed basis. This statement is based on the fact that particles can be measured as a percentage either by number or by weight. Figure 3 shows an example of how easy it is to misunderstand the two ways of expressing the percentage of particles in a sample of air. The figure shows a situation in which a filter is used for removing particles of a certain size or larger. The filter has the ability to eliminate 97% of all particles measured by weight. In practice this means that the filter used will remove only 2% of all the particles by number.

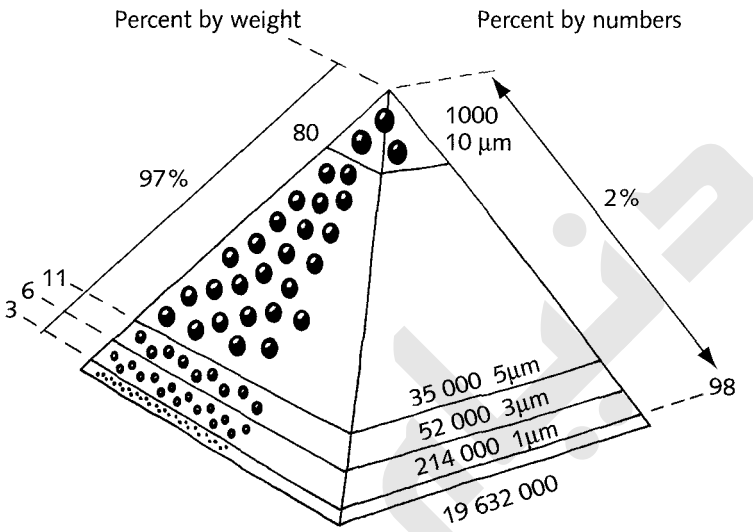

Figure 3. The pyramid of particles. This figure shows schematically the particulate content of a hypothetical air sample with respect to differences in particle size. To the left of the pyramid the scale shows the percentage distribution of particles in the sample by weight. To the right of the pyramid, the scale represents the percentage distribution of particles in the sample by number. If a filter is used to remove particles in the air before entering a cleanroom or a clean zone, and this filter is assumed to remove 97% (by weight) of all particles, this assumption will lead to the elimination of 2% (by numbers). The figure shows how important it is to differentiate between percentage by weight and percentage by number when discussing the effectiveness of a filter

2.3.3 Chemical contaminants

Chemical contaminants are all the other contaminants that do not comprise of solid material, i.e. particles. These are either liquid or gaseous. Chemical contaminants can affect a production in different ways, since they can be either inert, toxic, reactive or possibly explosive.

All chemical contaminants can be harmful or hazardous to the product, the production process and personnel present during the production, but must be defined for each and every individual system. Examples of this type of contaminant are unwanted liquid or gaseous chemicals present in different media (water, steam, pressurized gases), which after some time of exposure to the equipment, can dissolve or in other

ways change the integrity and/or performance of seals placed in pipe couplings used in the production process.

2.3.4 Physical risk factors

The list of contaminants is completed by the incorporation of physical risk factors. By including this last factor it is easier to get a better holistic view of the entire contamination control area. These physical risk factors summarize all the other factors, excluding solid, liquid or gaseous material, that can be hazardous to product, process and personnel. Physical risk factors include temperature, humidity, pressure, different types of radiation as well as static electricity and vibrations.

Good investigation practice (in many cases called hazard analysis) includes recognition of different factors in and around a process that can have a negative influence on the outcome of the production. In order to obtain a process that is working well and in accordance with the requirements, all materials, items and occurrences that have a negative impact must be kept to an absolute minimum.

2.3.5 Sources of contamination

The sources of contamination can be presented in different ways. One way is to divide the various contaminants in relation to where they actually come from, for instance outdoor or indoor environments (see Table 5).

2.3.6 The dispersion and spread of contaminants

Different contaminants will have different impacts on production processes and products. It is therefore of major importance to be fully cognizant of all aspects of the production process and of how to control them. There are many ways that different contaminants can come into contact with a product or process equipment. The following list contains some of the major sources of contamination that pose a risk to the production process:

- Personnel
- Incoming ventilation air
- Machinery and other equipment for production
- Raw material and semi-finished material
- Packaging material
- Different media used in the production process as well as chemicals used for cleaning

Table 5. Sources of contamination

Indoor environment		**Outdoor environment**	
People	Skin scales, Microorganisms Tobacco smoke Hair Textile fibres	Natural processes	Wind Fire Natural cycle of plants in nature
Cleaning Maintenance	Choice of material Choice of chemicals	Manmade processes	Motoring Combustion of fossil material Waste disposal
Machinery Equipment	Spray painting Welding Grinding		
Contruction material	Fibrous insulation material Microorganisms in wet wood		

- Textiles, in garments and other equipment
- Office equipment and office material
- Visitors
- Maintenance personnel, particularly external personnel

In order to eliminate possible contamination of the product or any stage of the production process, it is necessary to study the process that is actually in use and to identify any theoretically possible areas of contamination entry. Investigation of the existing process occurs most commonly when contamination has already been detected or when there is a need to upgrade an existing process by improving efficiency or cleanliness. Studying the areas of theoretical contamination is the method most commonly used when studying a totally new process, i.e. one that has not yet been commissioned for use. Personnel frequently gain information regarding contamination from trial and error studies that have been carried out previously and this information can be useful in further analyses.

2.3.7
How can contaminants be transferred from their sources?

As a general rule one can state that contaminants can come into contact with a product in two ways, either by their creation within the process system or by transfer from the outer environment into the inner production environment.

It is often quite difficult to avoid the creation of contaminants within the production process itself. The best way to minimize this type of problem is to choose the different components used in the production process with great care. If this is done and the required standard of cleanliness has still not been reached, it will be necessary, in the majority of cases, to use techniques that are able to collect the created contaminants (for instance by using different filters) within certain areas of the production equipment. In this way it is possible to keep the collected contaminants as far away from the critical parts of the total process equipment as possible. If, even after such preventive measures have been taken, the problem with contaminants created within the system still exists, it may be necessary to consider stopping the production process after a predetermined time period for cleaning and in some cases sterilization as well. After cleaning and sterilization the process can be restarted again.

The transfer of contaminants from the outer environment into the inner and more critical process environment is one of the most commonly encountered routes of contamination of a product. However simple the method of contamination might be, in terms of control it is one of the most important problems which have to be addressed. The air surrounding a production system can play a major role in the actual transfer of contaminants from the outer to the inner environment of a process. Transfer of contaminants can take place via the activities of personnel working with the process and/or via the introduction of contaminated media, such as water, steam and pressurized gases. Another way that contaminants can enter a process system may be through the introduction of different chemicals for cleaning. The use of cleanrooms, clean zones and the entire area of contamination control is the basis for the minimization of this type of contamination transfer.

When working in a cleanroom the personnel are normally dressed in specific garments, often called a personnel filter. Cleanroom garments are used in order to minimize the num-

Table 6. Particles generated by the clothing and activities of personnel

Activity	Normal street clothing	Laboratory garment	Cleanroom garment
Sitting still	448000	142000	14920
Sitting, moving arms	4450000	462000	48600
Standing, rotating	2240000	390000	31700
Walking rapidly	5380000	1285000	157000

ber of contaminants dispersed by personnel (in the form of skin scales and fibres from personal clothing) that can come into contact with more or less critical parts of a production process. Table 6 gives some examples of how many particles are generated per minute from personnel dressed in different types of garments and also when performing different types of physical activities.

Independent of the type of contaminant or how they may enter the production process there is a very useful general rule to be stated:

In connection with the higher demands for cleanliness and hygiene one must always make sure that the transfer of routinely occurring and critical contaminants must be avoided from the outer to the inner and more critical environment.

2.4 Cleanliness requirements

When trying to establish a certain level of air cleanliness in a room two major considerations have an impact on the final result. These two considerations are, the formation of contaminants inside the room as well as the removal of contaminants from the room. The formation of contaminants within a room is in turn dependent on the activity taking place within the room, including the movement of personnel and the formation of contaminants from the process. The removal of contaminants from a room is dependent on both the amount of air added through ventilation as well as the cleanliness level of the introduced air.

The overall cleanliness of a room is, in simple words, actually a balance between the introduced air and the develop-

ment of contaminants. Furthermore, local cleanliness in a room is dependent on the pattern by which the air inside the room is moving.

Different types of filter are used to remove contaminants from the air to be introduced into controlled environments. Despite the development of finer and finer filters for air filtration together with an increased use of this type of filter, it is still the case that ventilation systems in many factories do not work as they are intended to. Many reports from the United States state that as many as approximately 10% of all newly installed ventilation systems did not work as they where intended to. These reports also state that it is not only vital to install appropriate filters on the incoming ventilation air, but also to perform different tests on the air on a regular basis, in order to determine whether the efficiency of the total system is as good as intended.

In order to obtain as accurate an overall view as possible of the different requirements of the various steps of a production process, it is necessary to define what is critical and also to establish what the result might be if the critical cleanliness level is not reached. Further critical steps in the process must then be added to ensure that the total and overall requirements for cleanliness and quality are fulfilled.

In other words, it is interesting to study the process as a whole (in a more holistic or macroscopic sense) while at the same time studying in greater detail (from a microscopic viewpoint) the smaller components that together create the total system. One way to perform this type of study or estimation is to use what is called the balance theory. With this theory it is possible to analyze the process as a whole as well as to analyze all the various smaller steps in a process with regard to what type of contaminants are entering and leaving the system. This principle can be used on the process as a whole as well as on the smaller component steps of the process.

When performing this type of investigation it is quite interesting to ask some or all of the following questions:

- What is the actual meaning of the word ›clean‹ in this process or process step?
- What overall process requirements and process parameters

must be fulfilled or controlled in order to obtain the level of total cleanliness that is needed?
- How can the correct overall cleanliness actually be achieved?
- What type of analytical tools can be used, apart from studying the cleanliness of the final product, to evaluate whether the correct and required cleanliness is achieved?

In many cases it is difficult to state the ideal cleanliness requirements for a process, especially if these requirements are based on the measurement of inert or ›dead‹ particles. This is one of the reasons why the industry in general first sets a standard cleanliness level for particles in the air and then by various methods defines which process steps are most critical and which are not so critical. In such a way the transfer of contamination from personnel and/or the equipment can be kept to a minimum.

If the critical contaminants are ›live particles‹, i.e. microorganisms, other questions have to be asked. In comparison with inert particles, which cannot reproduce within a production process, microorganisms are a much bigger problem. Microorganisms have the ability to reproduce quite rapidly if their basic demands for nutrition, moisture and temperature are fulfilled. As a result of their ability to adapt to current environments it is likely that some of these three demands will become of less importance than others. In practice this means that in the case of certain microorganisms, as long as the surrounding atmosphere contains sufficient moisture, there is a critical risk that they will reproduce and grow to large numbers in a very short period of time.

2.5 Microorganisms

Microscopic living organisms are found in large numbers in most environments. Microorganisms can be found in soil, freshwater ponds and lakes, oceans, food and drinking water as well as in the air surrounding us. Due to the fact that the environmental conditions are favourable for both survival and reproduction, it is to be expected that microorganisms will be found on different surfaces in the human body, both internal and external. They colonize the epidermal surfaces, the mouth

and nose and the intestinal tracts and are found in greatest numbers in areas of the body where there is hair growth and which are moist. Most of these microorganisms are commensals and are quite harmless to man and other higher animals.

Microorganisms can be divided into different groups in several ways. A totally non-scientific way to divide them according to where they are found or what they might be used for, is as *essential*, *useful*, *harmless*, *harmful* and finally *dangerous*. Essential microorganisms are responsible for the breakdown of organic material in nature. These types of microorganisms are used in industrial situations to break down contaminants, for example in the purification of waste water. The second group, useful microorganisms, are often used in the production of commodities such as beer, wine and other alcoholic beverages, milk-based products and antibiotics. The microorganisms that are considered to be harmless are those found within the human body and in the intestinal tracts and are in part responsible for maintaining a healthy internal environment. Harmful microorganisms can have quite significant and negative effects on various products including food and beverages, cosmetics and a variety of pharmaceutical products. This group of microorganisms is mainly destructive and is responsible for spoilage of the final product, making it either look bad, taste bad or smell bad. The final group of microorganisms, the so-called dangerous organisms, are often referred to as pathogenic microorganisms and can cause disease in man.

As stated earlier the human element in a clean and hygienic production environment is often considered to be the greatest generator of contaminants and the number of microorganisms from this source can vary quite dramatically depending on the origin of the sample. This is demonstrated by the following examples:

- The outer layer of human skin can host up to 1×10^6 microorganisms per cm^2
- Human saliva can contain up to 1×10^9 microorganisms per ml
- Nasal wash (from a healthy person) can contain 1×10^6 microorganisms per ml

- The aerosol produced by sneezing (if no barrier is used e.g. a handkerchief) can contain 100000 microorganisms
- Human excrement can contain 1×10^{12} microorganisms per g. This figure corresponds to approximately 50% of the dry weight of the excrement

The above classification of microorganisms and descriptions of their usual habitats, is given in a non-scientific manner. More scientifically, microorganisms can be divided into subgroups such as bacteria, algae, fungi, protozoa and viruses.

2.5.1 Bacteria

The term ›bacteria‹ covers a large group of microorganisms comprising of several thousand species of single-celled organisms. Bacteria are found everywhere, in the air, in water, in soil and also in and on higher organisms both dead and living. Bacteria are probably the most common microbiological contaminant in cleanrooms.

In order to survive and reproduce bacteria need water. Despite the need for water many bacteria have the ability to survive in very dry environments, which in practice means that they can be widely dispersed by the flow of the surrounding air. Some bacteria are aerobic, which means that they require oxygen in order to survive and reproduce. Anaerobic bacteria however, are able to survive and reproduce in the absence of free oxygen.

The surrounding temperature is a major influence on the growth and development of different bacteria and their optimal temperature requirements vary between species. Normal human body temperature is usually the optimal temperature for growth of pathogenic species. When subjected to lower as well as higher temperatures their ability to reproduce will decrease. Bacteria are generally quite sensitive to heat and many bacteria cannot survive temperatures of 60 °C or higher. Some bacteria have the ability to form endospores, often simply referred to as spores. Spore-forming bacteria often belong to *Bacillus* and *Clostridium* species. The spores are usually very resistant to environmental conditions and can survive boiling water for several hours. Certain of the very resistant strains can also resist drying.

Most of the disinfecting agents used for inactivation of

microorganisms have no effect on spores. In the dry state spores can survive for decades. When the environmental conditions become favourable (with optimal nutrition, moisture and temperature) the spores will develop into the vegetative state and will be able to reproduce once again when the conditions have become optimal.

When an object, process flow or a product is to be sterilized, this means that the surface and/or gas or liquid must be freed from all living organisms. Sterilization must include methods which ensure the elimination or inactivation of both vegetative organisms and spores.

Bacteria are by far the largest group of microorganisms which exist both in the surrounding environment and in association with man. This is the reason why this group of microorganisms constitutes the largest and most common contamination hazard affecting production processes.

Bacteria exist in many different morphological forms: rods or bacilli (bacillus, singular); spheres or cocci (coccus, singular); and spirals or spirilla (spirillum, singular) such as curved rods (vibrios) and ovoid spheres (coccobacilli).

2.5.2 Algae

Algae are somewhat different from other microorganisms in that they have the ability to carry out photosynthesis, which in short means the conversion of light into chemical energy; in this respect, algae resemble plants. Photosynthesis is also carried out by some species of bacteria. The close resemblance between algae, protozoa and plants has created some confusion as algae can exist in a variety of forms from microorganisms of a few micrometers in size to sea-dwelling plants (seaweed) of several hundred meters.

Since algae require both light for photosynthesis and water, they are found in environments where the supply of these is plentiful. Algae are in general not a problem within contamination control and cleanroom technology, but can be a problem for instance in recirculating water systems.

2.5.3 Fungi

Fungi can occur in two forms, molds and yeasts. They are most commonly found on food that has been stored for too long at too high a temperature. The fuzzy mass that is seen on food is called the mycelium and consists of a multicellular

filament of hyphae. Spores are formed on the external areas of the hyphae and are highly specialized cells having several different functions such as reproduction, dissemination and protection.

In comparison to bacterial spores where one spore-forming bacterial cell is capable of forming one spore, a single mold hypha may produce thousands of spores, each of which can be released into the air. If these mold spores land where there is an optimal supply of nutrition and water they will eventually develop into new mycelia.

Mold spores are somewhat more resistant to environmental changes than the vegetative cells that make up the hyphae. Mold spores however, cannot withstand changes in the environmental conditions to the same degree as bacterial spores. In practice this means that conditions that are capable of destroying bacterial spores will easily eliminate mold spores as well.

Molds are the second most commonly found microbiological contaminant within the area of contamination control and cleanrooms and can become a serious threat. The nutritional requirements of molds are generally simpler than those of most bacteria and they also have the ability to grow in the absence of significant amounts of water.

Yeasts are the second form of fungus and are single-celled organisms which are either spherical or ovoid in shape with diameters ranging from 5 to 8 µm. When viewed under a microscope yeast cells resemble bacterial cells, but are larger in size. Yeasts also form spores as one mean of reproduction, but more commonly they reproduce by budding. Yeasts also have very specialized nutritional requirements for development and growth. It is these special requirements, together with the need for water, which make yeasts rare contaminants in contamination control and cleanrooms. They may however, grow in some products which contain high levels of carbohydrates and water.

2.5.4 Protozoa

Protozoa are commonly found in natural water environments, such as ponds, lakes and rivers. These single-celled organisms are more complex in structure than members of the other groups of microorganisms. They feed on smaller

organisms and in turn become food for larger organisms, thus establishing a position in the food chain. Since protozoa are nearly always associated with naturally-occurring water environments they are of little concern within the field of contamination control and cleanrooms.

2.5.5 Viruses

Viruses are quite unique among the microorganisms, as they cannot be classed as living organisms in the generally accepted sense. They are quite simple structures, frequently consisting of nothing more than a single molecule of either DNA or RNA contained within a protein coat, thus making them much smaller than other types of microorganisms.

In order to reproduce a virus requires a host cell and for this reason viruses are known as obligate intracellular parasites. Once a suitable host cell is found, the virus injects its DNA or RNA content into it. Within the host cell, the injected nucleic acid is replicated, i.e. the DNA or RNA is copied. After replication of the nucleic acid, the virus uses the host cell organelles to produce the protein coats it requires to enclose the nucleic acid. Finally the newly formed viruses are released into the surrounding environment.

The replication process for viruses within host cells can take from approximately 20 min to several hours depending on various factors. From a general point of view, viruses are not normally considered to be a greater threat within the area of contamination control and cleanroom technology.

2.5.6 Growth of microorganisms

In general microorganisms reproduce in such a manner that each cell (called the mother cell) divides and forms two identical new cells (called daughter cells). This process can take place every 20 min if the surrounding environment is favorable. Exceptions do, however, exist. Table 7 gives some examples of the time taken by different types of microorganisms to divide and form new cells, often referred to as the generation time (g).

In general microorganisms reproduce and thus can increase in number very rapidly. But like all other organisms, microorganisms only have a finite life span. The life cycle of microorganisms can be divided into some general stages, such as the *lag phase*, the *logarithmic growth phase*, the *stationary phase* and

Table 7. Generation times of various microorganisms

Microorganism	Generation time (min)
Bacillus stearothermophilus	11
Escherichia coli	20
Lactobacillus acidophilus	75
Mycobacterium tuberculosis	360
Treponema palladium	2000

the *declination phase*, the latter is sometimes referred to as the logarithmic death phase.

These different phases can be studied quite simply by introducing microorganisms into an isolated vessel containing a suitable growth medium, normally a water-based solution containing nutrients. After the introduction of a known number of microorganisms into this solution the overall growth will follow a specific pattern (Figure 4). The time for each phase will of course vary, depending on the type of organism to be cultured, the type of growth media used, temperature, etc.

The lag phase, which is the first phase the microorganisms enter, is the period during which the cells adjust to the surrounding medium. The time length of this phase is also dependent on the percentage of viable cells in the inoculum. The cells do not divide during the lag phase, but are still metabolically active in order to adapt to their environment.

The next step, the logarithmic growth phase, starts after the cells have adjusted to the environment, and it is during this time period that the cells divide in order to increase their numbers. During the logarithmic phase the cells reproduce in a geometric manner, i.e. one mother cell becomes two daughter cells. These newly formed daughter cells become two mother cells and in turn divide to form two new daughter cells each and so on. In practice this geometric pattern results in one cell becoming two, two cells becoming four, four becoming eight, eight becoming sixteen and so on (Figure 5A). If the number of cells formed are plotted in relation to time on a logarithmic scale a straight line will be obtained (Figure 5B).

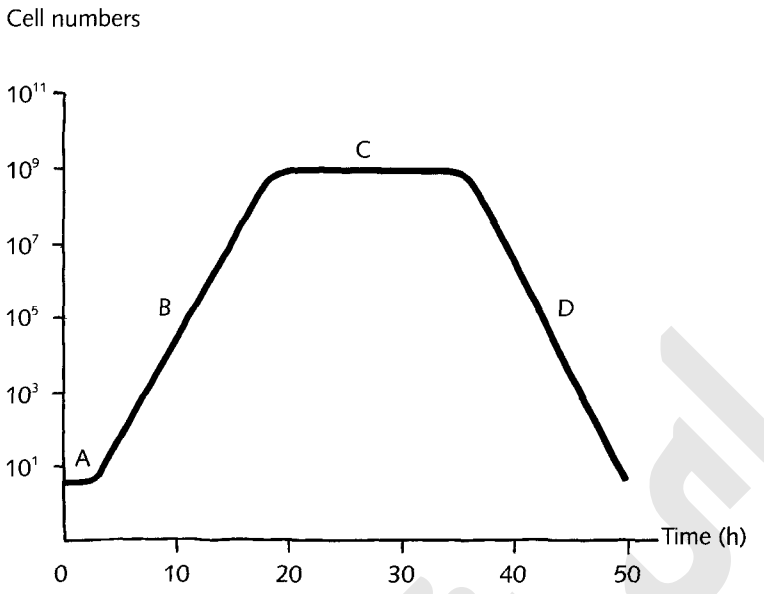

Figure 4. Microbial growth pattern. The figure shows the total growth curve for microorganisms in a system with limited amounts of water and nutrients. A is the lag phase, B the logarithmic growth phase, C the stationary phase and finally D the declination phase

The straight line obtained in this linear – logarithmic diagram has given the phase its name. The linear relation is also used to estimate the average time needed for a cell to divide. This time is normally called the generation time (g) for a particular microorganism.

The stationary phase in a growth curve of a microorganism is the third phase shown in Figure 4. During the final part of the logarithmic growth phase, the slope of the curve will decrease and finally become parallel to the *x*-axis. The number of cells in the growth medium will become stable during this phase, i.e. the number of newly formed cells will correspond to the number of cells that are dying. The reason for this new situation is that during the logarithmic phase, the cells are making use of the nutritional ingredients in the growth medium. The nutrients are used to produce energy to either maintain the cells or produce building blocks for the production of new cells.

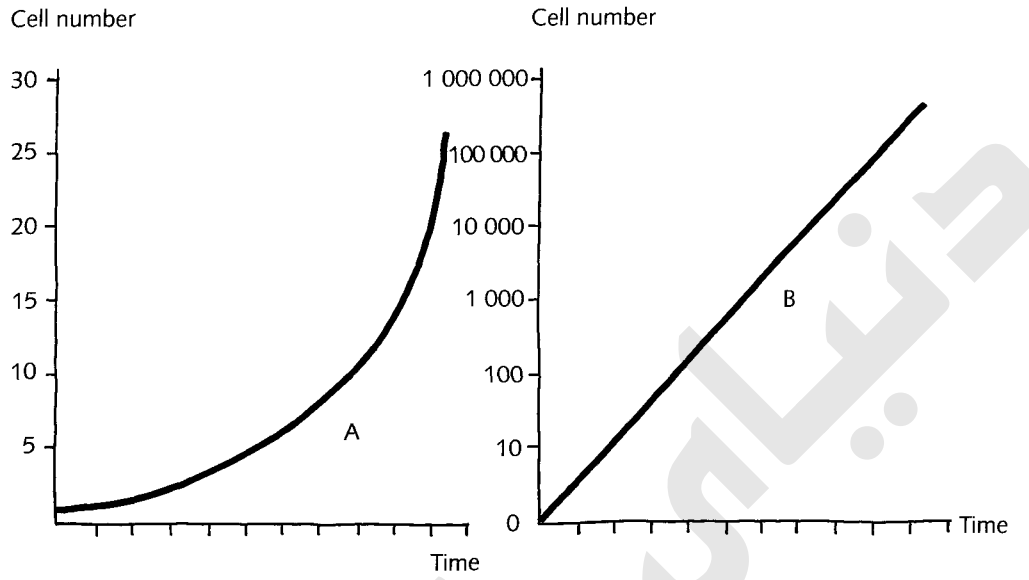

Figure 5. Representation of microbial growth. The curves representing microbiological growth are often presented in a linear logarithmic scale. In an ordinary scale (linear–linear scale), the growth pattern of the organisms will result in a curve which rapidly inclines (A). In a linear–logarithmic scale, the rapid increase in the number of organisms is represented by a straight line(B)

During this activity the concentration of nutrients in the medium will decline dramatically. At the same time, the cells in the growth medium produce large amounts of waste products which are transferred from the interior of the cells to the surrounding growth medium. The waste products often have a toxic effect on the cells when depletion of nutrients leads to the build-up of waste products in the growth medium during the stationary phase. After some time in the stationary phase, the system will enter the final stage, namely the declination or logarithmic death phase.

The declination phase is a phase in the life cycle in which the surrounding environment for the cells is quite unfavorable. The nutritional situation is very poor and the toxic waste products are increasing to levels that make further growth impossible. A new geometric system is then observed, but in reverse to the logarithmic growth phase: 64 cells become 32,

32 cells become 16, 16 cells become 8 and so on. The declination phase continues until the number of viable cells in the growth medium is very small. The final condition is thereafter constant for a period of time.

During the final phase of the growth curve some living cells are still observed in the growth medium. Some of the viable microorganisms have the ability to cope with extremely high levels of toxic waste products and some still have the ability to reproduce under these conditions, although with an extended generation time. After several days, weeks and even months it is still possible to find single cells that have survived.

A very important point to be made here is that it cannot be assumed that after a fixed period of time the system will be completely non-viable, i.e. that the growth medium is totally free of all living microorganisms. If according to the definition of sterility, a system totally free of any form of living organism is required, active measures (physical or chemical) must be taken in order to destroy any viable organisms.

Some microorganisms have very steep declination curves, in practice this means that only a minimal number of microorganisms will be present after 48 h or longer.

2.6 Control of microorganisms

In many cases there is a need to keep microorganisms under strict control. Many years of scientific research has been devoted to the development of different chemical and physical techniques for this purpose. Most of these can be described as antiseptic, disinfection, sanitation, decontamination and sterilization techniques.

A chemical is said to be antiseptic if, by one mechanism or another, it reduces the probability of organisms reproducing or in any other way being active. This can be achieved either by inactivating or killing the organisms. Antiseptic agents are mainly used to control organisms which might be present in living tissues, for example in hospitals for preventing infections in wounds.

The term disinfecting means, in the medical area, the complete eradication of all organisms which may pose a threat to health, namely those organisms which are normally recognized as pathogens. Within industry the term disinfection has

a somewhat different meaning. The industrial meaning of the word disinfection is generally defined as a process by which the majority, but not all, of the total microbiological contaminants are eradicated resulting in a cleaner but not sterile environment. Disinfection can be achieved by the use of chemicals or with the aid of physical methods and is traditionally used in industry for the elimination of non-living material, mostly different types of inert material.

In practice sanitation consists of two processes. The first involves cleaning, which means that all larger and thereby visible material is removed. Cleaning is then followed by reduction of the number of microorganisms (disinfection) to an acceptable lower level. The sanitation process is mostly applied to non-living material, often in the preparation of clean utensils and surfaces.

Decontamination means reducing the microbiological content of an object to a lower level, but still not to the level of sterility.

Finally, sterility is the only absolute term in the area of contamination control and in some publications is defined as the total absence of living organisms. The term ›sterilizing‹ includes all processes, chemical as well as physical that are designed to destroy all forms of life. In this case ›life‹ refers to microorganisms in particular.

As stated previously, methods used to control microbiological life forms can be divided into two major groups: chemical and physical. Table 8 shows some examples of methods.

2.7 Conclusions

Within the area of contamination control there are many different types of contaminants. Table 9 shows some examples. From the table it can be seen that contaminants vary from one branch of industry to another. Variation also exists within a single industry. It is also possible to find contaminants that can be both useful and harmful within a single process. This is the case when microorganisms are used as an active ingredient within a production process, for example when producing beer, wine and different bio-active substances within the pharmaceutical industry. Due to this fact, it

Table 8. Methods used in the control of microbiological contamination

Physical methods		**Chemical methods**	
Heat	Humid or wet heat Dry heat	Sterilization	Formaldehyde Glutaraldehyde Ethylene oxide
Radiation	Ionizing radiation Non-ionizing radiation	Disinfection	Quaternary ammonium salts Phenols Alcohols Chlorine/iodine Hydrogen peroxide
Filtration	Retaining the organisms at certain predetermined locations in the process system		

is important to decide which contaminants are critical, and at the same time to ensure that the analytical tools and control systems which are being used to control them, are correctly planned, performed and evaluated.

Table 9. Examples of sources of contamination affecting different industries

Particles	**Type of industry** **Electronics**	**Pharmaceutical**	**Food and beverage**	**Optics**
Non-viable	Textile fibers Sweat from hands Skin scales Solder aerosols Fats	Pyrogens Toxins and dust Allergens Cross contaminants	Toxins Taste deterioration	Dust Fats
Live	Sulfuric acid producers Acid producers General infections	General infections Pathogens Skin scales	Pathogens General infections	Molds

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3 Testing methods

3.1 Introduction

The fact that a production area or even a cleanroom looks clean does not necessarily mean that it *is* clean. Different analytical techniques are used in order to detect and analyze any contaminants. Traditional systems usually measure particulate contaminants, but within the area of analysis in contamination control there is also a need to study air movements, particularly to identify critical turbulent air currents known as vortexes. Vortexes in a cleanroom or a clean zone are often considered to be quite critical, since contamination will be concentrated at the centre of the vortex. To handle a product close to a vortex without any form of protection will increase the probability of the product being damaged.

When deciding what measuring or analytical technique is to be used within a cleanroom it is of vital importance to consider the overall system, i.e. to make the decision by taking into account the interrelationship between the different steps or components in the system. A single technique cannot normally be applied to the whole process. Instead each component or process step must be considered individually with respect to its requirements for contamination control. This is why it is important to have an adjusted and standardized working and analytical plan. Even when establishing the specifications of the requirements, the analytical and measuring tools used must be of a standard suitable for the task. The holistic view cannot be neglected, it is of utmost importance.

3.2 Analytical methods

Analytical methods used within the area of contamination control are usually of two types, either manual or automatic. Both have advantages as well as disadvantages. The advantage of a manual method is the fact that the contaminants can normally be observed with the aid of a microscope. Tradition-

Table 10. Methods of contamination analysis for different sample types

Manual methods	Automatic methods
Air and other gases	Air and other gases
Surfaces	
Media and other liquid products	Media and other liquid products

ally a manual method is performed in such a way that a given volume of the test sample (air or some other gas, water or some liquid product) is allowed to pass through a fine membrane filter which traps the particles present in the test sample. The particles which have been collected in this manner are then examined and identified under a microscope.

This type of manual analytical technique is used not only to count the number of particles collected, but also to identify their nature, thereby providing information with which to identify their source. Identifying the origin of the particles in this way, can be very useful in devising methods to eliminate the source of contamination.

Manual methods is not the most efficient for providing quantitative data, particularly when counting particles of different sizes. This is a specialized task and requires skilled and highly trained personnel to be engaged in the counting process.

One other disadvantage of manual methods is that they test what are known as discrete samples. This means that when analysing a water sample for instance, the results of the test only apply to the volume of water used in the analysis at the time of sampling, and give no indication of the water quality either before or after the sample was withdrawn.

In automated methods the material to be analysed is usually passed through a measuring chamber fitted with sensors which will detect a particular characteristic of the particles passing through it, for instance their number. The passage of particles through the measuring chamber will result in a signal, which is then forwarded to a computer where the signals are converted to produce a display. The advantage of an automated method is that it is easily reproducible and analyses can be performed continuously for long periods of time. One of the disadvantages of an automated test however, is that the

results can only be displayed via a computer, which in practice means that it is not possible to observe the physical characteristics of the contaminants which may indicate their site of origin.

Table 10 shows the type of samples that can be analyzed by either manual or automated methods. In general it is easier to perform automatic analysis on media that can be pumped, i.e. from its main source to the measuring device. Contamination of surfaces is more difficult to measure by automated techniques as testing has to be carried out *in situ*. Methods have now been developed to analyze particulate matter on surfaces automatically using scanning techniques, a method that can be described as semi-automatic.

3.3 The surrounding air

The exchange of air in a room is often calculated by measuring the turnover of air with the addition of a tracer gas. When adding a traceable gas, for instance dinitrogen oxide, which is considered harmless, easily accessed and quite inexpensive, it is possible to track the decrease of the trace substance in the surrounding air as it follows a decaying slope. It is essential that the tracer gas is easily mixed with the room air and that a homogeneous mixture is formed within the room air.

The trace gas method is standardized and based on the fact that a known flow of trace gas is added through an air duct. The concentration of the trace gas in the air in the room is then followed and recorded in relation to time.

Trace gas is often also used to study the dispersion of different contaminants in a room. In this case, the trace gas is added to the air in the room at the site of the presumed source of contamination. After this has been done, the amount, i.e. concentration of the trace gas, is analysed at different points in the room with the aid of an IR (infrared) analyser. The sample points are often referred to as protection points. In many cases there is a need not only to determine the maximum levels of the trace gas but also to calculate the mean value.

All of these different types of analysis can quite simply be amplified with other types of test in order to visualize how contaminants are dispersed in a production room. Visualization experiments should be documented in order to be of

practical use to the personnel working in cleanrooms or clean zones. Visualisation studies are conveniently performed using isothermally generated smoke. The use of smoke and smoke generators results in a visual picture of the dispersion routes of contaminants and these are easily documented with the aid of a camera. Methods using smoke are only visual methods and provide no information regarding the concentration of contaminants, in this case the smoke particles, at point of the sampling.

3.4 Cleanrooms

The need for cleanrooms and the degree of cleanliness in the room is dependent on the nature of the process to be carried out therein. In this respect it is vital to consider the degree of protection that is required for the products, processes and personnel, together with all the other components that together create the overall demand for cleanliness. To ensure adequate contamination control in a particular environment, means from a practical point of view, that it is necessary to control the number of air changes in the room, the room temperature and humidity, the pressure difference between the room and adjacent rooms, the particle content in the room, the materials used to construct the room and equipment, the number of microorganisms present, the contamination level of garments used and the adequacy of filters for air ventilation. Other factors that should be taken into consideration are hygiene of personnel, choice of equipment and the transportation routes of both personnel and materials.

3.4.1 System for air handling

A cleanroom is normally ventilated in order to remove the different contaminants present in the room. This is done not only to create an environment that is safe for the product and the process, but also to create a working environment that is satisfactory for the personnel. The addition of air ventilation to a cleanroom can be accomplished by two different methods, the traditional airflow system or the unidirectional airflow system.

When using the traditional airflow system, which is the system normally used, the air is introduced through one or more HEPA filters (or other filters) placed at different posi-

tions in the room. Unidirectional airflow systems (often referred to as LAF or UDF systems) in practice means that the air is introduced through a HEPA filter (or other filter) that covers the entire ceiling (for vertical flow cleanrooms) or the entire wall (for horizontal flow cleanrooms). These two systems will be discussed in greater detail later.

3.4.2 Classification

The cleanliness of a cleanroom is based on the number of particles in suspension per unit volume of air. The classification used may describe the cleanroom as class 100, class 1000 or class 10 000, for example. This classification refers to the maximum acceptable number of particles of a specified size or larger per cubic foot of air measured. Other classification systems are available and in some industries employing cleanroom technology class names such as class 1 or class 4 or class A may be used to describe the highest level of cleanliness. Regardless of the nomenclature that is used, objective measuring of contaminants per unit volume of air must be performed in order to classify different cleanrooms. The different standards and normative classifications used are dependent on the nature of the processes carried out within the room.

Within the microelectronic industry a very high level of cleanliness is needed in order to produce components of high quality. Cleanliness in the pharmaceutical industry is to a great extent controlled by authorities, governmental or others, to ensure that cleanrooms, process equipment and products are free from harmful contaminants.

The US Federal Standard 209 is one of the most frequently used standards for the classification of cleanrooms. The pharmaceutical industry, health care sector and other areas also use complementary norms and standards such as GMP or Good Manufacturing Practice, to complement the US Federal Standard 209 with respect to hygiene levels.

3.4.3 Control aspects

A variety of programs are used to ensure maximum control of potential contamination in cleanrooms and clean zones. Such a program can for instance include the following requirements:

- Classification of a cleanroom or a clean zone in operation and at rest

- Classification of LAF-protected areas
- Control of HEPA filters and the sealing of the filter to the surrounding frames
- Control of HEPA filter for pin holes
- Introduction of contaminated air into LAF-protected areas
- Air movement visualization together with photographic documentation

›Classification of the cleanroom in operation and at rest‹ aims to test and classify the basic cleanliness of the room itself. The contamination level is tested at the start of the process to be carried out in the cleanroom and then at specified re-testing times. Particle counters are used for this purpose.

›Classification of LAF-protected areas‹ aims to ensure that the LAF-protected area or the clean zone fulfills the requirements of the appropriate cleanliness class. The tests are performed at the initiation of the clean zone and at specified re-testing times. Particle counters are used for this purpose.

›Control of HEPA filters and the sealing of such a filter to the surrounding frames‹ is carried out in order to study the downstream environment, i.e. the clean side of the HEPA filter. This test is performed in such a way that the upstream side of the filter, i.e. the dirty side of the filter is challenged with particles, normally DOP-gas (di-octyl phthalate). This test should reveal whether or not the filter itself and/or the sealing between the filter and the surrounding frames are leak-proof. Leakage is normally observed at points where the filter is sealed to the equipment or the frames, rather than from the filter material itself. Normally an aerosol photometer is used as the analytical tool when the DOP challenge is used. The smoke or air generated by the leak and containing small DOP particles suspended in it, usually contains a very high concentration of particles. DOP is used because it has the ability to form small particles of uniform size. Traditionally leakage is often found where there are screws, welding joints, HEPA filters and filter packs and where cables intersect.

›Introduction of contaminated air into LAF-protected areas‹ is a test carried out in order to identify any leakages in vital construction parts of the cleanroom and to ensure that the general design of the LAF equipment is such that it

provides adequate protection against the introduction of contaminants. This type of analysis is normally performed at start-up and at times when the equipment needs redesigning. The results of this indicate the likelihood of vortex formation. Creation of vortexes will allow any contaminants in a cleanroom to be transported into the zone when clean work is undertaken.

Visualization of air movement is carried out using smoke and shows the movement of air in and around critical work stations; the test results are often photographed or videotaped.

3.5 General particle analysis

When performing a general test for particles the crucial information required is the number of particles in the air, liquid product or on a surface i.e. the concentration of particles: the number of particles per unit volume of air, etc. Furthermore, it is also necessary to know the size of the particles in the sample being analyzed, together with their morphology or shape. Morphology is not traditionally used as a standard analytical tool but is important at the research and development stages. Table 11 gives examples of some analytical methods used to measure various properties of contaminating particles.

3.6 Measurements according to US Federal Standard 209 E

US Federal Standard 209 E describes various methods that are available to characterize the air cleanliness of a cleanroom. The document is limited to the cleanliness of air and does not consider cleanliness standards for equipment or different types of media (water, pressurized gases or steam) that may be taken in and used within the cleanroom.

It is also important to understand that this standard does not characterize airborne particles, from either physical, chemical or radiological standpoints. The particles are not even divided into the two categories of ›dead‹ or ›live‹. A particle is just a particle as defined earlier according to US Federal Standard 209 E. Furthermore, it is stressed in this standard, that there is no universal relation between the concentration of airborne particles in air and the concentration of live airborne particles, i.e. microorganisms.

Table 11. Analytical methods used in the characterization of contaminating particles

Method of analysis	Concentration	Size	Morphology
Capture of particles on a membrane filter	Measuring the weight of the collected particles by weighing the filter before and after collection	Light microscopy of the sample after collection on a membrane filter ≥ 5 µm	Stereo microscopy
Light adsorption		Light reflection, in order to count the particles of sizes between 0.01 µm and up to 100 µm	Scanning Electron Microscope (SEM)
Light reflection	Optical particle Counters (OPC) for measuring particles of sizes between 0.1 µm and up to 100 µm Condensation Nucleus Counters (CNC) for measuring particles of sizes between 0.01 µm and 0.1 µm		

According to US Federal Standard 209 E there are different methods that can be used for measuring particles suspended in air. These are the microscopy method, and utilization of Discrete Particle Counters (DPC) such as the Optical Particle Counter (OPC) and the Condensation Nucleus Counter (CNC).

The microscopy method is based on the principle that a certain and predetermined volume of air is forced to pass through a membrane filter placed in a filter holder (Figure 6). The filter is connected to a pump that draws the surrounding air through the filter at a certain volume per unit time and after a predetermined volume of air has passed through the filter, the membrane filter material with the collected particles is taken out of the filter holder and placed under a microscope. The filter is viewed under the microscope and the number as well as size of the different particles that have been collected is counted. The filter material can also be photo-

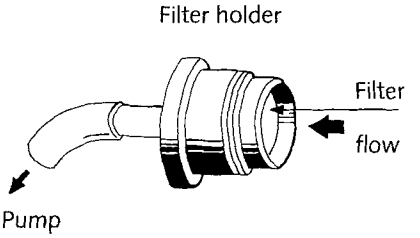

Figure 6. Collection of particles from the surrounding air. Microscopic analysis of particulate contaminants are generally performed after collection of the particles on a membrane filter

graphed so that the number, size and morphology of the collected particles can be recorded for future analysis. This technique is normally used for determining the number of large particles, i.e. when the particles to be analyzed have a size of 5 µm and larger.

The second alternative stated in the US Federal Standard 209 E is measurement of particle concentration by utilization of so-called Discrete Particle Counters, DPC for short. There are two alternative methods of performing such a test. The first of these methods involves the use of what is normally called Optical Particle Counters, OPC for short. The principle behind optical particle counters is that the air in a cleanroom is pumped through a measuring chamber, where it passes through a beam of light (Figure 7). If the particle is of sufficient size, it reflects the light when passing through this beam. The light reflections formed are collected by the optical equipment and transferred to a photo detector where they are transformed into electrical signals.

In practice this means that each particle passing through the measuring chamber, will if large enough, create a light reflection that is observable by the photo detector. Particles can thus be counted by number. Since the flow rate of the pump is known, the number of particles in the air per unit volume can easily be calculated. Moreover, the intensity of the reflected light from the particles will vary depending on the size of the particle, i.e. the area of the outer surface of the particle. This method therefore measures not only the concentration but also the size of particles entering the measuring chamber.

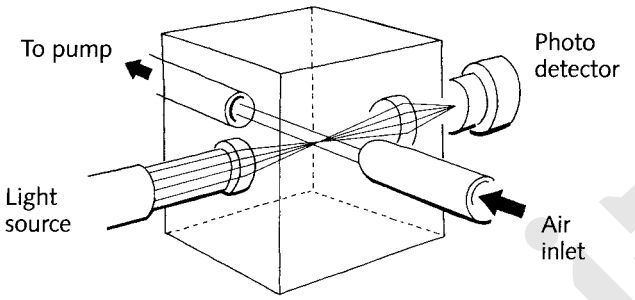

Figure 7. Schematic representation of an Optical Particle Counter (OPC)

Optical particle counters are mainly used for detection of particles in the size region 0.1 μm to 100 μm.

The lower limit of the OPC is 0.1 μm. The Condensation Nucleus Counter (Figure 8) can be used to detect particles of a smaller size. The CNC can detect particles of sizes from 0.01 μm and upwards. The principle of the CNC is quite like the one used for the OPC. A known volume of air is drawn into a measuring chamber by means of a pump. Before entering the measuring chamber the air is allowed to pass through a vessel containing vaporized alcohol which is maintained at a temperature which approximates that at which it condenses. When the particles in the air enter the alcohol chamber,

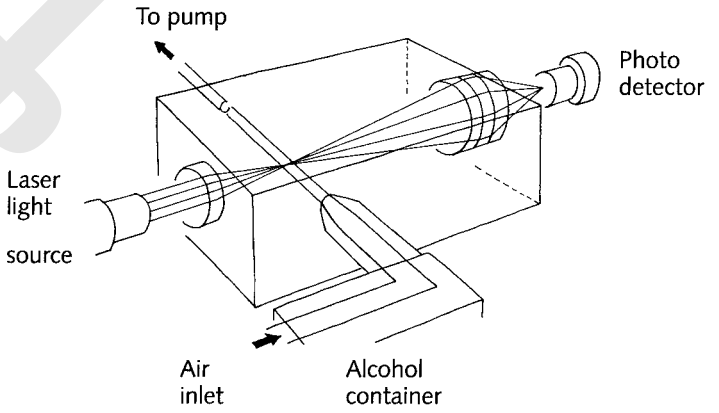

Figure 8. Schematic representation of a Condensation Nucleus Counter (CNC)

particles which are colder than the alcohol vapor will cause the vaporized alcohol to condense on the particle surfaces. This condensation will create a thin layer of liquid alcohol on the surface of the particle, and hence increase its diameter. When the particles covered with a layer of vaporized alcohol are introduced into the measuring chamber, those of sufficient size will reflect the incoming light and will thus be detected by a photo multiplier. In this way particles with a size of 0.01 µm and greater will be detected. The exact size of the particles cannot be determined since the size of the outer alcohol layer is an unknown parameter. The results from a test performed with a Condensation Nucleus Counter will therefore be presented as the number of particles with a size greater or equal to 0.01 µm. The number of particles in the size range 0.01 to 0.1 µm is often referred to as Ultrafines (according to US Federal Standard 209 E).

The Optical Particle Counter (OPC) and the Condensation Nucleus Counter (CNC) are in general called Discrete Particle Counters (DPC). When used together the number of particles calculated by the CNC minus the number of particles calculated by the OPC will give the number of particles that are smaller than 0.1 µm, i.e. the number of Ultrafines or ultrafine particles.

3.7 Measurement of hygienic parameters in a cleanroom

The measurement methods described above are used mainly to calculate the number of particles suspended in the air in a cleanroom i.e. the concentration of airborne particles. In many cases it is necessary to differentiate between the number of ›dead‹ and living particles, i.e. the number of microorganisms. This is of particular importance within the pharmaceutical and the food and beverage industries and in these cases it is necessary to analyze the number or concentration of living particles, i.e. particles that have the ability to reproduce and thereby multiply. The following are among the methods used to measure the hygiene standards in a cleanroom during a production process:

- Measurement of particles in air, according to the methods described above

Table 12. Procedures for analyzing microbiological contaminants

Strategy	Detailed plan	Capture of organisms	Evaluation
What organisms is to be studied? What test method is to be used? What is to be monitored: Premises? Equipment? During operation or at rest? Random sample or conventional monitoring?	What room is to be controlled? Exactly where in the room is the measurement to take place? What is taking place in the room during measurement? Number of operators? Etc. Date and time for the test Temperature and humidity (deviations)	Growth Identification	Report

- Active as well as passive analysis of airborne microorganisms
- Establishing microbiological cleanliness of surfaces

When classifying a cleanroom the US Federal Standard 209 E is often utilized as there is no corresponding standard for airborne microorganisms at present, i.e. there is no standard for measuring the CFU (Colony Forming Units) in air.

When analysing microorganisms the need for accurate and basic planning is essential. Table 12 gives some examples of the questions that must be taken into account in this type of the analytical procedure.

3.8 Microbiological monitoring of air

It is of vital importance to characterize the air movements in the area that is to be monitored. The various measurements should include the concentration of airborne particles and airborne microorganisms as well as the microbiological cleanliness of surfaces. The parameters of the analysis must be thoroughly defined before any determinations are made. The following parameters should be measured with respect to potential contaminants of microbiological origin:

- The maximum level in the room
- Levels associated with different types of activity
- Contamination in a critical area of the production

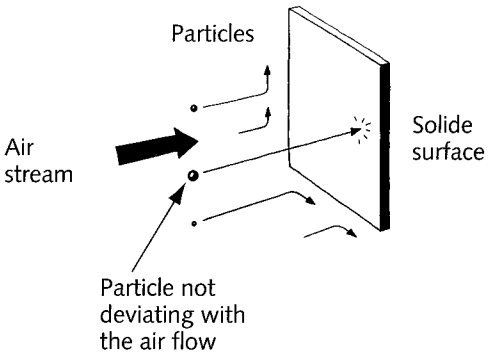

Figure 9. The principle of impaction

- Contamination in a critical process step
- The presence of particles of specified sizes
- The presence and absence of specific microorganisms

By defining the purpose of the measurements e.g. identification of a particular organism, the type of nutrient requirements, cultivation temperature and incubation temperature that should be used for the current microbiological analysis will become obvious.

Microbiological sampling is the only method by which the microbiological load of an area can be determined. After cultivation, the various microorganisms can be identified and this should provide information regarding the source from which they may have been dispersed. Airborne contaminants (microorganisms) are not usually suspended in the air as single cells, but are attached to ›dead‹ particles which are usually of sizes larger than 10 µm. In a cleanroom most of these larger particles originate from the personnel that are present in the room.

Collection of airborne particles and culture of microorganisms can be carried out using a filter, through the process of impaction (Figure 9), and by fallout techniques. Once the microorganisms has been collected, it is incubated for 3–5 days at a temperature of 30–35°C.

3.9 Active sampling

The *active* sampling of particles (microorganisms) in the air can be achieved by filtration and impaction. In the filtration technique (Figure 6) a certain volume of air is forced through an analysis filter, i.e. a very fine filter and the microorganisms are captured on the surface of the filter material. After allowing a predetermined volume of sample to pass through the filter material, the filter is either placed directly onto growth medium or is dissolved and the material in the resulting solution is then cultured on a solid agar plate. This technique has several advantages:

- Higher air velocities can be used during sampling, i.e. larger volumes can be sampled
- Isokinetic sampling can be performed
- Filter materials that are manufactured from gelatine-type material can be used. These filters can be dissolved after use and the resulting solution obtained in order to determine the total number of microorganisms using other techniques rather than the number of CFU

However, the filter method has some disadvantages. The optimal filtration time (sampling time) is usually 5 min and during this time there is a risk of the filter drying out and thereby damaging the microorganisms that have been captured on it.

During impaction, the theory of which is shown diagramatically in Figure 9, a stream of air under high velocity is allowed to hit a solid surface, thereby forcing the airstream to change its direction. Due to the momentum of particles present in the airstream (momentum = mass of the particle × its velocity), particles of a great enough mass will not follow the airstream when it is diverted. These particles will instead travel straight forward in the direction of the surface. If the particles come into contact with the surface it is possible that they will adhere to it.

The Andersen Sampler (Figure 10), the Reuter Centrifugal Sampler (RCS Sampler; Figure 11) and the Slit Sampler (Figure 12) are the most commonly used sampling devices and are all based on the principle of impaction.

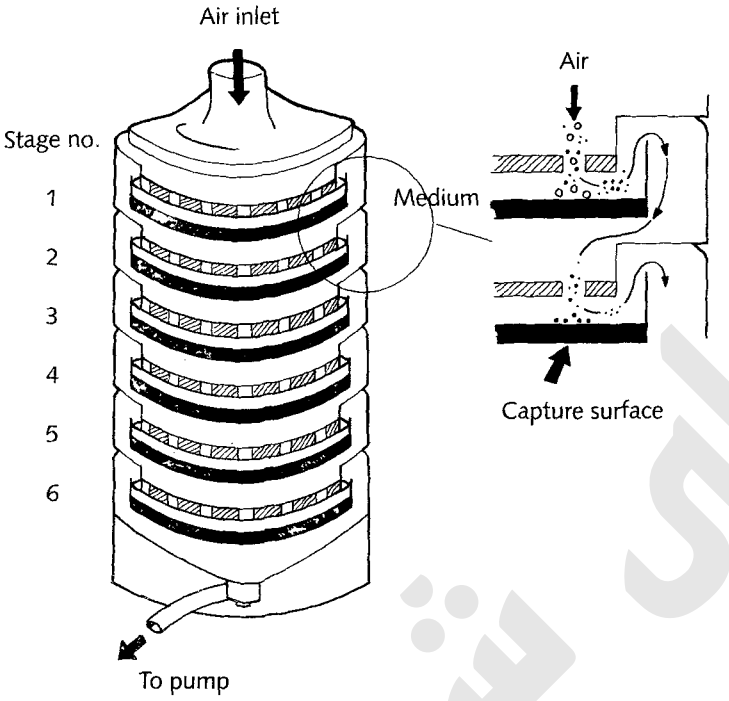

Figure 10. The Andersen Sampler

3.9.1 The Andersen Sampler

The Andersen Sampler, will effectively collect microorganisms and at the same time separate the different particles according to their size (actually their mass) The equipment consists of a series of perforated disks which are each placed directly over an open agar plate. The perforations in each disk are of the same diameter but are different between disks, such that the diameter of the holes decreases from one disk to the next. The perforations ensure that the air is forced through the equipment at a predetermined velocity. The smaller the diameter of the perforations, the higher the velocity of the air.

Due to the momentum of the particles suspended in the air to be analysed, larger particles will be trapped in the agar plates at the top of the equipment, whereas smaller particles will be trapped in the agar plates placed further down. After sampling for a predetermined time, the equipment is taken

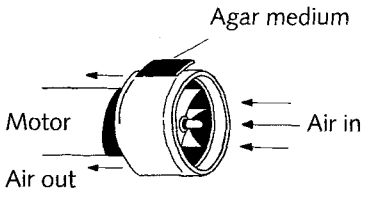

Figure 11. The Reuter Centrifugal Sampler (RCS)

apart and the agar plates located at different levels in the equipment are then incubated. The results of this type of analysis are expressed as the number of CFU per unit volume of air, and also provide information regarding the size of the captured particles.

3.9.2 The Reuter Centrifugal Sampler

In the Reuter Centrifugal Sampler (RCS Sampler) the air to be analysed is drawn into the equipment and redirected towards a collection surface with the aid of a small centrifugal fan. The collection surface consists of small segments of agar placed in small grooves in a flexible plastic strip. This plastic strip fits into a slit placed around the fan and thus forms a flexible, circular collection surface for any microorganisms that may have been present in the air sample. The fan is

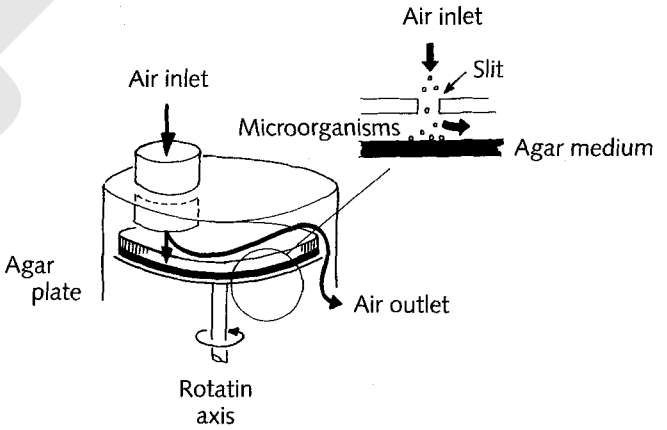

Figure 12. The Slit Sampler

turned on and after a predetermined time the plastic strip is taken out of the RCS Sampler and incubated.

3.9.3 The Slit Sampler

In a Slit Sampler the air to be analysed is forced at high velocity towards the solid surface of an agar plate. The air enters the equipment through a very thin slit placed in close proximity over a circular agar plate. The entrance for the air is arranged in such a way that the air will only come in contact with the agar plate in a radial manner, i.e. the air that is entering is only exposed to the agar at the centre point of the plate and then radiates outwards. The agar plate is then placed on a rotating disk fitted with a timer, which in practice means that the Spit Sampler can be used not only to collect micro-organisms, but also to record the time at which they were collected.

With the use of a Slit Sampler it is possible to study the microbiological content of the environmental air in relation to the different activities which may be taking place within a cleanroom, for example.

3.10 Passive sampling

Passive sampling of particles (microorganisms) in air is carried out using fallout techniques. These techniques are based on the sedimentation of particles suspended in the air into open agar plates (growth media) (Figure 13). Fallout techniques have several advantages:

- No sophisticated equipment is necessary
- The plates are easily placed at the sampling points
- The plates can be exposed for longer time periods
- The methodology can be utilized for routine analysis in cleanrooms

However, one disadvantage exists, the method cannot be used for quantitative analysis.

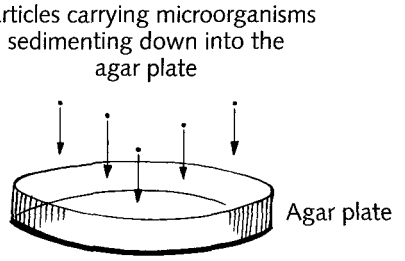

Figure 13. Fall-out method. A fall-out plate containing agar is placed on a surface. The plate is exposed to the air for a predetermined time and particles suspended in the air settle on the plate as a result of gravity. After sampling, the plate is incubated to allow the micro-organisms which have been collected to develop

3.11 Sampling surfaces in cleanrooms and clean zones

Surface sampling is carried out mainly within the bio-industries in order to control surface cleanliness. Various surfaces within a room can be contaminated by sedimentation of particles suspended in the air, as result of contact by operators involved in the production process and/or by the use of unsuitable cleaning methods and cleaning materials.

Different types of sampling techniques can be utilized, ranging from conventional *contact plates*, *swab techniques and analysis* through to *casting agar medium directly onto a surface.*

Contact plates are prepared by setting the growth medium in a solid frame, so as to achieve a convex appearance, similar to a stamping pad (Figure 14). By pressing this raised agar medium towards the surface to be tested, some of the material that is loosely attached will be freed and transferred to the growth medium on the contact plate. Any microorganisms that have been collected in this way will grow on the contact plate after a period of incubation. The results from such an analysis are expressed as the number of colonies per plate or the number of colonies per unit area, CFU per unit area. The contact plate method has the disadvantage of only being suitable for use on flat surfaces.

Since the contact plate method is not suitable for testing surfaces that are not completely flat, swab techniques are used in these instances. A swab is a solid pin equipped with a porous tip which may be manufactured from cotton or from a more soluble material such as alginate, for instance. The swab

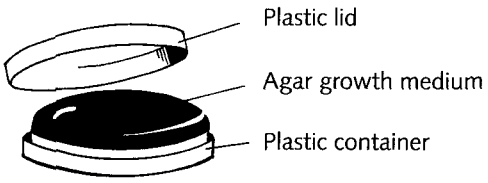

Figure 14. Contact plate

is often wetted with a sterile solution before being gently wiped over the test area, thus collecting particles (micro-organisms) from the surface onto the wetted tip. After contact with the test surface, the swab is either placed in growth media or used to coat the surface of an agar plate. If the tip of the swab is made from alginate, the whole tip can be dissolved and the entire solution obtained in this way can be incubated on an agar plate.

On some occasions when the surfaces to be monitored for microbiological cleanliness are particularly difficult to access, the swab method is not suitable and it may be more practical to use the casting method. Using this method, the surface to be tested is completely covered with growth medium containing an indicator that changes colour in the presence of any microbial activity. Due to practical reasons, the technique of casting is only suitable for smaller items, since the entire surface that has been coated with agar medium has to be placed in an incubator in order to facilitate the growth of microorganisms.

Different surfaces in cleanrooms must be monitored on a regular basis to ensure a consistent standard of cleanliness. Floors, walls, horizontal surfaces and also surfaces on equipment must be tested. In addition to the surfaces mentioned above, it is also necessary to test the surface cleanliness of garments worn by personnel.

It is a general rule that all types of critical surfaces must be tested on a regular basis. In order to perform these tests with maximum efficiency, it is of vital importance to know what type of work is undertaken in the room, the nature of the air movements in the room and also to relate this work to the overall production process.

3.12 Conclusions

Detection and analysis of particulate contaminants is of prime importance and there are several methods available to accomplish this. Various levels of cleanliness can be specified, for example the number and corresponding size of particles on surfaces and/or suspended in air, other gases and liquids.

Three methods exist for monitoring the concentration of particles in a cleanroom: collection on a filter material followed by examination under a microscope, Optical Particle Counting (OPC) and Condensation Nucleus Counting (CNC).

Microbiological monitoring of air and other gases and liquids is normally carried out by collecting and culturing the microorganisms present in the sample. Microorganisms in air can be sampled by filtration, impaction or by fallout methods.

4 Cleanrooms and clean zones

4.1 Introduction

Despite the fact that cleanrooms often are considered as one of the most important tools in modern contamination control, they have existed for more than 100 years. Originally, cleanrooms were used within the hospital environment in order to keep seriously ill patients isolated from other patients, thereby decreasing the risk of spreading infection. When it was discovered that infectious diseases were transported by air, the use of this type of isolation technique meant not only that patients could be separated but also that the environmental conditions could be more easily controlled.

Today contamination control and cleanrooms are used within nearly all branches of industry.

Government legislation along with increasing demands for more sophisticated products such as those produced by the microelectronic industry where the miniaturization of component parts is now commonplace, has played a major role in this development.

Contamination control has become increasingly important in industry because clean air and manufacturing conditions are essential in order to meet the demand for products of higher quality and sophistication, and to keep the percentage of rejected products to an absolute minimum.

4.2 Definition of a cleanroom

Put simply, it can be said that a cleanroom is a room that is clean. The question that always follows this definition is – How clean is clean? Different standards and practices define cleanliness in various ways. The three most frequently used standards are the US Federal Standard 209, British Standard 5295 and ISO ›209‹.

US Federal Standard 209 defines a cleanroom and/or clean zone in the following way:

›*A room in which the concentration of airborne particles is controlled to specific limits*‹.

British Standard 5295 gives the following definition of a cleanroom:

›*A room with control of particle contamination, constructed and used in such a way as to minimize the introduction, generation and retention of particles inside the room and in which the temperature, humidity and pressure shall be controlled as is necessary*‹.

Finally the new ISO Standard ›209‹, which is actually a series of standards, defines a cleanroom in the following way:

›*A room in which the number concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation and retention of particles inside the room and in which other relevant parameters, e.g. temperature, humidity and pressure are controlled if necessary*‹.

4.3 Classes of cleanroom

The above definitions indicate that there are different types of cleanrooms and also different levels and gradings of air cleanliness within such rooms. This is why the different standards are broken down into cleanroom classes in order to clearly specify the standard of air cleanliness in a room. The US Federal Standard 209 classification is the one most often used. In this classification the numbers of particles per unit volume of air in the room are studied. The particles that are counted and incorporated in these classifications are of different sizes. The most commonly specified size range is 0.5 µm or larger and the concentration of these particles are normally expressed as the number of particles per cubic foot of air (e.g. *x* particles of size 0.5 µm or larger per cubic foot of room air).

Table 13 shows the classification of particle size according to the US Federal Standard 209 D (this is the older version of this standard and is no longer valid). This classification has been used for many years by a large number of industries requiring high standards of cleanliness and is used here because it clearly illustrates the size limits within each class.

The classes have different names ranging from class 1, class 10, class 100 and further on up to class 100 000. The figures given in Table 13 indicate the maximum number of particles of a certain size or larger (measured in µm), permitted within

Table 13. Cleanroom classification according to US Federal Standard 209 D

	Class limit (measured particle size) (equal to, or larger than the stated size, measured in micrometers)				
Class	0.1 µm	0.2 µm	0.3 µm	0.5 µm	5 µm
1	35	7.5	3	1	–
10	350	75	30	10	–
100	–	750	300	100	–
1000	–	–	–	1000	7
10000	–	–	–	10000	70
100000	–	–	–	100000	700

1 cubic foot of air in the room. The classification which is currently used, US Federal Standard 209, version E, is shown in Table 14.

The cleanliness of a cleanroom is also classified by criteria of occupancy. The reason for this being that the number of particles will vary depending on what is actually taking place in the room when testing is carried out. The particle concentration will vary depending on whether (1) the room is totally empty, (2) the room contains all the necessary equipment but personnel are absent or (3) the room is being used for normal production, i.e. the room contains the full complement of equipment and personnel necessary to carry out a specific task. The concentration of airborne particles is mostly dependent on the volume and cleanliness of the incoming ventilation air, the activity which is taking place within the room, and also to a certain extent, the pattern of the airflow in the room.

It is therefore essential to take into consideration the nature of the activities taking place within a cleanroom. The concentration of particles will be lower in a cleanroom that is empty and in which no activities are taking place. The creation of contaminants within such a room is minimal and the total particle concentration is dependent only on the incoming air quality and quantity. The quality of the incoming air is in turn dependent on the efficiency of the various filters used for

cleaning the ventilation air before it is allowed to enter in to the room.

4.4 Occupancy states

When process equipment and, more importantly, people are present in a cleanroom, the concentration of particles and other contaminants will increase. This, in turn, will lead to a change in the classification of the room. The US Federal Standard 209 E and also the ISO ›209‹ series of standards use the same wording and definitions for the various occupancy states valid for cleanrooms. The three occupancy states are: *As built*, *At rest* and *Operational Cleanrooms*

- An ›As built‹ cleanroom, is characterized as a cleanroom or facility that is complete and ready for operation, with all services connected and functional, but without equipment or personnel in the facility
- An ›At rest‹ cleanroom, is characterized as a cleanroom or facility that is complete, with all services functioning and operable as specified but without operating personnel in the facility
- An ›Operational‹ cleanroom, is characterized as a cleanroom or facility in normal operation, with all services functioning and with equipment and personnel, if applicable, present and performing their normal work functions in the facility

4.5 The meaning of the cleanroom classification

When defining the cleanliness class in a cleanroom it is not sufficient just to state the class of the room. Questions that might arise from such a description might be for instance: What does this class actually mean? What type of work is undertaken in this cleanroom? What will happen if an increase in the cleanliness of the room is required and what changes would have to be undertaken in order to fulfill this new requirement?

Cleanrooms are usually classified according to the type of industry in which they are being used and the nature of the work being carried out in them.

Class 1 This cleanroom class is mainly used within the

microelectronic industry when manufacturing integrated circuits which requires submicron resolution.

Class 10 This cleanroom class is mainly used within the semiconductor industry using band widths below 2 µm.

Class 100 This cleanroom class is, according to many, the most useful critical cleanliness class. Cleanrooms class 100 are often, incorrectly, known as sterile rooms and are used when ›bacterial free‹ and/or ›particle free‹ environments are required. Cleanroom class 100 is used in aseptic manufacturing within the pharmaceutical industry, for example. This cleanroom class is frequently used during the manufacture of implants or when performing surgical operations including transplantation; during the manufacture of integrated circuits; and also during isolation and treatment of patients who are especially sensitive to bacterial infections, e.g. after bone marrow transplantation.

Class 1000 This cleanroom class is mainly used when producing high quality optics; when carrying out mounting work and testing of gyroscopes for aircraft; and also when mounting high quality miniature bearings.

Class 10000 Cleanrooms of class 10 000 are used for mounting procedures in hydrolic or pneumatic equipment and on some occasions are also used within the food and beverage industry. Class 10 000 cleanrooms are also commonly used within the pharmaceutical industry.

Class 100000 This cleanroom class is used by many industries, for example when working with optical products, when building large electronic systems based on smaller components, when building hydraulic and pneumatic systems and also within the food and beverage industry. The pharmaceutical industry also makes frequent use of this class of cleanroom.

4.6 US Federal Standard 209 E

The US Federal Standard 209 E is an American standard with the purpose of supplying industry with a standardized classification system for air cleanliness in cleanrooms or clean zones. The concentration of particles within the air is measured and as stated earlier this includes all particles, ›dead‹ as well as live. The objective of this Standard is to supply defined methods for measuring particle concentrations including that of so-called ›ultrafine‹ particles.

A new amendment in the 209 E Standard (published in 1992) is that the SI system is used in defining particle concentration and it is now expressed as particles per cubic meter of air. The particle sizes are the same as within the older version, 209 D, which are shown in the Table 14. The old class names, class 1, class 10, class 100 and so forth, have been changed and replaced with numbers 1, 1.5, 2 etc.

The new class names and the new classification table according to the Federal Standard 209 E is not as straightforward as the older version of the Standard whose classification names corresponded to the maximum number of particles with a size of 0.5 µm or larger that was acceptable in a particular class. However, under the new standard 209 E, it is acceptable to use either the new class names, class 1, class 1.5, class 2 etc. preceded by ›M‹ to indicate that the measurement is now in cubic meters, or the old class names, class 1, class 10 and class 100 etc. The major difference between the two versions of Federal Standard 209 is that the new version use SI units and air cleanliness is now expressed in cubic meters rather than cubic feet.

Using this standard, requirements for intermediate classes can be approximated with the following formula:

$$\text{Number of particles per m}^3 = 10^M \, (0.5/d)^{2.2}$$

where

M is the numerical designation of the class based on SI units
d is the particle size in µm

The US Federal Standard 209 E can also be expressed in the volumetric unit ft^3 according as shown in Table 15.

In the same way as above intermediate classifications can be calculated according to the formula below:

Number of particles per $ft^3 = N_c\,(0.5/d)^{2.2}$

where

N_c is the numerical designation of the class based on imperial (US customary) units
d is the particles size in µm
The full content of the US Federal Standard 209 E covers the following:

- Scope and limitations
- Reference documents
- Definitions
- Airborne particulate cleanliness classes and U descriptors
- Verification and monitoring of airborne particulate cleanliness
- Recommendations for change
- Conflict with reference documents
- Federal Agency interest
- Appendix A: Counting and sizing airborne particles using an optical microscope

Table 14. Cleanroom classification according to US Federal Standard 209 E

	Limit of concentration of airborne particles (with sizes equal to or larger than the particle size stated per m^3)				
Class	0.1 µm	0.2 µm	0.3 µm	0.5 µm	5 µm
M 1	350	75.7	30.9	10.0	–
M 1.5	1240	265	106	35.3	–
M 2	3500	757	309	100	–
M 2.5	12400	2650	1060	353	–
M 3	35000	7570	3090	1000	–
M 3.5	–	26500	10600	3530	–
M 4	–	75700	30900	10000	–
M 4.5	–	–	–	35300	247
M 5	–	–	–	100000	618
M 5.5	–	–	–	353 000	2470
M 6	–	–	–	1000000	6180
M 6.5	–	–	–	3530000	24700
M 7	–	–	–	10000000	61800

Table 15. Cleanroom calssification according to US Federal Standard 209 E expressed in imperial units

	Limit concentrations of airborne particles with sizes equal to or larger than the particle size stated per ft^3				
Class	0.1 µm	0.2 µm	0.3 µm	0.5 µm	5 µm
M 1	9.91	2.14	0.875	0.283	–
M 1.5	35.0	7.50	3.00	1.00	–
M 2	99.1	21.4	8.75	2.83	–
M 2.5	350	75.0	30.0	10.0	–
M 3	991	214	87.5	28.3	–
M 3.5	–	750	300	100	–
M 4	–	2140	875	283	–
M 4.5	–	–	–	1000	7.00
M 5	–	–	–	2830	17.5
M 5.5	–	–	–	10 000	70.0
M 6	–	–	–	28 300	175
M 6.5	–	–	–	100 000	700
M 7	–	–	–	283 000	1750

- Appendix B: Operation of a discrete-particle counter
- Appendix C: Isokinetic and anisokinetic sampling
- Appendix D: Method for measuring the concentration of ultrafine particles
- Appendix E: Rational for the statistical rules used in Federal Standard 209 E
- Appendix F: Sequential sampling: An optional method for verifying the compliance of air to the limit of airborne particulate cleanliness classes M 2.5 and cleaner
- Appendix G: Source of supplemental information

4.7 British Standard 5295

British Standard 5295 is divided into five parts which aim to simplify the specification procedure regarding the utilization of cleanrooms and clean air devices. The five parts of this standard are as follows:

Part 0 General introduction and terms and definitions for cleanrooms and clean air devices

Table 16. Classification of cleanrooms and clean air devices according to British Standard 5295

	Maximum permitted number of particles per m^3 (equal to, or greater than, stated size)				
Class	0.3 µm	0.5 µm	5 µm	10 µm	25 µm
C	100	35	0	–	–
D	1000	350	0	–	–
E	10000	3500	0	–	–
F	–	3500	0	–	–
G	100000	35000	200	0	–
H	–	35000	200	0	–
J	–	350000	2000	450	0
K	–	3500000	20000	4500	500
L	–	–	200000	45000	5000
M	–	–	–	450000	50000

Part 1	Specification for cleanrooms and clean air devices
Part 2	Method for specifying the design, construction and commissioning of cleanrooms and clean air devices
Part 3	Guide to operational procedures and disciplines applicable to cleanrooms and clean air devices
Part 4	Specification for monitoring cleanrooms and clean air devices to prove continued compliance with BS 5295.

The specifications for cleanrooms and clean air devices as mentioned in Part 1 above is detailed in Table 16.

In a similar manner to US Federal Standard 209 E, British Standard 5295 identifies three states of operation. These states are called:

- As built
- Unmanned
- Manned

›As built‹ means a room that is complete but that has not yet been used. Unmanned is operational but not in use and finally

the occupancy state ›Manned‹ refers to a cleanroom in full operational use.

4.8 ISO ›209'

US Federal Standard 209 is a standard that is valid in the USA which means in theory, that it cannot be implemented in countries outside the USA. However despite this, it is widely used. One of the reasons for this is that GMP, Good Manufacturing Practice, actually incorporates Federal Standard 209 E, thus those working to GMP guidelines will also be following US Federal Standard 209.

Many countries do not use imperial units such as the cubic foot and for this reason a new cleanroom standard was developed known as ISO '209‹. This new ISO standard is not a US standard but a world standard which uses SI units. ISO ›209‹ is the working name for this standard, which is actually made up of standards ISO 14644 and ISO 14698.

These two standards, ISO 14644 and 14698, contain a series of documents which are shown below:

ISO 14644–1	Cleanrooms and Associated controlled environments Part 1: Classification of air cleanliness
ISO 14644–2	Cleanrooms and Associated controlled environments Part 2: Specification for testing and monitoring to prove continued compliance with ISO 14644–1
ISO 14644–3	Cleanrooms and Associated controlled environments Part 3: Metrology and test methods
ISO 14644–4	Cleanrooms and Associated controlled environments Part 4: Design, Construction, and start-up
ISO 14644–5	Cleanrooms and Associated controlled environments Part 5: Cleanroom operations
ISO 14644–6	Cleanrooms and Associated controlled environments

	Part 6: Terms and definitions
ISO 14644–7	Cleanrooms and Associated controlled environments Part 7: Enhanced clean devices
ISO 14644–8	Cleanrooms and Associated controlled environments Part 8: Molecular contamination
ISO 14698–1	Cleanroom technology-Biocontamination control Part 1: General principles
ISO 14698–2	Cleanroom technology-Biocontamination control Part 2: Evaluation and interpretation of biocontamination data
ISO 14698–3	Cleanroom technology-Biocontamination control Part 3: Methodology for measuring the efficiency of processes of cleaning and (or) disinfection of inert surfaces bearing biocontaminated wet soiling or biofilms

The scope of the various currently available standards presented above are detailed below.

ISO 14644–1 Classification of air cleanliness

This standard covers the classification of air cleanliness in cleanrooms and associated controlled environments. This classification is in accordance with the standard as specified and is only concerned with the concentration of airborne particles.

It must be stressed that this standard cannot be used to characterize the physical, chemical, radiological, or viable nature of airborne particles.

ISO 14644–2 Specification for testing and monitoring to prove continued compliance

This part of the overall standard specifies requirements for periodic testing of cleanrooms and associated controlled environments to prove their continued compliance with ISO

14644–1, and for the designated class of environmental cleanliness.

ISO 14644–3 Metrology and test methods
This standard specifies the various methods and techniques which can be used to characterize and monitor various parameters in cleanrooms and other controlled environments

ISO 14644–4 Design, Construction, and start-up

ISO 14644–5 Cleanroom operations
This international standard specifies the basic requirements for operating cleanrooms and other controlled environments. It is primarily intended for those who are planning to carry out work in a cleanroom or any other controlled environment.

ISO 14698–1 General principles
This standard describes the principles and basic methodology for a formal system to assess and control biocontamination where cleanroom technology is applied. The standard is used in order to allow reproducible monitoring of biocontamination and also to allow selection of appropriate protective measures.

ISO 14698–2 Evaluation and interpretation of biocontamination data
This part of the standard concerning cleanroom technology and biocontamination control describes basic principles and methodological requirements for all microbiological data evaluation. The standard also covers the estimation of biocontamination data obtained from sampling for viable particles in zones at risk, as specified by the selected system.

ISO 14698–3 Methodology for measuring the efficiency
This standard concerns the efficiency of various processes that incorporate one or more of the following actions: rinsing, cleaning, disinfection, combined cleaning and disinfection by either biochemical or mechanical means. Methods of measuring the efficiency of the processes of rinsing and/or cleaning

Table 17. Classes of air cleanliness according to ISO 14644-1

Classification number (N)	Maximum concentration limits (particles/m^3 of air) (for particles equal to and larger than the sizes shown below)					
	0.1 µm	0.2 µm	0.3 µm	0.5 µm	1 µm	5 µm
ISO 1	10	2				
ISO 2	100	24	10	4		
ISO 3	1000	237	102	35	8	
ISO 4	10000	2370	1020	352	83	
ISO 5	100000	23700	10200	3520	832	29
ISO 6	1000000	237000	102000	35200	8320	293
ISO 7				352000	83200	2930
ISO 8				3520000	832000	29300
ISO 9				35200000	8320000	293000

and/or disinfection and/or combined cleaning and disinfection of wet soiled surfaces on which microorganisms may be present either with or without the formation of a biofilm, is explained in relation to the application of cleanroom technology.

4.9 Classification of airborne particles according to ISO 14644-1

The International Standards Organization has produced a worldwide standard of cleanroom classification. The classes of air cleanliness specified in this standard are shown in Table 17.

This table is derived from the following formula

$$C_n = 10^N \times (0.1/D)^{2.08}$$

where

- C_n represents the maximum permitted concentration (particles/m^3 of air) of airborne particles that are equal to and larger than the specified particle size. Cn is rounded up to the nearest whole number
- N is the ISO classification number, which shall not exceed 9. Intermediate ISO classification numbers may, if desired, be specified, with 0.1 as the smallest permitted increment of N
- D is the considered particle size stated in µm

Different international standards are compared in Table 18.

Table 18. Comparison of various international standards

USA Fed Std 209 D[1]	USA Fed Std 209 E[2]	Britain BS 5295[3]	Australia AS 1386[4]	France AFNOR X44101	Germany VDI 2083[5]	ISO 14644-1[6]	Japan JACA 24[7]
						1	1
				–	0	2	2
1	M 1.5	C	0.035	–	1	3	3
10	M 2.5	D	0.35	–	2	4	4
100	M 3.5	E	3.5	4000	3	5	5
1000	M 4.5	G	35	–	4	6	6
10000	M 5.5	J	350	400000	5	7	7
100000	M 6.5	K	3500	4000000	6	8	8
		M			7	9	–

1 Measured as number of particles ≥ 0.5 μm/ft^3
2 Measured as number of particles ≥ 0.5 μm/m^3
3 Measured as number of particles ≥ 0.5 μm/ft^3
4 Measured as number of particles ≥ 0.5 μm/m^3
5 Measured as number of particles ≥ 0.1 μm/m^3
6 Measured as number of particles ≥ 0.5 μm/m^3
7 Measured as number of particles ≥ 0.1 μm/m^3

4.10 Cleanliness testing within cleanrooms

Measurement of cleanliness in a cleanroom should follow certain guidelines in order to give as thorough and correct result as possible. The process of monitoring a cleanroom can be divided into stages such as *preparation*, *measurement* and finally *evaluation* and *reporting*.

4.10.1 Preparations for measurements

Before the standard of cleanliness of a cleanroom can be monitored, a decision must be made as to which parameters should be evaluated. In practice, this means that the following considerations should be taken into account.

- The size or area of the cleanroom should be defined
- Appropriate instrumentation should be used to carry out these measurements
- The required class level of the cleanroom should be determined
- The number of sampling points within the room should be determined
- The volume size of the samples to be monitored should be ascertained

- The time required to carry out one test should be calculated
- The total time required to complete all the tests necessary for the room to comply with the requirements of the chosen class designation should be calculated

4.10.2 Measurement and evaluation

Once the above-mentioned considerations have been taken into account, the monitoring process can begin as shown below.

- Carry out various tests and measurements
- Evaluate the data obtained in relation to the requirements of the chosen standard
- Finally, using the results of the tests, a decision can be made as to whether the cleanroom meets the standards required

4.10.3 Reporting

After measurement and evaluation, the information gathered should be documented. The documentation can include the following for example:

- Identification and location of the cleanroom (clean zone)
- Specification of the particle counter used together with its calibration
- Measurement of background for the particle counter
- Date and time when testing was performed
- The occupancy state of the cleanroom (clean zone) – as built, at rest or operational
- Type of test performed – whether it is for verification or monitoring
- The target cleanliness of the cleanroom
- The particle size area that is measured
- The incoming airflow to the discrete particle counter
- The location of the test sites
- Test scheme and protocol
- Raw data for every test site (if necessary)

4.10.4 Control program

With the implementation of a control program it is often possible to eliminate errors before the system is put into use. This can save both time and money. If errors are built into the system process failures will eventually occur and these are then difficult to analyse and define. A good control program

Table 19. Principles of a contamination control program

Controlled environment	Cleanroom
DOP testing of HEPA filters	Number of turn overs of air in the room
Air speed control	Temperature and humidity
Pre-study with smoke	Differential pressure
Induction test	DOP test
Classification	Classification
Smoke visualization (photo or video)	Smoke visualization (photo or video)
Approval	Approval

is therefore a necessary working tool in order to reach the required goal of products and process equipment that are efficiently protected against contamination caused by particles.

The various steps in a contamination control program for a clean area, cleanroom or clean zone are shown in Table 19.

4.11 Classification of pharmaceutical cleanroom

In the standards mentioned previously, i.e. the Federal standard, the British standard and also the new ISO ›209‹ standards, there are no guidelines for the concentration of viable particles, i.e. microorganisms. It should be noted that there are no universal guidelines or equations available to calculate the proportion of viable and non-viable particles present in the air; the absence of such guidelines is emphasized in US Federal Standard 209 E. In other words no mathematical formula exists which can be used to calculate the concentration of microorganisms in a sample of air whose volume and total particle content are known.

For this reason more specific guidelines relating to the number and level of viable microorganisms in the air were required and have now been produced. These guidelines are incorporated within Good Manufacturing Practice (GMP) for instance. GMP guidelines also state the need to take into account the different occupancy states of a cleanroom or a clean zone. The occupancy states are the same as those defined in the Federal Standard and the ISO standard, namely ›at rest‹ and ›operational‹. Under GMP guidelines, it is essential that the standard of air cleanliness should be tested under

both occupancy states in order that the cleanliness standard of the cleanroom is known at all times. The hygiene classification used in GMP follows the guidelines specified in the supplement, ›Annexe to the EU Guide to Good Manufacturing Practice – Manufacture of Sterile Medicinal Products‹.

The following text is an extract from the above-mentioned supplement and is relevant to the design and operation of pharmaceutical cleanrooms.

The manufacture of sterile products should be carried out in clean areas, entry to which should be through airlocks for personnel and (or) for equipment and materials. Clean areas should be maintained to an appropriate cleanliness standard and supplied with air which has passed through a filter of an appropriate efficiency.

The various operations of component preparation, products preparation and filling should be carried out in separate areas within the clean area. Manufacturing operations are divided into two categories; firstly those where the product is terminally sterilized and secondly those which are conducted aseptically at some or all stages.

Clean area for the manufactures of sterile products are classified according to the required characteristics of the environment. Each manufacturing operation requires an appropriate environmental cleanliness level in the operational state in order to minimize the risk of particulate or microbial contamination if the product or material being handled.

In order to meet ›in operation‹ conditions these areas should be designed to reach certain specific air-cleanliness levels in the ›at rest‹ occupancy state. The ›at rest‹ state is the condition where the installation is complete with production equipment installed and operating but with no operating personnel present. The ›in operation‹ state is the condition where the installation is functioning in the defined mode with the specific number of personnel working.

This supplement divides the various cleanrooms into grades, namely:

Grade A which is a local zone for high-risk operations for example a filling zone for open ampoules and vials that are aseptically closed at a later time. This grade is often achieved by the use of various

types of benches such as the LAF-bench or safety benches (see Section 4.14). The laminar or unidirectional airstream in such a system should be homogeneous with an air velocity of at least 0.3 m/s for vertical systems and 0.45 m/s for horizontal systems.

Grade B — is used during aseptic manufacturing and filling and also as the background for grade A cleanrooms.

Grade C and D — are clean areas that are used for less critical steps during production of sterile products.

Table 20 shows the maximum number of particles permitted per cubic meter of air in the different grades of cleanroom described above.

In order to reach the cleanliness levels required in grade B, C and D cleanrooms, the number of changes of air in the room should be greater than 20, i.e. the number of times per

Table 20. Maximum number of particles/m^3 permitted within each grade of cleanroom according to GMP

Grade (GMP)	**Maximum permitted number of particles/m^3 equal to or above**			
	At rest[2]		Operational	
	0.5 µm	5 µm	0.5 µm	5 µm
A	3500	0	3500	0
B[1]	3500	0	350000	2000
C[1]	350000	2000	3500000	20000
D[1]	3500000	20000	Not defined[3]	Not defined[3]

1 In order to reach the B, C and D air grades, the number of air changes should be related to the size of the room and the equipment and personnel in the room. The air system should be provided with appropriate filters such as HEPA for grades A, B and C.

2 The guidance given for the maximum permitted number of particles in the ›at rest‹ condition corresponds approximately to the US Federal Standard 209 E and the ISO classification as follows: grades A and B correspond with class 100, M 3.5, ISO 5; grade C with class 10000, M 5.5, ISO 7 and grade D with class 100000, M 6.5, ISO 8.

3 The requirement and limit for this area will depend on the nature of the operations carried out.

hour that the air in the room is exchanged for clean air. Furthermore, in order to reach the desired cleanliness class, HEPA-filters are used. Cleanroom classes A, B, C and D, as classified according to GMP, can be compared with US Federal Standard 209 D classes 100 to 100 000 by the number of particles of a particular size that are permitted per cubic foot of air ›at rest‹ within each grade. These figures are shown in Table 20.

In practice the different GMP grades in Table 20 are used as follows:

- Grade A is used in a LAF (UDF)-protected area. Grade A is normally used for filling procedures with products that are at risk of contamination and are normally sterilized at the end of the procedure. On the other hand, when used for operations requiring aseptic production, grade A is normally used for aseptic preparation and filling.
- Grade B cleanrooms are used to provide background cleanliness for grade A areas.
- Grade C is used when preparing solutions at risk of contamination and for filling ampoules or vials, both types of product being sterilized at the end of the procedure. Grade C is used in aseptic production for the preparation of solutions to be filtered.
- Grade D is used for the preparation of solutions and components for subsequent filling, for the production of tablets, for washing and subsequent handling of components after washing. Grade D is also used for the final step in bulk production.

Furthermore, GMP recommends levels of microbiological control for cleanrooms with the occupancy state of ›operational‹. Table 21 shows the maximum number of viable microorganisms permitted at various locations, measured as Colony Forming Units (CFU).

Table 21. Maximum number of CFU permitted in each grade of cleanroom

	Maximum number of viable organisms[1]			
Grade	**Air sample** CFU/m^3	**Settling plate** CFU/ 90 mm/4 h	**Contact plate** CFU/ 55 mm	**Glove print** CFU/ 5 fingers
A	< 1[2]	< 1[2]	< 1[2]	< 1[2]
B	10	5	5	5
C	100	50[3]	25	–
D	200	100[3]	50	–

1 Recommended limits for contamination may be exceeded on isolated occasions and this situation would necessitate only an examination of the production conditions and the control system. If the frequency of these occasions is high or an upward trend of these occurrences is observed, then action should be taken.
2 Low values shown here are only reliable when a large number of samples is taken.
3 For grades C and D settle plates may be exposed for less than 4 h.

4.11.1 Factors determining the cleanliness of a cleanroom

In clean environments all exposed surfaces should be smooth, hard and inert and ideally should be continuously tested in order to reduce the risk of spreading and/or collecting contaminating particles. The levels of contaminants including microorganisms, should be as low as possible. All of these requirements must be met in order to clean and disinfect a cleanroom efficiently. In order to avoid the accumulation of dust and other types of contaminants in addition to facilitating cleaning and disinfection, there should not be any area within a cleanroom that cannot be accessed and cleaned. Some areas that might have an impact on the overall cleanliness in a cleanroom are as follows:

- The cleanliness of the incoming ventilation air
- The volume of the incoming ventilation air
- Activities taking place within the cleanroom, i.e. the presence of production personnel and the nature of the tasks they carry out during production
- The pattern of air movement within the cleanroom

The cleanliness of the incoming air through the ventilation system can be controlled with the aid of various types of filters. The filters used within contamination control have

Table 22. Types of air filter and resultant air quality

Filter type	Tested with	Minimum removal	Measured particle size (μm)
Absolute filter	DOP	99.97%	
High Efficient Particle Air Filter (HEPA)	DOP	99.97%	0.5 and greater
Ultra Low Penetrating Air Filter (ULPA)	DOP	99.999%	0.12 and greater

different contamination removal ratings resulting in different qualities of the air supplied (see Table 22).

4.12 Different types of cleanrooms

In practice there exists two general types of cleanroom, differing only in their methods of ventilation. The first type is normally known as a conventionally-ventilated cleanroom, but in some instances is called turbulently ventilated or a cleanroom with non-unidirectional airflow patterns. The other type of cleanroom employs unidirectional airflow, and in the literature is sometimes referred to as an LAF-room or cleanroom, with laminar flow. On some occasions the latter type of cleanroom is also known as an ultra clean cleanroom.

4.12.1 Conventionally-ventilated cleanrooms

In this older and more traditional type of cleanroom the air is introduced through a ventilation system entering at different locations in the roof, for instance. The ventilation system traditionally consists of a fan, filters, and some kind of battery that can be used to adjust the heat and in some cases also the humidity of the air. The treated air is then transported to the point of use through a distribution system.

In many cases this type of ventilation system is similar to a traditional ventilation system used in offices and other areas where ventilation is usually required. However there are some differences between a traditional office ventilation system and the ventilation system used for a conventionally-ventilated cleanroom (see Figure 15). One of the major differences is the volume of air introduced into a cleanroom. In order to create the desired cleanliness of air within a cleanroom, larger vol-

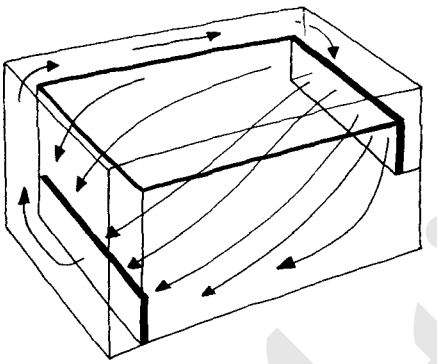

Figure 15. Conventionally-ventilated cleanroom which uses the principle of dilution

umes of air are filtered into it than those required for a traditional office environment. In addition, the air entering a cleanroom passes through a high-efficiency filter, for instance a HEPA-filter or a filter with a similar or more efficient filtering capacity, which is positioned so that the ventilation air passes through the filter just before entering the room. This is often called filtration at point of use.

Within a conventionally-ventilated cleanroom the filtered and thereby cleaned air is continuously added to the room. The ventilation in conventionally-ventilated cleanrooms is based on a turbulent mixture of the clean air together with the contaminated air. This system thus continuously decreases the number of contaminants in the air by the act of dilution.

4.12.2 Unidirectional flow cleanrooms

The term unidirectional flow (UDF) means that not only filtered, clean air is introduced into the room but also that the pattern of air movement through the room is at all times, maintained in a unidirectional flow. As the clean ventilation air is used, it is forced to flow in a single direction through the room. This type of cleanroom was previously known as a cleanroom with laminar flow or, an LAF cleanroom. In theory ›laminar flow‹ should actually describe the overall pattern of air movement through the room and since it is exceptionally difficult to create such a laminar flow, and even impossible when equipment and personnel are *in situ*, the terminology

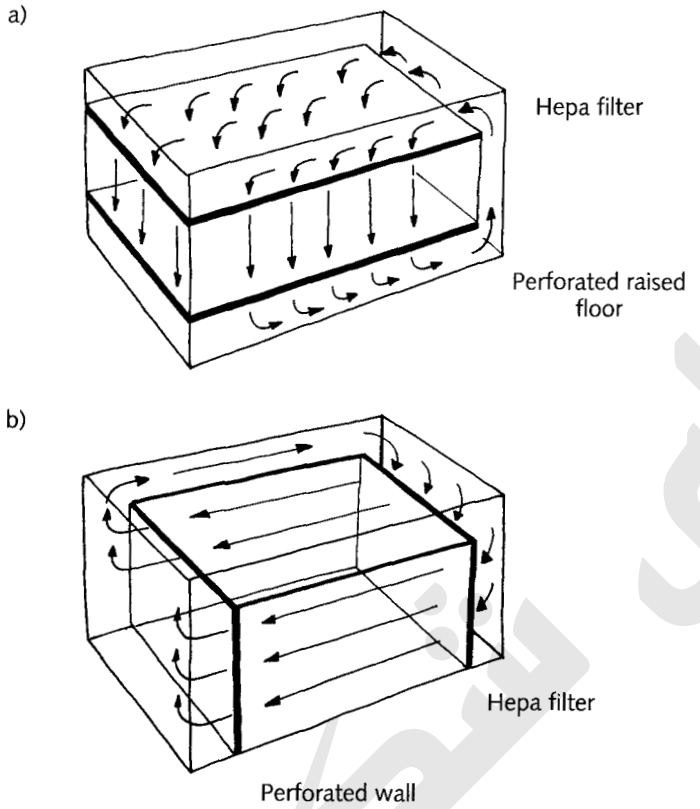

Figure 16. UDF cleanroom with (a) vertical and (b) horizontal airflows

(LAF) was considered misleading and was replaced with the term ›unidirectional flow‹. The speed of movement of the air in such UDF cleanrooms is in the range of 0.3 to 0.45 m/s.

The purpose of a unidirectional airflow is, as compared with conventionally-ventilated cleanrooms which employ the dilution effect, that the contaminants in the air should be picked up by the unidirectional airstream and in that way actively transported out of the room. The airflow in a unidirectional flow cleanroom could actually be looked upon as a piston forcing the contaminated air to leave the room. Unidirectional flow cleanrooms are found in two versions, vertical as well as horizontal. In a vertical unidirectional flow cleanroom the air moves in a vertical pattern and in a horizontal

flow unidirectional cleanroom the air is travelling in a horizontal pattern. Both these flow patterns are illustrated in Figure 16.

Traditionally, in a vertical unidirectional flow cleanroom, the air enters through filters which almost entirely cover the ceiling area. The air then travels downwards at high speed collecting contaminants in the room air en route and is finally forced out of the room through perforations in a specially designed raised floor. In a horizontal unidirectional flow cleanroom on the other hand, the situation is somewhat different as the incoming air enters the room through filters which cover almost the entire surface of one wall. The air then travels through the room in a horizontal pattern and leaves via a perforated wall on the opposite side of the room.

4.12.3 Airflow and air quantity

In a conventionally-ventilated cleanroom the cleanliness of the air in the room is dependent on the number of times it is exchanged for fresh and cleaner air. In a unidirectional airflow cleanroom the cleanliness of the air in the room is dependent on the cleanliness of the introduced air and also the speed at which this clean air passes through the room.

In order to determine how and to what extent the room is ventilated there are two ways of expressing cleanliness in relation to air quantity and airflow. In conventionally-ventilated cleanrooms the addition of air to the room is normally expressed as the number of turnovers of air in a given room, measured per hour. In offices and other ventilated buildings, the turnover of air is approximately between two and three times per hour. This means that the air in such rooms will be exchanged for clean air two to three times every hour. When considering traditional cleanrooms with ventilation patterns of the conventional type, the exchange rate is normally between 10 and 15 air changes per hour. When working to GMP guidelines in class B, C and D cleanrooms, an average turnover rate of 20 air changes per hour is recommended.

The total volume of air that is introduced in a unidirectional flow type of cleanroom is several times higher, in general between 10 and 100 times, than the air volume introduced into a conventional cleanroom. This is one of the reasons why unidirectional flow cleanrooms are more expensive both to

buy and to use. The ventilation in a unidirectional flow cleanroom is not defined by the rate of air exchange in the room per hour but rather by the velocity of the airflow passing through the room. Traditionally the flow of air through such a room lies in the region between 0.3 and 0.45 m/s.

4.13 Cleanrooms and clean zones

In the past cleanrooms were manufactured and used in accordance with the cleanliness standards that prevailed at the time, but it became more difficult to meet the standards of cleanliness that the newer, more sophisticated production processes required if the outer environs of the cleanrooms were not also controlled for contamination.

This is one of the contributory reasons for certain industries building cleanrooms within already existing cleanrooms. The high standard of cleanliness demanded by the industries of today has also increased the cost of constructing and maintaining cleanrooms. This has led to the cleanrooms of today being constructed with clean areas or so-called clean zones placed within them. In principle this means that areas with extremely high cleanliness levels can be located within smaller areas of the cleanroom. By implementing this type of concept it is possible to solve the practical problems of cleanliness and at the same time to keep the costs at a reasonable level.

The clean zone (which can be achieved by using a special type of bench or by just using an extra fan and filter solution, for instance) offers an environment with a higher cleanliness level in which work of the most critical nature can be undertaken.

4.14 Working in clean zones

Clean zones within cleanrooms often consists of different types of benches such as open benches or LAF units and safety benches as they sometimes are called. Open benches or LAF units are used to provide complete protection to the product being handled. This means that an LAF unit is used when the products that are being handled need complete protection. Open LAF units exist in two versions depending on the flow pattern of the introduced air: horizontal units have a horizontal flow pattern whereas vertical units have vertical flow pattern (see Figure 17).

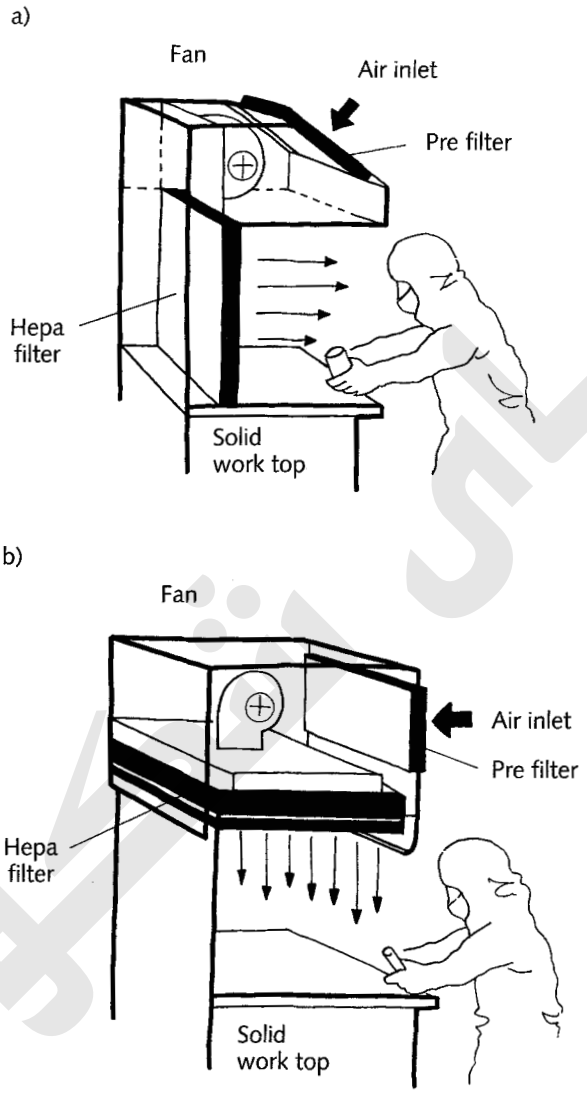

Figure 17. Schematic representation of open LAF-units, (a) horizontal and (b) vertical

Safety benches are divided into different classes, class I, II and III and are used for the protection of personnel, although certain types also afford protection to the product being handled.

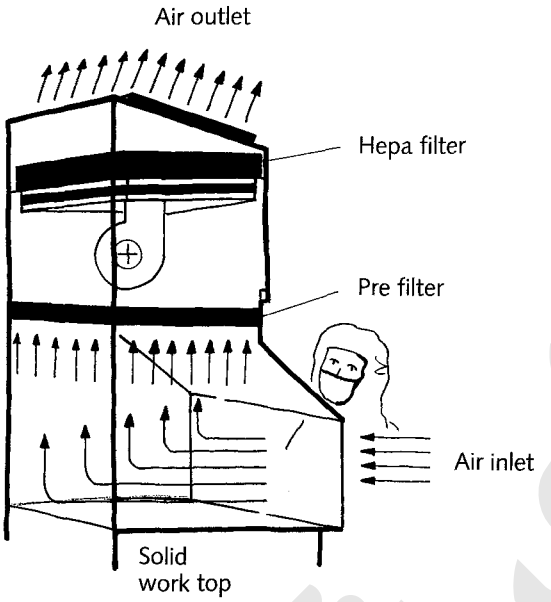

Figure 18. Schematic representation of safety bench class I

Safety bench class I (shown in Figure 18) is used for the protection of personnel only. The various contaminants in the outer environment where the operator is situated, will flow into the area where work on the product is being carried out. The class I bench therefore, offers protection only to the operator and not to the product.

Safety bench class II (Figure 19) however, provides protection for the product as well as for personnel, this is achieved in the following manner. The air within the bench cannot escape and reach the operator and at the same time contaminants in the air surrounding the operator cannot enter the critical area within the cabinet and therefore cannot contaminate the product.

Safety bench class III is a totally sealed system, sometimes referred to as an isolator. In an isolator there is a total barrier between the outer and the inner environment and this barrier is so tight that nothing can either leave or enter the enclosed system. In some cases safety benches class III are also called enhanced clean devices or glove boxes etc. (see Figure 20).

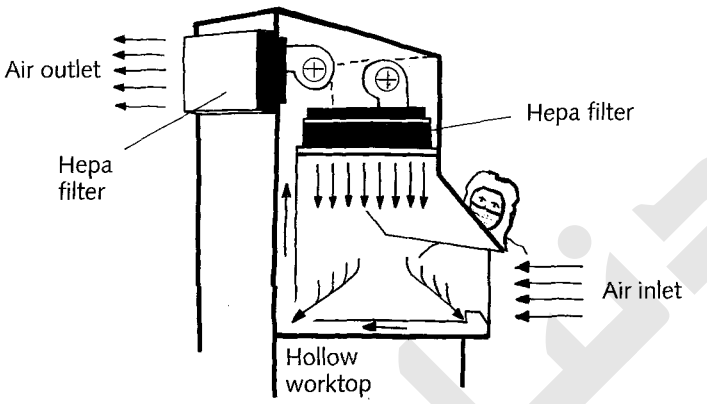

Figure 19 Schematic representation of safety bench class II

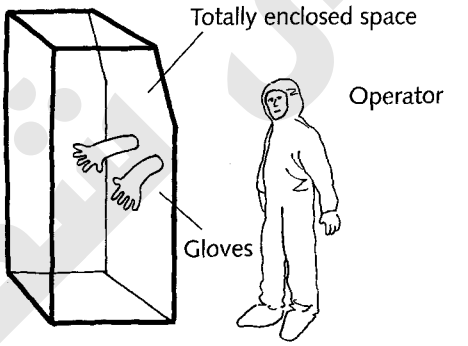

Figure 20 Schematic representation of safety bench class III

The use of isolators or enhanced clean devices as they are called in ISO ›209‹, has increased significantly over recent years especially when dealing with products or a production process that might be toxic or hazardous to the operator. Working in such a safety cabinet is, as expected, much safer than working with a class I or II safety bench, however there is a major drawback, namely the flexibility. An isolator is less flexible than other types of safety benches mostly because all the materials to be handled have to be placed in the cabinet through an air lock before work can commence.

4.14.1 Working in cleanrooms and clean zones

When working in a cleanroom or in a clean zone it might be assumed that there is no risk of contamination or at least only a minimal risk. This assumption is understandable since the air inside the cleanroom or the clean zone is so rigorously controlled that the risk of contamination must be extremely small, at least from a theoretical point of view. This however, is not absolutely true. The observance of certain precautions when working both in a cleanroom and in a clean zone are of major importance for the successful outcome of a production process. Since the practical working techniques are of major importance for the maintenance of cleanliness both in clean zones and cleanrooms, there are some general rules which should be observed in this type of work. The rules for working in a clean zone can be summarized as follows:

- Ensure that the clean zone functions in a satisfactory way. In practice this means checking that the airflow is correct and that the filter used to clean the incoming air has retained its integrity.
- All necessary movements inside a clean zone must be performed in a slow and controlled manner. This means that the operator must work according to a controlled pattern when moving hands and arms during work. It is possible for vortexes to be formed when hands are introduced inside the critical area of a bench. This is why it is not good practice to place the hands towards the air stream in front of or above a sensitive area. Vortexes formed in such a way can in the worst cases, extend outside the area of containment, thereby allowing contaminants from outside the bench to enter the critical zone (see Figure 21).
- It is important that all movement of hands, arms and various items or objects in and out of the cabinet space are performed slowly and with low frequency. If there is a risk of vortexes formation during such an operation it may be preferable to take materials into the clean zone of the bench through an air lock.
- Items placed on the working surface of a clean bench should not be too large in size. Equipment and materials should not be placed close to the inner side walls of a cabinet and should be spread out evenly over the entire

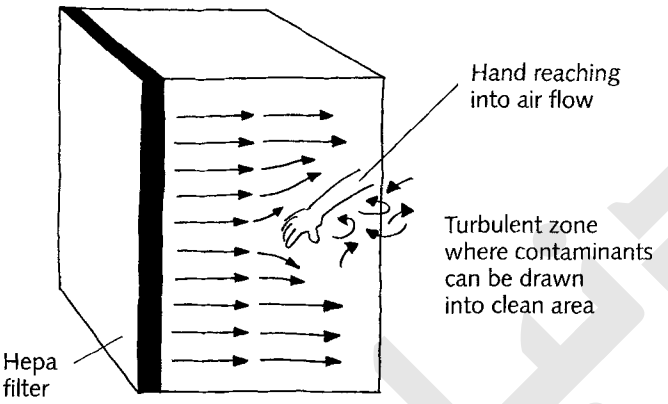

Figure 21 Vortex formation generated by a hand. The air that is moving towards the hand will deviate from its flow pattern, thereby resulting in vortex formation on the ›shadow-side‹ of the hand. This vortex consists of turbulent air which can force contaminants from outside into the clean zone

surface of the bench. Every item placed in a cabinet will give rise to vortex formation (see Figure 22).

- Larger items that have to be taken into the cabinet should preferably be placed on a stand so that they are located approximately 5–10 cm above the surface of the bench. This will allow free passage of air not only round the sides and top of the item but also underneath it.
- The total area of the bench does not account for the total operating area. The surface close to the front of the bench where the operator stands is a critical area. If the bench is equipped with side walls, these are also critical parts. One of the most critical areas of a bench is that close to the filter. The aforementioned areas are often called free zones and materials, items etc. should not be placed in these zones.
- The bench should not be used for storage. On completion of a task, the bench should be left as empty as possible.
- Access to the cabinet from the exterior should be through as small an opening as is possible. A maximum opening size is often given by the manufacturers of clean zones. In some cases an alarm is incorporated into the system to warn the operator when the access opening is too large. If a safety cabinet has too large an access to the external environment,

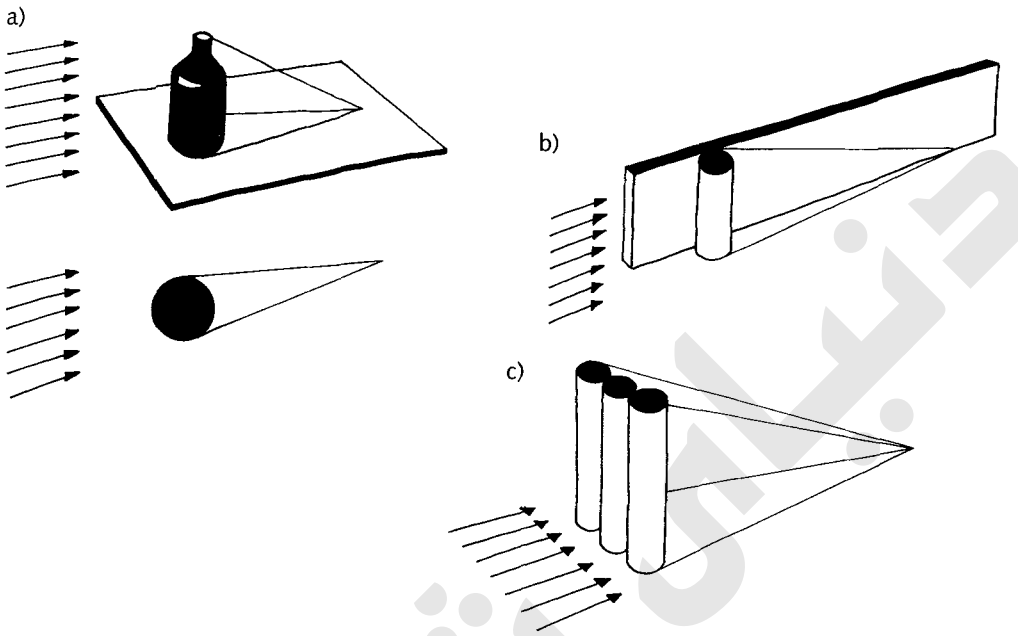

Figure 22. Vortex formation by objects. The following figures show schematically how the air movement in a horizontal LAF bench can be affected by an object placed in the airstream. (a) A single object placed in a horizontal airflow will change the flow pattern of the air. A turbulent zone will be formed behind the object. The larger the object the longer and larger the turbulent zone will be. A turbulent zone is generally considered to be three times as long as the diameter of the object. A bottle with a diameter of 10 cm will thus create a vortex zone as long as 30 cm. (b) The turbulent zone will be three times as long as the total width of several objects placed close together in a LAF bench. This is the reason for having a suitable distance between adjacent objects in a clean zone. (c) An object that is placed close to the wall of a LAF-unit will hinder the airflow between the object and the wall. The turbulent zone thus created can be as much as six times the width of the object, and poses the risk of attracting contaminants in the air outside the bench, into the clean zone

this can comprise the cleanliness within the cabinet as a result of activities taking place outside the bench, for instance.

- A comfortable chair should be available when working at a clean bench. The chair must, of course, be approved for use in cleanrooms and it should be adjustable so that the operator is in the most appropriate position not only for sitting but also for keeping their arms in a comfortable position so that mistakes resulting from tiredness can be avoided.

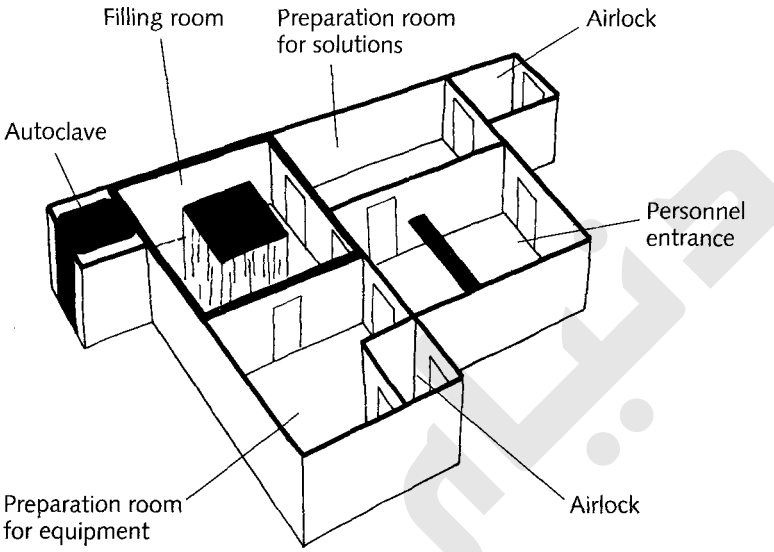

Figure 23. Cleanroom layout for the production of finally-sterilized products

4.14.2 Maintenance and safety

The airflow velocity together with the effectiveness of the filter system has a direct impact on the overall efficiency of an LAF unit. The filter used in such an operation will gradually become blocked by the contaminants collected from the air, which in turn will block the passage of air through the filter. In order to extend the life of a HEPA filter used in such applications, various types of pre-filter are often used. The pre-filter will collect the majority of particles and the HEPA filter will then only be challenged by a reduced number of particles. In this system, the pre-filter will become blocked and therefore replaced first. As a result of such a blockage, the speed of the fan forcing the air through the filter will have to be monitored and possibly adjusted in order to maintain the correct airflow through the LAF unit. Since the source of air for LAF units and/or safety benches used in cleanrooms, is the surrounding clean environment, air filters in such benches generally have a fairly long life, so that changing a filter is often a rare event.

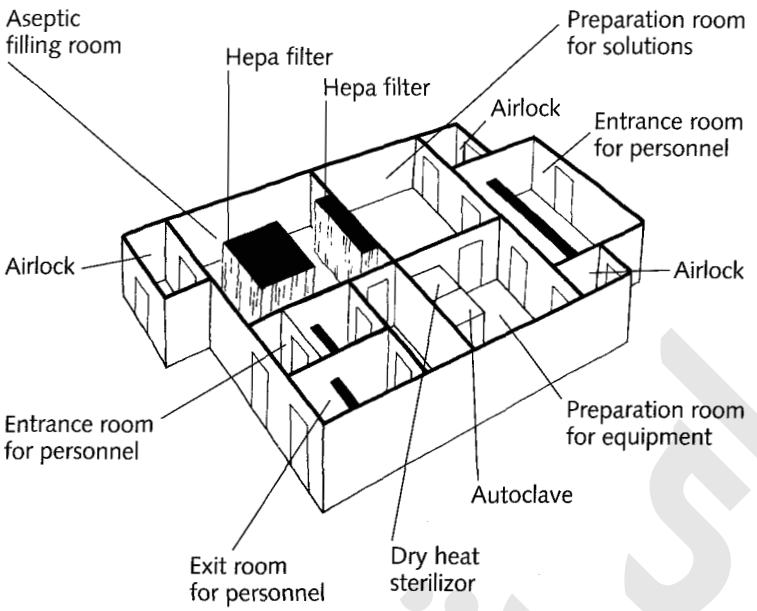

Figure 24. Cleanroom layout for the production of aseptically filled products

4.14.3 Systems for cleanroom production

To create a clean environment for a production process, a cleanroom meeting the requirements for a particular standard of cleanliness is needed, and this can be achieved with an holistic approach and an ›aerial‹ overview of the situation. Figures 23 and 24 show schematically two possible ways that cleanrooms and clean zones can be arranged to obtain the required cleanliness levels. The two examples shown in these figures have been taken from the pharmaceutical industry, but they could equally apply to any other type of industry. The differences in the two examples shown depend on the nature of the product to be manufactured and on the conditions required for the manufacturing process.

Figure 23 shows a pharmaceutical process in which a prepared product is filled into vials, ampoules or bottles. The production takes place in a cleanroom, meaning a facility with low levels of ›dead‹ particles and microorganisms. After the product has been transferred into vials, they are sealed. The filled vials are then placed in an autoclave and heated to a sterilization temperature. During this process any viable mi-

croorganisms are killed. This type of production process is normally called production of ›finally-sterilized‹ products.

Figure 24 shows another type of production system used within the pharmaceutical industry. This production process is often described as aseptic production and is used for products that cannot be sterilized in an autoclave as a final step. Examples of such products are those containing active substances that might be harmed or destroyed during heat treatment. In order to ensure the sterility of the product it is filtered through a sterile filter which is very fine and able to trap microorganisms. After filtration the product is dispensed into bottles or ampoules which are then sealed. After sealing, the vials leave the production room through an air lock.

Both of these two production systems take place in cleanrooms. The cleanrooms are used with an overpressure (a recommended value for the overpressure is 15 Pa higher than the pressure in the outer less clean environment). The added ventilation air is filtered to the required level of cleanliness. Within the rooms there are also clean zones, for example clean air devices containing fans and filters or LAF or safety benches.

Figures 23 and 24 provide examples of the different requirements of a cleanroom with respect to personnel entering and leaving. In the system shown in Figure 23 for producing ›finally-sterilized‹ products, there is only one entrance to the room, and therefore personnel entering the cleanroom in fresh protective clothing will come into contact with personnel who are leaving the cleanroom and may have contaminated clothing. In this case staff will have to be particularly conscientious about changing their cleanroom garments and about their movements within the room. In the case of aseptic production (Figure 24) there is a separate entrance and exit in the cleanroom. This system is the preferred alternative as there is a definite demarcation between clean and contaminated areas. However the latter system takes up considerably more space and this may increase the costs of the entire production system.

4.15 Conclusions

A cleanroom is a room that is clean, but whose cleanliness is defined by various standards.

The principles of contamination control and cleanroom technology are to define classes of air and surface cleanliness. These different classifications are specified in order to fulfill the different requirements of various production processes and it is these requirements which form the basis on which the decision regarding the type of cleanroom or clean zone to be used is made. Cleanrooms are generally of two types depending on how the air is introduced into the room.

Conventionally-ventilated cleanrooms employ a dilution effect and cleanrooms with a unidirectional airflow employ the principle of a piston forcing contaminants out of the room.

Unidirectional airflow cleanrooms are found in two versions depending on the flow type. These are horizontally-ventilated rooms and vertically-ventilated rooms.

It is more common today, to find clean zones or cleaner areas within a cleanroom. This means that levels of extremely high cleanliness will be restricted to zones in the room where they are required for a particular task and that the remainder of the cleanroom will be kept at a lower cleanliness level. This combination of a clean zone within a cleanroom is the most cost-effective option.

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5 Cleaning and decontamination

5.1 Introduction

It may be difficult to motivate staff, and at times seem even unnecessary, to discuss and plan the cleaning procedures for cleanrooms and other rooms in the vicinity. One often hears comments such as ›you can't see the contaminants‹ or ›it looks as clean after cleaning as before‹. Both these comments illustrate the general attitude towards the task of decontaminating cleanrooms and clean zones. A cleanroom is not just one part of an industrial activity, it is and should be viewed as, the central part of a total system in which a series of critical process steps have to be carried out. This means that not only should the cleanroom itself be an important consideration but also those rooms which are situated outside the cleanroom.

The cleanliness and the overall quality of the outer environment is therefore of great importance in relation to the cleanliness level of the cleanroom itself. This is why the outer environment also has a major impact on the quality of the work which is to be undertaken in the cleanroom.

This is why it is important that all clean and hygienic operations should be studied from an holistic viewpoint. The questions addressed by an holistic overview should include among others, (1) are personnel adequately trained to carry out their tasks? (2) Are they motivated to do a good job? and (3) Are they fully aware of the consequences associated with not working in accordance with the instructions?

5.2 The purpose of cleaning

The purpose of cleaning cleanrooms and clean zones is to *release*, *collect* and *remove* all undesired contaminants from surfaces of varying importance (with the regard to cleanliness) in the room or zone.

5.3 Standards and practices

Standards for cleaning, decontamination and surface cleanliness in industries with requirements for high levels of cleanliness are documented in various standards and recommendations, such as the ISO Standard 14644–5, VDI 2083 and IES-RP-CC-018.2. Furthermore, different national standards are available regarding cleaning and disinfecting of cleanrooms. One national standard, the Swedish Standard SS 2678 produced by the Swedish Mechanic Standardization Organization applies mainly to the mechanical industry, but also gives guidelines that might be useful in other branches of industry. The German organization VDI has produced a document VDI 2083, which contains classifications for surface cleanliness that are similar to the standards for air cleanliness defined in US Federal Standard 209. The Institute of Environmental Sciences and Technologies (IEST) in the United States has produced a document containing various recommendations entitled ›Cleanroom Housekeeping – Operating and Monitoring Procedures‹ (IEST-RP-CC-018.2; RP = Recommended Practices, CC = Contamination Control).

5.4 Cleaning

Cleaning as a whole can be divided into two categories, preventive cleaning and active cleaning. Preventive cleaning is undertaken in order to avoid and (or) minimize the creation and transportation of contaminants from the outer to the inner environment and also to avoid the dispersion of contaminants inside a critical area such as a cleanroom. Preventive cleaning also includes all actions taken in order to facilitate active cleaning. Active cleaning on the other hand, is described as the actual release, collection, transportation and removal of contaminants from the critical zone.

5.4.1 Preventive cleaning

Preventive cleaning is also used as a preparatory measure for active cleaning. Preventive cleaning includes the following for instance:

- All the land areas outside the actual production facility should have permanent paving in order to minimize the amount of contamination taken into the production building on shoes.

- In order to avoid the use of anti-freeze on roads, pavements and parking places during cold weather, it may be advisable to install heating coils in all areas leading to the production building.
- A floor grill should be placed just outside the door to the entrance of the facility. The floor grill should be large enough and particularly deep enough to collect large amounts of soil.
- So-called entrance carpets located in the entrance halls of the building should be made of synthetic fibres. These specially designed carpets should be as wide as the entrance door and they should also be large enough to allow a person to take at least three steps on them (in practice this means that the entrance mats should be at least 2 m long).
- Within the facility tacky mats are usually utilized. This type of mat has a sticky surface which attaches to and thus removes contaminants from the soles of shoes, wheels and any object that passes over it.

Preventive cleaning is carried out both outside and within a cleanroom. All surfaces within a cleanroom should be smooth and even in order to facilitate active cleaning. This also applies to seams in the floor covering. If the floor is not covered with a single layer of floor covering, the seams should be filled with a suitable material. Corners between the floor and walls should have rounded edges so that it is easier to wipe them clean.

All floors should be kept as free as possible from foreign material in order to facilitate active cleaning. These means that chairs, tables etc. should preferably be mounted on the wall, however, from a practical point of view, this is not always possible.

Drains are practical in many ways but should be avoided since they often pose a considerable risk to hygiene. Cracks and gaps in and between walls and floors should be avoided. Visible and free hanging pipelines should also be avoided and if possible be located in areas outside the cleanroom so that they will be easier to service and maintain, even during cleanroom operations.

5.4.2 Active cleaning

Active cleaning can be divided into two procedures, cleaning and decontamination. Cleaning is performed in order to remove contaminants that are visible to the naked eye whereas decontamination refers to the elimination of all other contaminants, i.e. those that are not visible to the naked eye. Active cleaning can be described as production cleaning or maintenance cleaning.

Production cleaning takes place in premises used only by personnel and in those used for production and include cleaning on and around production machinery and tooling. Maintenance cleaning incorporates degreasing, industrial washing and removal of surface coatings such as pigments or oxides. Only production cleaning will be discussed here since this type of cleaning is usually carried out by the personnel working in the cleanroom, i.e. the operators.

5.5 Cleaning responsibility

It is generally accepted that the personnel who work on a production process in a cleanroom, are also responsible for all aspects of cleaning resulting from the process. The same personnel also attend to the daily cleaning of the room, tables and other items. Major cleaning or special cleaning is in some cases performed by another category of personnel. It is not important which category of staff undertake the cleaning procedures but it is essential that those involved in any cleaning process are fully aware of what is required and are willing to do the job to the best of their ability.

5.6 Classification of surface cleanliness

It is a good practice to classify the cleanliness of areas and surfaces within a particular cleanroom in relation to the nature of the products manufactured and processes carried out therein. Applying these classifications is useful in developing adequate cleaning strategies for cleanrooms.

Surfaces in cleanrooms can in general, be divided into three basic categories, *critical surfaces*, *general cleanroom surfaces* and *surfaces of changing rooms and air locks.*

- Critical surfaces are surfaces at and around the point of manufacturing or production and are areas where con-

taminants can gain direct access to the product or process. Unidirectional flow equipment such as the various types of benches and workstations can be used in order to control the cleanliness of these surfaces. These surfaces are the most critical and should therefore be kept the cleanest.

- General cleanroom surfaces are all the surfaces within a cleanroom that are not considered to be critical with regard to contamination. In practice this means that general surfaces are not those located at the point of production or localized by unidirectional airflow. These surfaces should be cleaned on a regular basis to prevent transfer of contamination onto critical surfaces.
- Surfaces of changing rooms and airlocks are the most contaminated areas as a result of the high level of activity that occurs within them. Frequent cleaning is therefore necessary in order to minimize the level of contamination and also to reduce the possibility of contaminant transfer into the cleanroom.

5.7 Basic cleaning

Once surface cleanliness has been classified, the methods of cleaning must then be defined. There are basic methods for attaining the required levels of cleanliness and these can be divided into three categories, namely basic *gross cleaning*, *intermediate cleaning* and *precision cleaning*.

- Gross cleaning involves the removal of larger particles, of sizes 50 µm and larger. This type of contaminant is often found on floors and is introduced into the cleanroom from changing areas and airlocks. This type of contaminant can be generated from materials broken or spilled during the production process, or from construction processes and various maintenance activities.
- Intermediate cleaning involves the removal of smaller particles, typically in the size range of 10–50 µm. When intermediate cleaning is performed in cleanrooms this usually involves cleaning of walls, benches and hallways. The above-mentioned particles are often still intact after gross cleaning. In other words, intermediate cleaning provides the next level of cleanliness after gross cleaning.
- Finally, precision cleaning is used to remove the remaining

particles from surfaces. These particles are of sizes 10 µm and smaller. Precision cleaning is generally carried out on or in close proximity to critical surfaces where products are stored and/or handled.

5.8 Cleaning program

When setting up a cleaning program the classification of cleanrooms as well as the rate at which these rooms are contaminated, must be taken into consideration. The cleaning schedule must be specified to ensure that cleaning procedures are carried out frequently enough to maintain the required cleanliness standard of a cleanroom once it has been achieved. The requirements of cleaning should take into account the product and the process handled in the cleanroom so that it can be decided whether cleaning should be carried out on a daily, weekly or other periodic basis.

The following should be considered when preparing a general cleaning program:

- Classify all existing surfaces as critical, general and other surfaces
- Determine the optimal cleaning and surface treatment methods needed to obtain the desired cleanliness levels
- Determine the frequency of cleaning required to maintain the desired cleanliness levels for each surface type
- Prepare the cleaning schedules
- Determine which part of the cleaning schedule is to be performed by the cleanroom operators and which is to be performed by cleaning staff
- Choose the correct material, machines, cleaning solutions and surface treatments to be used for the methods specified.
- Train all personnel, both operators and cleaning staff, to the levels required for their specific task in the cleaning program
- Provide adequate storage facilities for the cleaning materials chosen
- Decide how cleaning should be monitored
- Decide what action to take in the event of discrepancies
- Finally, organise all documents and schedules so that these can be easily reviewed and managed

5.9 Control of cleaning methods

Control of cleaning is often performed by what is called visual inspection or visual control. Visual inspection can be described as just looking at a surface to see if it is clean. Since the naked human eye is only able to observe particles of 40 µm or greater in size, visual inspection is normally not sufficient for control of cleaning. In practice this means that written instructions are required for the control of cleaning and that these instructions should be closely adhered to. It is important that all cleaning checks are thorough and recognized by the personnel. There are other methods for controlling surface cleanliness but these are often quite time-consuming and can sometimes take several days before completion of the analysis. Most of these other methods are based on microbial growth using samples collected with contact plates and swabs.

5.10 Cleaning techniques

Most of the contaminants associated with a cleaning process are surface contaminants. These particles are microscopic and in many cases were originally airborne and then came into contact with a surface. Airborne particles are often found floating around quite freely in the air and can settle on surfaces, if they are of a mass great to be affected by gravity.

When particles come into contact with a surface there is a chance that they will bind quite strongly to the surface. The smaller the particle the larger the force that holds the particle attached to the surface (see Table 23). Particles that should be removed during a cleaning process are those found on machinery and other equipment, on products, within the production process, on floors, walls and ceilings and on personnel present in the production room.

5.11 Cleaning methods

Cleaning can be carried out using either *dry* or *wet* methods.

5.11.1 Dry methods

Dry methods include vacuum cleaning and dry tooling. A vacuum cleaner has several advantages including the fact that it is simple and quite inexpensive to use, but it also has

Table 23. Relationship between the size of a particle and its strength of adherence to a surface

Particle size (μm)	**Air speed (m/s)** (required to remove 50% of all particles)
50	< 30
30	60
15	100
5	–

drawbacks such as its inability to collect small particles and to retain any particle once it has been collected.

Figure 25 shows the uptake efficiency of a vacuum cleaner in removing particles from a floor or a mat, in relation to the size of the particles. Nearly 100% of particles greater than 100 μm in size, but only 10% of particles with the sizes around 10 μm are removed from the surface. A vacuum cleaner is unable to take up particles of 10 μm as efficiently as it collects those of 100 μm in size because the smaller particles are bound more strongly to the surface.

The use of dry cloths and mops is also included under the heading of dry cleaning methods. Developments in the textile industry have led to the creation of new materials which can be used for cleaning without the use of water or any other liquid. These dry methods collect and remove particles by

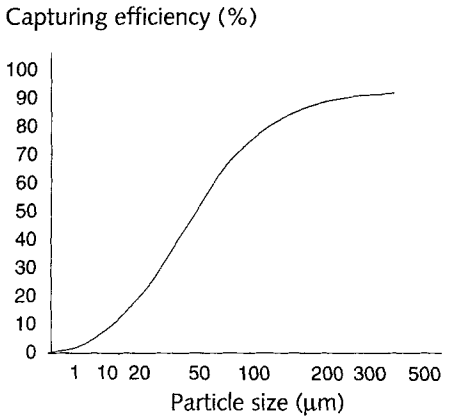

Figure 25. Overall effect of a vacuum cleaner

means of the static electricity generated by the action of rubbing the cleaning material over the surface. Some materials used in the manufacture of cleaning cloths are fibrous and mesh-like in nature and it is this unevenness in the fibers which is responsible for tearing particles away from a surface and then enmeshing them.

Adhesion methods are also used in dry cleaning. In domestic situations rolls of adhesive material are used to remove unwanted particles from clothes and other fabrics. This technique is also used within the field of contamination control and cleanroom technology in the form of rolls or mats, called tacky rolls and tacky mats.

Tacky mats are available as single-use or reusable and cleanable mats. Single-use tacky mats are often composed of 50 to 60 layers of sticky paper placed on top of one another. When the exposed paper surface has been filled with contaminants, the outer and used layer is simply torn off thereby exposing a new and clean surface ready to be used. Tacky mats that are cleanable comprise of the same type of cleanable plastic material as the adhesive rolls mentioned above. These tacky mats are cleanable and can be used for a long time. One advantage of the cleanable mat is that these can be placed over significantly broader and larger surfaces, for example an entire corridor. In practice, this means that personnel walking along such a corridor will have the particulate contaminants attached to the soles of their shoes easily removed.

5.11.2 Wet cleaning methods

As previously mentioned the smaller the particles the more strongly they are bound to a surface. It is also a well-known fact that the longer particles are attached to a surface the harder they are to remove. The different forces keeping the particles attached to a surface are dependent on particle size, the size of the contact surface between the particle and the surface, the surface structure of both the particle and the surface to which it is attached and the conductivity of both the particle and the surface. Other factors that effect the force between the particle and the surface are, the charge between them, the humidity in the contact zone, the temperature and the length of contact time.

Humid or wet methods for cleaning are complementary to

vacuum cleaning. Smaller particles attached to a surface are very hard to remove by vacuum cleaning, since the vacuum cleaner has not enough power to remove the particle from the surface. By using humidity in the form of water for instance, it is possible to remove the particle and thereafter capture it in a cloth or mop.

In wet or humid methods the cloth or mop is used together with a liquid cleaning agent. The cleaning agent is used in order to dissolve or remove particles and spilled chemicals, and may also eliminate microorganisms. There are also cleaning agents which reduce and minimize the risk of building up static electricity.

5.12 Cleaning solution

In order to dissolve solid contamination and chemicals the following principles are used: water-soluble contaminants are dissolved with water whereas contaminants soluble in organic solutions will be dissolved in hydrophobic solutions, thus water and organic solvents will be used to remove these contaminants respectively. One major factor to take into account is that the cleaning solution must never affect the surface to be cleaned in a negative way. Furthermore, when using water as the cleaning solution there is a risk of corrosion of certain metallic surfaces.

If the solid material on a surface is not soluble in the cleaning solution another type of cleaning action must be undertaken.

A particle attached to a surface in a dry state will be forced to move away from the surface when molecules of the cleaning solution enter the space between the particle and the surface. In many cases water is the basic ingredient in a cleaning solution. Various chemicals are added to the water in order to decrease the surface tension of the solution and thus facilitate the removal of the particle from the surface. Decreasing the surface tension of water will enable the cleaning solution to separate the particle from the surface. When the cleaning solution is introduced between the particle and the surface, the particle will be slightly lifted from the surface thereby decreasing the force that adheres it to the surface, thereafter, the particle can more easily be taken up in the cloth or mop.

Cleaning solutions are comprised of different chemicals that are mixed together in certain proportions to obtain a specific effect during cleaning. Surfactants, alkaline substances, sequestering agents and alcohols are amongst the most commonly used chemicals. Surfactants are molecules containing two moieties with different chemical properties which are held together by covalent bonding. One part is hydrophilic or ›water-loving‹ and the other is hydrophobic or ›water-hating‹.

Because of their molecular structure, surfactants are used to lower the surface tension of a solution. Surfactants are also able to solubilize fat and disperse pigments. When used to solubilize fats for example, the hydrophobic area of the molecule will be attracted to the fat, thereby exposing the hydrophilic area to the surrounding water. In this way the fat will be solubilized in water and removed from the surface.

Alkaline solutions are able to solubilize most proteinaceous materials and certain other organic substances.

Sequestering agents, such as EDTA (Ethylene Diamine Tetraacetic Acid), are used in cleaning solutions in order to remove various metal salts.

Finally, the properties of alcohols make them useful ingredients in cleaning solutions because they can dissolve fats, act as stabilizers for the solution, act as a preservative and therefore increase the shelf-life of the product and also ensure that the solution maintains its effectiveness at lower temperatures.

A cleaning solution must fulfill certain requirements. It must not be hazardous in any way and therefore should be non-toxic and non-flammable, it should be fairly quick drying and the solution must not affect the surfaces to be cleaned in a cleanroom in a negative way. The cleaning solution should be clean, i.e. should not contain any particles and should not leave any residual material on the surfaces after they have been cleaned, it should be effective in either solubilizing the contaminant or removing the contaminant from the surface in a solid state and should also be commercially available at a reasonable price.

The equipment used for cleaning should be carefully chosen before it is used in a cleanroom. It should be ensured that

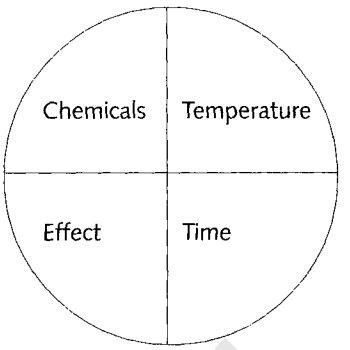

Figure 26. The Zinner circle

neither the cleaning equipment nor the cleaning materials are sources of contamination and therefore should not shed or distribute particles. Both the cleaning equipment and materials should be kept clean, in some cases by sanitization or even sterilization.

5.13 The Zinner circle

The Zinner circle shown in Figure 26 illustrates the four major components that together create the entire process used to remove particles from a surface, these are *chemicals*, *temperature*, *time* and *effect*.

›Effect‹ describes the physical force required to remove particles from a surface. The ›Chemical‹ component encompasses the principles described earlier in the section ›Cleaning solutions‹ (see Section 5.12).

Temperature can also be used in order to remove particles from surfaces, as in general the higher the temperature the easier it is to remove the undesired particles. Treatment time is the last component of the Zinner circle. For example, in some cases cleaning is more efficient if the cleaning solution is allowed to remain on the surface for some time.

Effective cleaning can be achieved by varying the proportions in which the techniques shown in the four areas of the 360° Zinner circle are used, depending on the cleaning method used.

The physical ›effect‹ component used during a cleaning procedure can consist of the following, for example:

- Flushing or rinsing, which can create aerosols that are widely dispersed in the air in the room. For this reason flushing or rinsing should be avoided in a cleanroom.
- Wiping with a cloth or a mop is a very gentle method of cleaning and is most used commonly in cleanrooms.
- Scrubbing with a brush can damage surfaces thus creating and dispersing contaminants which can later be transported to other parts of the cleanroom. Scrubbing should therefore be avoided in a cleanroom.
- Blowing with pressurized air or other gases for instance, should not be used in a cleanroom since the contaminants are only eliminated to a certain degree from the surfaces and are then dispersed into the air in the cleanroom.
- Brushing if not carried out too vigorously, can be used to make certain types of contaminant accessible to a vacuum cleaner for instance. This procedure should be carried out with great care since there is a risk of contaminants being dispersed into the air in the cleanroom.
- Vacuum cleaning, as stated earlier, is an efficient method for collecting larger contaminants. If a vacuum cleaner is to be used in a cleanroom it should be either centrally located or the exhaust pipe fitted with a HEPA filter. Traditional vacuum cleaners used for domestic purposes should never be taken into and used in a cleanroom.

5.14 Elimination of microorganisms

Microorganisms that can be hazardous to the product, the process, the personnel or to users of the product must be controlled. Microbial contamination can be controlled either by disinfection or sterilization. The inactivation or killing of microorganisms follows a logarithmic pattern (see Figure 4) and is dependent on different factors, such as the type of microorganism present, the method of deactivation or elimination and the concentration of the agent to be used for the latter processes. Other factors which determine the success of inactivation/elimination of microorganisms include temperature and treatment time, i.e. the time for which a chemical or deactivating substance is allowed to act upon the suspected source of microorganisms.

Agents or chemicals used for disinfection are mainly util-

Table 24. Disinfectant chemicals in common use

Chemical substance	Effect on microorganisms
Aldehydes	Reacts with proteins
Alcohols	Denatures proteins
Phenols	Destroys cell membranes
Iodine and chlorine	Oxidizes organic material
Chloro hexidine	Destroys cell membranes
Quaternary ammonium salts	Destroys cell membranes
Peracids and peroxides	Strongly oxidative

ized in order to remove or deactivate microorganisms on surfaces, instrumentation and skin. Most of the available disinfectants are not powerful enough to kill microbial spores however. Table 24 gives examples of some commonly used disinfectants, sometimes called anti-microbiology substances.

An ideal disinfectant should have a rapid inhibiting effect on bacteria, molds, spores and viruses. The agent should have adequate stability and should not affect the surface to be treated. Furthermore, the agent should not be inactivated by other agents or contaminants present and it should be odorless, harmless to the environment, free from allergenic ingredients and also commercially available at an affordable price.

5.15 Cleaning program

A cleaning program for cleanroom areas should be specified and carried out in a sequence of stages:

1. Read the instructions for cleaning as stated in the SOP (Standard Operating Procedure)
2. Collect all materials for cleaning and check the performance and cleanliness of the equipment
3. Place all the material in the airlock or in the ›pass-through‹
4. Change into cleanroom clothing
5. Prepare the different solutions as stated in the SOP
6. Enter the clean area or cleanroom taking care not to disturb items in the airlock or the pass-through
7. Remove all parts from machinery and equipment that is

to be assembled at another location. Also remove raw materials and other items from the previous production
8. Transport all the machinery equipment that has been disassembled to the washing department
9. Remove all visible contaminants
10. In general, active cleaning should be initiated in the cleanest part of a cleanroom and should be directed towards the more heavily contaminated areas. This can be achieved by always working from the inside of the room towards the airlock. Always work from the top of the room downwards in long overlapping strokes
11. Clean the ceiling, the walls and the floor according to number 10
12. At the end of the cleaning procedure remove all cleaning materials from the room
13. Clean the changing room and the airlock
14. Clean the area outside the airlock

Areas for production can be divided into critical areas and more general areas. A critical area is one in which a specific production process is carried out and where the product is handled at a time when it is most vulnerable to contamination. The general areas in clean production include airlocks, toilets and dressing rooms.

The air handling system providing the cleanroom with filtered, cleaned air should always be running during cleaning procedures so as to reduce the likelihood of contaminants being dispersed into the room. The particles that are released during cleaning will be removed by the ventilation system. Personnel who carry out cleaning tasks should work to the same standards of hygiene as those involved in the production processes, this includes among other precautions, wearing protective clothing.

5.16 Risk factors associated with cleanroom decontamination

The following may affect conditions within a cleanroom during the cleaning process:

- The environmental conditions such as airflow, airborne particles, outgassing, viable organisms, electrostatic discharge, etc.
- Movement of personnel and materials
- The methodology used for cleaning
- Expansion and modification of the facility
- The frequency with which cleanliness levels are monitored

5.17 Conclusions

- Contaminants in a cleanroom are often not visible to the naked eye
- Contaminants are removed by the action of cleaning and by disinfection
- The term cleaning is generally used to describe the process whereby visible particles are removed from a surface
- Decontamination is generally used to describe the removal of contaminants that are invisible to the naked eye
- Instructions for cleaning should specify all the various steps in the cleaning program as well as all the materials to be used during this process
- Cleaning instructions should always be followed
- There are four general methods for cleaning:
  - vacuum cleaning, only effective on larger particles
  - wet or humid wiping
  - tacky rolling
  - dry wiping
- Particles are often strongly attached to surfaces. The smaller the particle, the greater the force between the particle and the surface
- Agents used for disinfection should be varied regularly. This is due to the fact that microorganisms have a great ability to adapt to different environments
- The exact amount of cleaning solution should always be added to the water when making up a cleaning solution. Water should never be added to a cleaning solution
- When wiping a surface it should always be done with long straight and overlapping strokes. Circular or scrubbing movements should never be used

- Always work from the cleaner area out towards the less clean areas
- Always work in the direction of the airflow in the room, i.e. from the filter in the room outwards and downwards
- Cloths should be folded in such a way that there is always a clean surface available for each stroke
- The cleaning solution should only be used with a cloth. It should not be poured out or sprayed directly onto the surface to be cleaned
- The HEPA filters should not be touched during cleaning
- A surface should not be disinfected unless it has first been cleaned

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6 Cleanroom garments

6.1 Introduction

Man is considered to be the greatest generator of contaminants in a cleanroom. This is due to the fact that skin scales are continuously being shed from the outer layer of skin on the human body and are dispersed into the surrounding environment. This is a naturally-occurring process and the rate at which these small skin particles are released is dependent on the nature of the activities being undertaken and for this reason cannot be completely controlled. Another source of contamination associated with humans is their everyday clothing, fibres and other particles from which may be dispersed into the room air. About 30 to 40 years ago it was proposed that human operators should be replaced with robots to undertake work in clean environments. However, since a fully automated system using only robots is less flexible, more costly (at least initially) and in certain instances difficult to manipulate, other solutions where sought.

Today the cleanroom environment is protected from contaminants generated by personnel with the use of special clothing, known as personnel filters or the garment system. These garments act as filters and retain any particles that are produced until the clothing is removed in an area where this poses no risk to the production process. The material used to manufacture this clothing prevents particles passing through it into the room to be dispersed into critical areas of a production process at a later time. Clean, and in some cases sterilized garments are required for work in a hospital in order to meet the required standards of cleanliness. The same type of garments as are used as in hospitals, have been adopted for use in the United States space program and have proved satisfactory. This development has led to many other branches of industry using specially designed garments or clothing systems in critical areas of production.

Two types of clothing system are usually specified when comparing different national standards. These are working clothes and protective clothes. Traditionally working clothes are defined as clothing used under normal working conditions. For example a traditional laboratory coat should be put on when entering the facility and used throughout the day. Protective clothing on the other hand, often consists of a close-fitting garment sometimes fitted with protective breathing equipment, which covers the entire body and is generally only used for short periods of time when a specific task is undertaken.

Garment systems for use in cleanrooms can actually be defined as both working clothes and protective clothing, depending on the nature of the work undertaken when the garment is worn. According to the older national standards, protective clothing is only used to protect personnel from the product or process. Contamination control and cleanroom technology demands protection for both personnel and products and processes. Depending on the nature of the product and the conditions under which it is manufactured, both product and production area should be protected from personnel, and in certain cases personnel should be protected at the same time from hazardous components in the process, for example when working with radioactive material, vaccines or cytostatic agent.

6.2 Garment systems for cleanroom use

Aprons, coats and coveralls are the most commonly used garments within contamination control. Normally, garment systems are defined rather than the individual items that make up the system. The separate items that make up a garment system should effectively cover the operator with a filter-like material to reduce the possibilities of contaminants leaving the body, being dispersed in the air and later coming into contact with critical parts of the process or the product.

Garment systems traditionally comprise a combination of the following parts:

- Trousers, jacket or coat
- Coverall

- Cap and cap with integrated hair protection; hair protection covering the entire head
- Face mask and beard protection
- Eye protection
- Helmet that allows no particles from the face to be dispersed into the air
- A hood that covers the entire head, gloves
- Specially designed underwear
- Shoes, shoe coverings and long-legged boots

In general different items or systems can be divided into three categories depending on how they are used. The clothing system can be said to be disposable, of limited use and reusable. The various items chosen for a garment system should actually protect both the worker and the process and product. The garment system should also be comfortable to wear and should be manufactured of a material with suitable properties. The construction and design of the clothing should also be appropriate, the durability should be adequate, the material should be washable and robust enough to withstand further treatment (if it is not the disposable type of clothing) and finally the clothing should be readily available at a reasonable price. Furthermore, the material from which cleanroom clothing is made may also incorporate static-dissipative fibres in order to reduce problems with static electricity.

6.3 Choice of material

The material used for cleanroom clothing must conform to certain requirements, it must be comfortable to wear, it should provide an adequate barrier between the wearer and the environment and should not release any particles that may contaminate the production area. Among the properties required of materials used for cleanroom clothing are, suitable length of fibres so as to reduce contamination by their dispersion into the air, resistance to wear, moisture absorbing capacity, ability to repel and also contain soil.

The textile fabric should be woven in such a way that it will provide maximum protection. For example, the weave should be strong and smooth and at the same time provide adequate water vapour permeability and should have adequate filtra-

tion properties. The material used for cleanroom clothing should be of a quality that can withstand repeated laundering and sterilization and also has suitable conductivity. Polyester is by far the most commonly used woven material. Laminated material, are mostly used in very high classification cleanrooms and often consists of a base (carrier material) of woven material that has been covered with a micro-porous surface material (non-woven).

6.4 Aspects of comfort

Beyond the aspects of protection there is a general requirement for clothing to be both smart and comfortable to wear. The term ›comfort‹ is often considered to be a subjective judgement, but comfort can be divided into components, which are, comfort with respect to *climate*, *movement* and *feeling*. Comfort with respect to climate depends on whether there is a heat equilibrium between the garment-wearer and the surrounding environment. 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. Finally to ensure comfort with respect to feeling, the garment should not be made of a material that will irritate the skin on contact.

To ensure that staff are willing to wear the garments, cleanroom clothing should be chosen to provide optimum protection and to be of acceptable appearance. Personnel should also be informed of why the garment is necessary and should be instructed in how to wear it. The overall purpose is to choose a garment suitable for the work to be performed. The main requirements in the design of cleanroom garments are that (1) there should be as few seams as possible, (2) the seams are made with the same thread as the material itself, (3) the garment should have no pockets or pleats and finally (4) the garment should be loose and comfortable.

Two items which are of importance in a cleanroom garment system are face masks and gloves. Face masks are mainly used for two reasons, to redirect and filter exhaled air. First, the air leaving the mouth should be directed away from the item which is being worked on and facial protection directs the air upwards, downwards and sideways. Second, the air leaving

the mouth and nose region will enter the face mask and will be filtered to a certain degree. Facial masks are selected according to the following criteria: they should not be made of a material that will disperse fibres into the environment, they should provide adequate filtration and also should be easy to use. If necessary they should be able to withstand sterilization and they should naturally be available at a fair price.

Gloves are important in many cleanroom applications and should therefore provide adequate protection and at the same time should not produce fibres which can be dispersed into the air. They should also be resistant to chemicals as well as to physical conditions which may be used in the production process. This means that gloves must be manufactured of a suitable material, should fit snugly but not be too tight and should be available in a variety of sizes. Furthermore gloves for certain applications should be washable and in certain instances able to withstand sterilization.

6.5 Construction of garment systems

Garment, as well as their use, are defined in certain standards and practices. The standards considered in this book are Good Manufacturing Practice (GMP) ›Garment System Considerations for Cleanrooms and Controlled Environments‹ (IES-RP-CC-003.2) and ›Cleanroom Gloves and Finger Cots‹ (IES-RP-CC-005.1).

Good Manufacturing Practice covers garment systems with regard to the different hygiene classifications. The recommended practice entitled ›Garment Systems Considerations...‹ is related to US Federal Standard 209. An earlier version of this recommended practice did not take in to account whether the particles to be contained by the garment system where live or dead. The newer version of this document contains several options for cleanroom use. The recommended practice 005.1 covers gloves and finger cots.

Table 25. Construction of a garment system according to GMP

GMP Grade	Garment system recommendation
Grade D (Class 100 000, ›At rest‹ state, US Federal Standard 209 D)	Hair and, where appropriate, 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 (Class 10 000, ›At rest‹ state, US Federal Standard 209 D)	Hair and, where appropriate, 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 fibers or particulate matter
Grade B (Class 100 US, ›At rest‹ state, US Federal Standard 209 D)	Headgear should totally enclose hair and, where appropriate beard; it should be tucked into the neck of the suit; a face mask should be worn to prevent the shedding of droplets; sterilized non-powdered rubber or plastic gloves and sterilized or disinfected footwear should be worn; trouser-bottoms should be tucked inside the footwear as well as garment sleeves into the gloves. The protective clothing should shed virtually no fibers or particulate matter and retain particles shed by the body

Table 26. Recommendations for cleanroom garments according to IEST-RP-CC-003.2

US Federal Standard 209 D	**Class 100 000**		**Class 10 000**		**Class 1000**	
US Federal Standard 209 E	**Class M 6.5**		**Class M 5.5**		**Class M 4.5**	
Cleanroom type	**Non-LAF[1]**	**Mixed**	**Non-LAF[1]**	**Aseptic**	**Non-LAF[1]**	**Aseptic**
Frock	R	R	R	NR	AS	NR
Two-piece suite	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 suite	AS	AS	AS	AS	AS	AS
Changes	2/week	2/Week	2/week	Every entry	2–3/ week	Every entry
US Federal Standard 209 D	**Class 100**		**Class 10**		**Class 1**	
US Federal Standard 209 E	**Class M 3.5**		**Class M 2.5**		**Class M 1.5**	
Cleanroom type	**Non-LAF[1]**	**Mixed**	**Non-LAF[1]**	**Aseptic**	**Non-LAF[1]**	**Aseptic**
Frock	NR	NR	NR	NR	NR	NR
Two-piece suite	AS	NR	AS	NR	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 suite	AS	AS	R	R	R	R
Changes	1/day	Every entry	Every entry	Every entry	Every entry	Every entry

R, Recommended; NR, Not recommended; AS, Application specific;
1 Conventionally-ventilated cleanroom; 2 Surgical mask recommended.

6.6 Good Manufacturing Practice and cleanroom clothing

Table 25 shows the GMP view on how to construct a garment system.

6.7 IEST-RP-CG 003.2

Table 26 shows the recommendations according to IEST-RP-CC-003.2.

6.8 Using cleanroom garments

Listed below is the order in which various procedures should be followed to ensure the effective use of cleanroom clothing.

- Take off everyday clothing when entering the production area
- Remove all jewellery and wash off all cosmetics
- Wash hands thoroughly. In some industries its permissible to use a humidifying ointment in order to avoid dry skin
- Place the cleanroom clothing in its wrapping on the bench between the clean and the non-clean zone in the dressing room, or hang them on a hanger in the room
- Cover all hair with a hairnet
- Wear gloves for dressing if necessary
- Put on the hood and the facial protection
- Remove the cleanroom clothing from its outer wrapping or take them down from the hanger
- Put on the body garment carefully. Make sure the body garment does not touch any surfaces. Secure all open ends at the wrists and arms
- Put on the footwear. Footwear must not touch any surface on the non-clean side of the room. Do not touch the underside of the shoes with hands
- Remove the dressing gloves and put on cleanroom gloves. Avoid contact between naked skin and the outer surface of the gloves. If necessary, disinfect the gloves before entering the cleanroom
- Enter the cleanroom after standing on a tacky mat to remove any contaminants adhering to soles of footwear

The procedures followed when entering the cleanroom should be given the same care and attention when leaving. This is important particularly if the cleanroom garments are to be re-used. Exit from a cleanroom follows the same general procedure as entry, but in the reverse order.

6.9 Clothing and personal items

Garments worn under cleanroom clothing will have an effect on the dispersion of particles and fibers into the outer environment, particularly personal clothing made of natural materials, such as wool or cotton. If these particles and/or fibers pose a significant contamination risk, specially designed cleanroom undergarments may be required. Cleanroom undergarments are made from closely woven, artificial fibers (for instance polyester) which have an adequate filtration capacity.

Personal items, such as rings, watches and chains, should be left outside the cleanroom in a secure area. Items worn on hands may puncture cleanroom gloves and those worn on the wrist, such as bracelets, may hang below the sleeves of the clothing, whilst items worn on the head e.g. earrings, may dangle outside face masks or hoods.

6.10 Processing of clothing and change frequency

Cleanroom garments become contaminated during use and if the garment is of the reusable type, it must be cleaned. Laundering as well as packaging the garment must be carried out in a cleanroom, with cleanliness levels equal or superior to those of the room in which the garments are to be worn. Garments will not only be contaminated with inert particles, but also with microorganisms which will be present on and in the fabric. If microorganisms are considered to be critical contaminants of the production process in progress, the process cycle in the cleanroom laundry should also include either disinfection, hot water cycles or sterilization.

The frequency of clothing change may vary according to the intended use of the room and the level of protection required. In general, the more sensitive the operation, the more frequent the changes of clothing and cleaning should be. Guidelines for change frequency are shown in Table 26.

6.11 Risk factors associated with cleanroom clothing

The following factors related to cleanroom clothing may have an influence on the operation or the quality of the environment in a cleanroom.

- The required level of containment, i.e. the items of clothing chosen
- The performance of the garment material
- Design and construction of the garment system
- Whether the garment is washable or disposable
- Nature of personal clothing worn under cleanroom clothing
- Time interval or the number of uses before laundering is required
- The choice of clothing laundry
- Packaging, storage and distribution of clothing
- Time interval or the number of laundry cycles before clothing should be renewed

6.12 Conclusions

In order to carry out work in a cleanroom or a clean zone there is often a need to use special garments, a personnel filter. Traditional working clothes protect only some parts of the body whereas protective clothing covers most parts of the body. The choice of garment and its use are regulated by different standards and practices, for instance Good Manufacturing Practice, IEST-RP-CC-003.2 and IEST-RP-CC-005.

7 Personal hygiene and personal responsibility

7.1 Introduction

As soon as personnel enter a cleanroom its level of cleanliness will change. People continuously release small particles from the outer layer of the skin into the surrounding environment. This process occurs because cells from surface layers of skin are continually replaced by new cells from the layers below. The number of particles released from the outer layer of the body will increase as a result of the abrasive action of clothes and jewellery for instance. As personnel become more active during the performance of their work, so the number of particles that are shed will increase.

7.2 Man as generator of contaminants

Table 27 shows the number of particles that are released from one person engaged in different activities.

Since it is not possible to dress personnel in a totally sealed micro-filter or to give the human form a fully aerodynamic shape, man will always have an impact on the surrounding environment. This fact is independent of whether the work performed is carried out in a class 10 000 or in class 1 clean-

Table 27. Relationship of activity to the number of particles shed from a human body

Activity	Number of particles generated (0.5 µm and larger per min)
Sitting or standing still	100000
Sitting, small movement of arms or head	500000
Sitting, moving arms, legs or head	1000000
Standing up	2500000
Walking slowly	5000000
Walking normally	7500000
Walking with speed (2.5 m/s)	10000000
Performing work-out	15000000–30000000

Table 28. Sources of contamination and their effect on their environment

Contamination source	**Size of generated particles** (μm)
Rubbing a painted surface	90
Folding a piece of paper	65
Rubbing an epoxy-treated surface	40
Writing with a ballpoint pen on ordinary paper	20
Rubbing the skin	4
Activity	**In relation to normal breathing**
Brushing one's clothing	1.5–3
Stamping one's foot (without a shoe cover)	10–50
Stamping one's foot (with shoe cover)	1.5–3
Take a handkerchief from a pocket	3–10
Normal breathing	1
Breathing from a smoker (after 20 min)	2–5
Sneezing	5–20
Rubbing hands or face	1–2
Walking together (4–5 persons)	1.5–3
Normal walking	1.2–3
Sitting still	1–1.2
Clean air zone with micro-filter	0.01

room. When working with extremely critical process steps trials have been carried out to replace man in the production process by automation and the use of robots. Although the use of robots and automation will reduce the level of particles released into the environment, it will not completely eliminate the generation of all particles. Contamination can be reduced by the use of appropriate buildings and ventilation systems to make the environment more suitable for various production processes, but most importantly contamination control and cleanroom technology is based on highly motivated and skilled personnel, dressed in appropriate garments who work according to defined procedures.

Table 28 shows different contamination sources and how these will give rise to elevated levels of particles in the surrounding environment.

Cosmetics often pose a problem within contamination control. Cosmetics are normally not allowed in a cleanroom. Table 29 shows how many particles are generated by each full application of various cosmetics.

Table 29. Number of particles found in cosmetics

Type of cosmetics	Number of particles per application (Particle size 0.5 µm and larger)
Lipstick	1100000000
Rouge	600000000
Powder	270000000
Eye shadow	82000000
Mascara	3000000000
In total for one application	5100000000

7.3 Smoking

Smoking is prohibited in controlled clean areas as well as in cleanrooms. After smoking particles remain in the respiratory tract and lungs and these will continuously leak out of the body into the surrounding air. In practice this means that a smoker will act as a powerful particle generator. Some companies insist that personnel should not smoke for at least 20 min before returning to their work place in the cleanroom area whereas other companies have no stated time period and only prohibit smoking in the cleanroom.

Some companies in the United States have rules stating that drinking a glass of water after smoking is sufficient to reduce the number of particles exhaled in the cleanroom area, which is surprising since according to medical reports a smoker will continue to generate particles for up to 12 h after the last cigarette.

7.4 Good Manufacturing Practice for personnel

The GMP contains information and requirements in relation to numbers of personnel and personnel hygiene. Only a limited number of persons should be in the production room at one time, this is of particular importance during aseptic production. Visual inspections as well as control of processes should be carried out from outside cleanroom areas wherever possible. External personnel, i.e. those not actively involved in the cleanroom process and who have no specialist knowledge of cleanliness requirements, for example building workers, service personnel etc., should be thoroughly briefed before being allowed to enter a cleanroom. These visitors

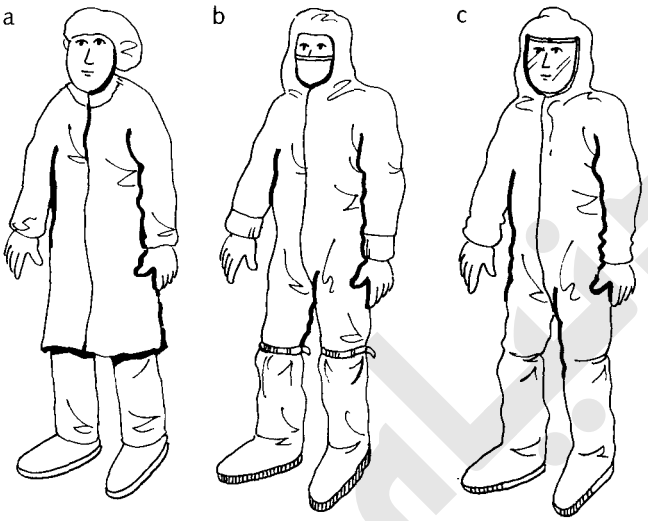

Figure 27. Examples of different types of cleanroom garment. (a) Garment for lower classification cleanrooms; shoe cover, trousers, apron, and complete hair protection. (b) Garments for use in higher classification cleanrooms, coverall, gloves, hood, facial protection and long-legged boots. (c) Garment as in (b) but with the addition of a helmet which replaces the face mask

should normally be accompanied by a member of staff who is familiar with the rules of hygiene in a cleanroom.

All personnel, including those involved in cleaning and maintenance, should be trained on a regular basis. They should also be trained in areas that are relevant to a production process. If the production involves sterile products staff training should also include hygiene and basic microbiology.

7.5 Education, training and control

Persons working in a cleanroom must be made fully aware of which procedures they are to follow and which activities they should avoid. An adequate training program should include the following:

- Basic training on cleanliness and hygiene
- Special training on certain parts of the process
- Personnel should be motivated to do a good job
- Personnel should be trained so that they are aware of and

can avoid any inappropriate activity. Personnel should be dressed appropriately and should also be completely aware of the reasons why special garments should be worn

- Personnel should be equipped with suitable work tools in order to be able to perform the task
- The overall environment should be constructed and maintained in such a way that it is pleasant to work in
- Personnel involved in a clean production should be aware that the nature of the work may place restrictions on their personal life, for instance not being permitted to smoke, drink or to eat during work

7.6 Instructions

Regardless of their background and training, all personnel working in a cleanroom must conform to specific instructions. In some cases these instructions can be written out with the help of personnel involved in the process, thus ensuring

that all personnel will be able to understand and follow them.

The reasons for following the specified rules and instructions must be explained together with the consequence that will result if the instructions are not followed accurately. All rules and instructions must be consistent and should be followed throughout the organization.

The fundamental issues described above are very important as it is impossible to monitor all the movements and activities of personnel working in a cleanroom, in contrast to the situation in a traditional workplace. It is for this reason that staff working in a cleanroom have greater responsibility than those working in other environments, to understand, accept and put into practice the various rules which apply to the different working steps used within a cleanroom.

7.7 Man and the process

A major requirement in a cleanroom is to keep the personnel as distant from the process being carried out as is possible. Isolating the process and using separate ventilation flows which continuously force contaminants away from critical parts of the process, contribute to meeting this requirement. Thus personnel need only be present in critical areas when there are disruptions in the system that need correcting, for example if ampoules or vials drop out of a filling line or if some other parameter needs adjustment. Correctly designed tools and other aids therefore become a prerequisite for keeping personnel intervention to a minimum. Assembly work and other construction work are critical steps in a process, which if carried out on a routine basis and according to instructions will reduce the risk of contamination. In other words a well-adjusted process requiring the minimum of interference is the aim of any manufacturer.

Service and maintenance personnel may also compromise the cleanliness in a room. This problem can be overcome in two ways. Either all the items that require maintenance should be placed in service environments outside the cleanroom or if the equipment cannot be removed from the cleanroom, maintenance procedures should be kept to minimum. Service personnel entering the cleanroom, especially those

from outside the facility, should be trained in the correct procedures to minimize contamination.

The pattern of airflow together with the dispersion of contaminants generated during different working procedures, can both affect the outcome of a process. A well-planned arrangement for the movement of materials and personnel in and out of a cleanroom, will ensure that procedures are carried out correctly on a routine basis. To establish whether procedures are being adhered to, hazard analyses can be carried out by visualization of air movements for instance. This can be achieved with the use of smoke and is most commonly performed under simulated conditions. This type of hazard analysis works well for processes that are sensitive to both particle and microbial contaminants.

To ensure that personnel, the production process and the cleanroom interact in an efficient manner, there is a need for well-trained cleanroom staff, a documented flow of material through the cleanroom, spacious storage areas, spacious and well-planned areas for storage of cleaning equipment in addition to spacious airlocks and dressing rooms.

7.8 Guidelines for work within a cleanroom

A summary of guidelines for work within cleanrooms and clean zones is presented below.

- Hands, nails and face should always be kept clean. The hygiene of personnel is of vital importance
- A correctly designed and chosen garment system should be used. The garment system for working in clean production is made up of different items. The system must be complete and worn in the correct manner in order to ensure total protection
- Spectacles must be cleaned and disinfected before entering the cleanroom. Unfortunately routine spectacle cleaning can be easily forgotten
- Components and tools must be kept as clean as possible
- Work should always be performed on clean surfaces
- Smoking and the use of snuff are not permitted. Both smokers and people using snuff will release many more millions of particles into the air compared to non-smokers/non-users of snuff

- Hair must not be combed or even touched within a cleanroom
- Cosmetics are normally not allowed within a cleanroom. All forms of cosmetics contain particles that are loosely attached to the skin. When talking, coughing, sneezing or touching an item such as a face mask, these particles will be dislodged from the skin and thus enter the surrounding air, where they can become a contamination risk at a later stage
- Nail polish is not allowed. Nail polish is generally harder than the nail itself, which means that after a certain time cracks appear in the polish and these can harbor dirt and microorganisms, which may proliferate
- Jewellery and watches are not permitted within a cleanroom, because these items are difficult to clean and can become a source of infection or contamination
- Eating or drinking is not allowed within a cleanroom. The same goes for chewing gum and candy
- Cleanroom garments should not be handled. Personnel should not touch the garment or scratch themselves as there is a high risk of hands or gloves contaminating the clothing. Handling cleanroom garments can also result in the particles which have been collected inside garments being dispersed into the surrounding environment
- All unnecessary activity should be avoided. Increased physical activity will lead to an increase in the number of particles dispersed from the surface of the body. Unnecessary movement of personnel can also cause the formation of vortexes in the surrounding air. It is recommended that work should be carried out with slow steady movements

7.9 Personal hygiene

Good personal hygiene cannot be stressed enough when working to high standards of cleanliness. Rules, recommendations and regulations for personnel hygiene are unfortunately not always easy to interpret, however there are some fundamental rules. Contamination is spread most frequently by handling materials and equipment, it is therefore essential that hands are washed frequently particularly after coughing, sneezing, visiting the toilet, smoking or using snuff and when

returning to the work station after breaks. Hands should also be cleaned thoroughly after handling contaminated material and animals. In some instances, depending on how clean the items are, it is a good practice to wash hands after using the telephone or computer.

Personnel hygiene in relation to good manufacturing practices can be summarized as follows:

- Each individual is responsible for their own personal hygiene and cleanliness
- All personnel affected by good manufacturing practice should be informed and trained in personal hygiene
- Personnel are one of the major sources of contamination within clean production
- Skin scales shed from the body are ›dead‹ particles, but bacteria and other microorganisms are frequently attached to them
- Since microorganisms are associated with the human body, both internally and externally, any form of contact with equipment or products should be avoided
- In addition to regular bathing or showering, hair should also be washed regularly. From a hygienic point of view, showering is preferable to bathing
- All hair should be fully covered with the hair protection including beards and moustaches
- Hands are a great source of contamination and are responsible for the transportation of contaminants from one place to another. It is therefore important that good hand hygiene is maintained with particular attention being paid to nails, cuticles and wounds on the hands
- As the mouth is also a major source of contamination, good oral hygiene is of vital importance. Shouting or raising one's voice should be avoided wherever possible, as should sneezing or coughing, or if this cannot be avoided, staff should turn away from the working zone
- Personnel working in areas where high standards of cleanliness are required, should be medically well

7.10 Contamination risks associated with personnel

The following risk factors associated with personnel can have an influence on the environmental quality of a cleanroom.

- Selection of personnel
- Education and training of personnel
- Safety aspects in cleanrooms
- Personnel practice and hygiene
- The medical condition of the personnel
- Which members of staff should enter the cleanroom
- Decisions on maximum occupancy
- Entry as well as exit procedures
- The passage of personnel in and out of a cleanroom

7.11 Conclusions

The need for good personal hygiene is self evident in cleanroom work. It is also obvious that use of the correct garments for different cleanroom operations is essential.

These aspects of hygiene apply not only to staff who work in a cleanroom but also to all personnel entering a clean area. Different categories of visitors must be made aware of the rules that exist to protect the clean area and must follow the same procedures as the regular cleanroom personnel.

8 Concluding remarks

Contamination control and cleanroom technology is a vast and interdisciplinary area. A summary of cleanroom technology including its three major components cleanliness, hygiene and quality, is given below.

8.1 Cleanliness

Cleanliness is a word that can have different meanings depending on the type of environment it is being used to describe. Cleanliness in a domestic environment means that everything is set to order, all surfaces have been wiped clean of dust, perhaps with a damp cloth and floors have been vacuum-cleaned.

To use the same tools and techniques in industrial areas may be sufficient in some instances but inadequate in others. For certain workshop industries the same level of cleanliness as that achieved in a domestic situation may suffice, but within the pharmaceutical and microelectronic industries for instance, these levels of cleanliness are completely inadequate in critical areas of the production process.

Cleanliness is therefore a relative term and depends to a major extent on the overall standards of cleanliness required for the process, the process room and the personnel.

8.2 Hygiene

Once achieved, good hygiene should be maintained in order to avoid contamination of the product. Thus establishing and maintaining good hygiene is cost effective, as a contaminated product would be difficult, if not impossible to sell. In many cases a discarded product cannot just be thrown away, it may have to be incinerated or destroyed in some other way, thus incurring costs for its disposal. However, the greatest cost would be to the reputation of any manufacturer who attempted to sell contaminated goods on the open market.

Microorganisms can proliferate and disperse at a great rate and for this reason it is important to implement good hygiene and hygienic processes in areas where the manufacture of products particularly sensitive to microbial contamination, takes place.

8.3 Quality

Quality is often considered to describe the reaction of a consumer or user to products on the market. In many cases quality is measured in terms of product capacity or product performance, for instance fuel consumption in a car, the speed of delivery of an order or the energy consumption of a building.

Quality however, covers a much more extensive area, but is generally associated with freedom from faults and defects. It is important that an operator or supervisor working within a production process, should establish a reasonable level of quality and document the methods by which it can be achieved. They should identify the areas and stages of the work process which are particularly vulnerable to contamination and should also specify procedures to be used in order to minimize contamination and maintain quality. Once these areas have been identified, it is the responsibility of the company management to take action to reduce these risks. Continuous monitoring and taking preventive measures in certain key areas will ensure the quality of the goods being produced.

8.4 General rules for cleanroom work

Every cleanroom must have its own documented rules and procedures. The following general rules apply to all cleanrooms but do not take into account the nature of the particular procedure that takes place within a specific cleanroom, which means that the choice of garments and cleaning equipment for example, are not stated in the rules.

1. Only the necessary personnel should be present in the cleanroom. The more personnel in the cleanroom the higher the contamination level.
2. Only trained personnel are allowed to work in the clean-

room and only those staff who have the necessary skills to carry out the particular procedures are allowed to enter the cleanroom.

3. Personnel are not allowed to take anything into the cleanroom that might be a source of contamination. Only items needed for the work within the room are permitted and these items should be prepared in such a way as to prevent them from generating contaminants. The following items are not allowed in a cleanroom:
   - Food, drinks, sweets or chewing-gum
   - Cans or bottles
   - Cigarettes, pipes and snuff
   - Radio, CD-player and portable tape recorders
   - Items made of wood, rubber, paper, leather, cotton and other natural materials
   - Paper that is not produced for cleanroom use, this includes newspapers, magazines, books and paper handkerchiefs
   - Lead pencils and erasers
   - Materials used for writing that can release particles, for instance fibre pens
4. Doors must never be left opened. Open doors can create loss of pressure within the room, which in turn will result in unwanted air movements within the cleanroom.
5. Doors should not be opened and closed rapidly. Opening and closing doors rapidly can increase the amount of air taken from a less clean area into the cleanroom. Doors can be equipped with a closing system or spring for instance, that will control the speed with which the door closes.
6. When passing through one of the two doors in an airlock system, personnel should only open the second door once the first door has been securely closed. So-called interlock systems make it impossible to open both doors at the same time.
7. Personnel should not be able to enter a cleanroom without first having passed through the changing area. The changing area is used not only for garment changing but also acts as a buffer zone between less clean external areas and the cleaner interior production area. Personnel are

not allowed to use exits, for instance emergency exits, leading directly from the production area to the outer less clean area because of the possible rapid transportation of contaminants between the exterior air and the interior cleanroom air.

8. The cleanroom should always be tidy and have a clean appearance. A cleanroom can never be clean if it is not tidy.
9. Personnel should always be correctly positioned in relation to the production process. For instance, it is bad practice to lean over the product as this would allow particles from the cleanroom garment, both ›dead‹ and those of microbial origin, to fall onto and around the product. When working in a UDF airflow, personnel should not be sited directly in the airflow as this would cause the incoming air to disperse any loose particles on clothing into the air. The methodology used in a cleanroom should be well planned so that the risk of contamination can be minimized.
10. Consideration should be given to the way the product is moved or in any other way manipulated. Remote methods of manipulation are preferred so that handling of the product can be avoided. Gloves are often used in cleanroom work but they can be a source of contamination.
11. When moving an item within the cleanroom the item should be carried so that it cannot make contact with clothing or other items.
12. Speaking should be avoided when working in close proximity to a product. Even if the operator uses a face mask, there is a risk that particles from the nose and mouth will pass either through or around the face mask and thus come into contact with the product. If sneezing or coughing cannot be avoided, personnel should turn away from the product. After sneezing or coughing, the face mask should be replaced with a clean one. A face mask should cover both mouth and nose.
13. Avoid touching surfaces within the cleanroom. Even if the cleanroom itself is clean, the surfaces within the room and the surfaces of the equipment may be contaminated. Hands should be held away from the cleanroom garment to reduce the risk of contaminating the gloves.

14. Cleanroom gloves should be cleaned on a regular basis to reduce the risk of transferring contaminants by touch. Sterile-filtered 70% ethanol or isopropanol can be used for this purpose. Either of these alcohols are acceptable because they do not leave any residues after drying.
15. Cloths used in cleanrooms should be used only once or for a predetermined number of times. After use a cleanroom cloth should be discarded. Wiping with a cleanroom cloth should be carried out in long straight, overlapping strokes.
16. Products left in a cleanroom should be contained in sealed cupboards or vials in order to minimize the risk of contaminants coming into contact with the items or the products. The best alternative is to keep items or products on an LAF-bench or security-bench.
17. Work in a cleanroom should be carried out with smooth, well planned movements. The concentration of airborne particles will increase drastically with rapid and irregular movements.
18. Material to be discarded should be collected in specially designed containers, which are removed from the cleanroom on a regular basis.
19. The cleanroom should be cleaned according to the appropriate protocol and in some instances should also be disinfected.
20. People entering the cleanroom must be aware of their responsibilities, be well trained in the different techniques used in the cleanroom and be aware of how all the systems within the cleanroom function.
21. Personnel must react in an effective way when an acute incident occurs, for instance if there is a fire, explosion or electrical fault either inside or outside the cleanroom. Staff should know what to do and should act in a calm manner if emergencies arise, such as breakage of a container of either toxic or non-toxic material, another member of staff being taken ill and other similar situations.
22. Persons entering a cleanroom must be fully aware of the garment system in use.
23. Whatever function they are fulfilling, all personnel working within a cleanroom must wear the appropriate cleanroom garment.

24. Sterile cleanroom garments used for aseptic production cannot be used a second time without prior washing and re-sterilization.
25. Personnel working in a sterile area must be fully aware of and comply with the various disinfection and sterilizing procedures.
26. Personnel working in a cleanroom must avoid walking back into the airlock.
27. Plastic bags for containment and transportation of used cleanroom garments should be available in the airlock. The used garments can thereby be transported in these bags to the washing-area without any risk of contaminating either the product or the personnel.
28. Comfort as well as the effectiveness of the overall system, will determine the number of persons that can be allowed into the airlock at one time.
29. No personal items, for instance wallets and bags, are allowed into the cleanroom or airlock.
30. Persons that are physically unwell, especially with disorders of the stomach, nose and throat should not enter clean areas.
31. All verbal communication with persons outside the cleanroom should take place via internal telephones or intercom systems for instance. Airlocks and pass-throughs should never be used as communication lines between the outer part of the production area and the cleanroom.
32. The wearing of cosmetics, make-up, rings, jewellery and watches is prohibited within a cleanroom.
33. Beakers, flasks and other equipment must be cleaned or sterilized according to the set procedures before they are taken into the cleanroom.
34. Only cleaning cloths made from materials with long fibers can be used in a cleanroom. Synthetic materials are most widely used. Mops, brushes and other items traditionally used in a domestic environment, should never be used in a cleanroom.
35. Personal hygiene, with particular emphasis on hand and oral hygiene, is of utmost importance for the successful outcome of work in a cleanroom.

Glossary

Aerobe	Organism only viable in the absence of oxygen
Aerosol	A colloidal system in which the dispersion medium is a gas, i.e. a liquid solution which has been atomised into a fine mist (small droplets)
Agar	A dried substance of algal origin having the property of melting at 100 °C and solidifying into a gel at 40 °C. Agar is not normally digested by most bacteria and is used as a medium in the preparation of solid cultures of microorganisms
Air changes	A value indicating the number of times per hour that the air is changed within a certain room or containment. A very commonly used indicator for conventional cleanroom that purge themselves
Airlock	Intermediate room that is normally ventilated, and used to minimize the transfer of airborne contaminants from one area to another
Air velocity	A value indicating the speed of the air movement in a room or a zone in the room. A very commonly used indicator of the ability of a unidirectional-flow cleanroom that purge itself
Alga	Any individual species of algae
Algae	A group of cryptogamic plants in which the body is unicellular. This group includes seaweeds and many unicellular fresh water plants, most of which contain chlorophyll

Alkali	A class of chemicals which form soluble soaps with fatty acids and also form soluble carbonates. These substances are mainly used as cleaning agents due to their ability to dissolve proteins and other organic material
Alcohol	Any of a class of organic substance formed from hydrocarbons by the substitution of one or more hydrogen atoms with an equal number of hydroxyl groups. Alcohols are mainly used as cleaning agents because they can solubilize fats and are potent disinfectants where microorganisms are concerned
Anaerobic	Lacking molecular oxygen
Anaerobic microorganisms	Microorganisms that have the ability to grow in the complete or almost complete absence of molecular oxygen
Anderson sampler	Sampling device for the collection of microorganisms based on impaction. The sampler has up to eight stages stacked in series, each stage consisting of a perforated plate. A Petri dish of agar growth medium is placed beneath each plate. Air that is drawn into the sampler impacts with the agar of each stage. Air velocity increases at each succeeding stage due to progressively smaller holes causing suspended particles to be distributed among the plates according to size. Larger particles are trapped in the upper stages; smaller particles in the lower stages
Anisokinetic sampling	The taking of air samples under conditions in which the velocity and direction of the air entering the sampler are different from the velocity and direction of the air in the room
Antiseptic	A substance that will inhibit the growth and development of microorganisms

	without necessarily destroying them
Antisepsis	The prevention of sepsis by the inhibition or destruction of the causative organisms
As-built cleanroom	A cleanroom or facility that is complete and ready for operation, with all services connected and functional, but without equipment or operating personnel
Aseptic	Free from infection or septic material
Aseptic filling	Sterile containers are filled with the sterile product and sealed with sterile closures. Frequently used with products that cannot be sterilized at the final stage of production
Aseptic technique	Techniques that are carried out to avoid infection. In other words aseptic techniques are used to prevent sterile material and/or products from becoming infected
At-rest cleanroom	A cleanroom or facility that is complete, with all services functioning and with equipment installed and operational but without operating personnel
Autoclave	An apparatus for effecting sterilization by steam under pressure, fitted with a gauge that automatically regulates the pressure and therefore the degree of heat to which the contents are subjected
Bacteria	Main group of microorganisms
Bactericidal	Capable of killing vegetative bacterial cells, but not necessarily bacterial spores
Bacteriostat	An agent that inhibits the growth of bacteria
Bacteriostatic	An agent that inhibits the growth or multiplication of bacteria
Biocide	An agent that kills organisms
Biosafety cabinet	Containment device for protection of operators and workers from hazardous microorganisms and also in some cases

	for protecting the work area from contaminants from the outer environment
Calibration	Determination of the accuracy of an instrument, usually by measurement of its variation from a standard, to a certain necessary correction factor
CCP	Critical Control Point
Centrifugal air sampler	A sampling device used for the collection of microorganisms suspended in air, based on impaction. The impaction force is obtained with a rotating fan which forces particles in the air onto an agar plate
CFU	Colony Forming Unit, e.g. the number of colonies found on an agar plate after incubation
Changing room	Room where people using a cleanroom may change into or out of cleanroom clothing
cGMP	Current Good Manufacturing Practice
Cleanroom	A room in which the concentration of airborne particles is controlled to a certain level. Control of cleanliness in such a room is often achieved by controlling the introduction, formation and retention of particles in the room. In many cases there is also a need to control temperature, pressure and humidity
Cleanroom technology	That area of contamination control which utilizes cleanrooms. Cleanroom technology also includes all the different actions and precautions taken in order to create a working environment in which the level of contamination is controlled
Clean zone	A defined space in which the concentration of airborne particles is controlled to meet a specified cleanliness class
CNC	Condensation Nucleus Counter
Colony	A collection or group of microorgan-

	isms derived from the proliferation of an isolated single organism or group of organisms
Complex former	A chemical agent with the ability to solubilize metal-containing oxides
Condensation nucleus counter	An instrument for counting small airborne particles, approximately 0.01 μm and larger in size, by detecting, with optical methods, droplets formed by condensation of vapor on the particles
Contact plate	A small plastic dish overfilled with growth medium so that the level of agar rises above the rim of the plate and used for surface sampling of microorganisms. To take a sample on a flat surface the convex agar surface is firmly pressed against the surface using a gentle rocking motion in order to secure complete contact
Contaminant	Something that contaminates
Contaminate	To soil, stain and/or infect by contact or association
Contamination control	The overall description of all actions taken in order to gain control of contaminants. Can be used either to protect the product being manufactured or to protect the personnel working in the production, or both
Cross-over bench	See Step-over bench
Declination phase	The stage in the growth curve of microorganisms, when they gradually begin to deteriorate
Decontamination	The freeing of a person or an object of some contaminating substance such as gas, radioactive material, microorganisms etc. Within the area of contamination control decontamination often refers to the reduction of numbers of living organisms to some lower population level, but not necessarily to zero

Discrete particle counter	An apparatus used for the numerical counting of discrete particles
Disinfect	To free from pathogenic organisms, or to render them inert. Within contamination control the word disinfect is often used in order to express the gross elimination of all organisms
Disinfectant	An agent that disinfects. The term is usually applied to agents used on inanimate objects
Disinfection	The act of disinfecting
DOP	Di octyl phthalate, a liquid that can be broken up into particles of minute size, e.g. smoke
DOP aerosol	Finely dispersed DOP particles in a gas
DPC	Discrete Particle Counter
Dry heat sterilization	Thermal sterilization at relative humidities less than 100%. It is less efficient than moist heat where the relative humidity is 100% and requires longer exposure times at higher temperatures
Dust	Solid material that can be found on surfaces or suspended in gases
D-value	The decimal reduction time. The D-value is defined as the sterilization time, for a physical or chemical agent, used to obtain a 90% reduction in the number of microorganisms (one log) present in a sample
Equivalent diameter	The diameter of a reference sphere having the same properties and producing the same response in the sensing instrument as the particle being measured
Facultative anaerobe	Microorganisms which are able to grow under either anaerobic or aerobic conditions
Fall out plate	A Petri dish containing sterile agar medium used to determine the presence of microorganisms in the air. A fall out plate is placed on a surface and exposed

	to the surrounding air. Particles suspended in the air will be deposited in the agar as a result of the force of gravity and air movements
FDA	Food and Drug Administration
Fermentation	An enzymatic decomposition, especially of carbohydrates as used in the production of alcohol, bread, vinegar and other food or industrial materials
Fiber	A fiber is defined (ISO 14644) as a particle that has an aspect (length-to-width) ratio of 10 or more
FMEA	Failure Mode Effects Analysis. A method of assessing risks
FTA	Failure Tree Analysis. A method for assessing risks
f-value	The time needed to reduce the total number of microorganisms in a certain medium at 121 °C (using an autoclave)
Garment system	A set of clothing which when worn together provides optimum protection to both wearer and the environment
Generation	The act or process of reproduction
Generation time	The time needed for the act or process of reproduction, e.g. the time needed for one microorganism to divide into two daughter cells
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice. A series of rules used to ensure that the product is always produced in the same way with respect to cleanliness, identity, effect and content
HACCP	Hazard Analysis Critical Control Point. HACCP is a risk analysis mostly used by the food and beverage industry
HAZOP	Hazard and Operability studies of the International Social Security Association. A method for assessing risks
HEPA	High Efficiency Particulate Air filter. A

	HEPA filter is defined as a filter for ventilation air with the ability to reduce the number of particles of 0.3 μm or greater in size in the air by at least 99.97% as measured with a DOP aerosol
Hydrophilic	Water wettable
Hydrophobic	Not readily wetted by water
Impaction	Physical phenomenon taking place when the movement of a particle suspended in a gas is stopped by an obstruction present in the gas stream. When a gas stream is forced towards a solid material, the gas itself will alter direction. Particles suspended in this gas stream, if they have a mass great enough, will not follow the gas stream due to inertia, and will continue in a tangential direction and finally hit the solid material. Impaction is often utilized in apparatus used for the collection of solid contaminants
Impinger	An apparatus used for the collection of contaminants in air and other gases when the gas is forced through liquid in a flask
Incubation	The process of maintaining warm conditions in which microorganisms or other cells can replicate when provided with appropriate media
Incubator	An apparatus for maintaining a constant and suitable temperature for the development of eggs, cultures of microorganisms or other living cells
Inert	Having no action. Not reacting with other elements
Inertia	Inability to move spontaneously. Inertia is used in many sampling techniques and is based on the inability of a particle to be collected due to both mass and

	speed of movement of the particle
Isokinetic sampling	The condition of isoaxial sampling in which the mean velocity of the air entering the probe inlet is the same as the mean velocity of the unidirectional airflow at the location
LAF	Laminar airflow. A theoretical situation where the air is moving in absolutely parallel streams. In practice very hard to achieve. LAF is not used frequently today. A more accurate term to use is UDF (Unidirectional Flow)
Lag phase	The early period in the growth pattern of microorganisms when growth or cell division is slow, following inoculation into a culture medium
Laminar flow	A flow of parallel streams in the same direction
Lethal	Deadly, fatal
Logarithmic phase	Logarithmic growth phase, the stage of active division in the growth pattern of microorganisms
Medium	A substance used for the culture of microorganisms
Microbiology	The science which deals with the study of microorganisms
Micrometer	One thousand of a millimeter, or one millionth of a meter
Mold	A large group of fungi
Moist heat sterilization	Sterilization by heat at 100% relative humidity
Monodisperse aerosol	An aerosol which contains particles that are all the same size
Morphology	Physical shape of an object
Non-unidirectional airflow	Air distribution where the supply air entering the room mixes with the internal air by means of induction. This type of air distribution results in dilution of the concentration of particles and/or other contaminants

Obligate anaerobe	Microorganisms that can grow only in the complete absence of molecular oxygen. Some obligate anaerobe microorganisms are killed by oxygen
OPC	Optical Particle Counter
Operational cleanroom	A cleanroom or facility in normal operation, with all services functioning and with equipment and personnel, if applicable, present and performing their normal work functions in the facility
Operator	Person working in the cleanroom performing work or carrying out process procedures
Oral	Pertaining to the mouth
Overpressure	A term used in a cleanroom if the pressure in the room is higher than in the surrounding environment. Overpressure is utilized to reduce the risk of contaminants entering critical environments
Parasite	An organism that derives its nutrients from other living organisms
Particle	Defined according most standards as an object of solid or liquid composition, or both, of a certain size. US Federal Standard 209 E specifies a size between 0.001 and 1.000 µm. ISO 14644 defines a particle as a minute piece of matter with defined physical boundaries
Particle concentration	The number of individual particles per unit volume of air
Particle size	The apparent maximum linear dimension of a particle in the plane of observation as seen with a microscope or the equivalent diameter of a particle detected by automatic instrumentation
Pasteurization	The process of heating milk or other liquids to a moderate temperature for a specified period of time. This exposure

	kills most species of pathogenic bacteria and significantly retards the development of others
Pathogen	Any disease producing microorganism or material
Pathogenic	Giving origin to disease or to morbid symptoms
Personal filter	A simple way of expressing a clothing system for cleanroom use
Point of use	The point where a particular activity is carried out. Typically the final filter of a ventilation system is point of use, i.e. the filter is an integral part of the roof
Positive ventilation	Ventilation pattern where a positive flow of air or other gas is used to purge the room or zone of contaminants
Pre-filter	A filter unit positioned in front of any other filter, used to reduce blockage of the main filter caused by contaminants. The pre-filter has in general a lower removal rating than the main filter
Protozoa	A major group of microorganisms comprising the simplest forms of life in the animal kingdom
Pyrogen	A substance that causes fever
Qualitative	A term used in relation to quality
Quantitative	A term used in relation to the measurement of quantity
RCS	Reuter Centrifugal Sampler. See Centrifugal Sampler
RODAC plate	A form of contact plate
RP	Recommended Practices, a series of documents published by the Institute of Environmental Sciences and Technologies (IEST) USA
Slit sampler	An impaction sampler for microbial analysis in which airborne particles are collected onto a slowly rotating agar plate making it possible to obtain the microbiological profile of a facility as a

	function of time. The impaction forces in such an apparatus are obtained by allowing the air to be analysed to pass through a very fine slit
Spore	A subcellular body produced by some species of bacteria which is considerably more resistant to harsh conditions such as heat, disinfectants and radiation, than the vegetative form
Stationary phase	The stage in the growth pattern of microorgansism where the number of new cells formed is equal to the number of cells that die
Step-over bench	Bench that is used when changing cleanroom clothing and which provides a barrier to the spread of floor contamination
Sterile	Cleanliness definition. Different definitions can be recognised: Free from living organisms or free from living microorganisms
Sterilization	Any process or activity leading to the complete elimination of microbial activity
Tenside	Surfactant. An agent, both hydrophobic as well as hydrophilic in nature. Used for cleaning purposes as it can be used to dissolve fats and make them water-soluble
Toxin	A poison. The term is frequently used to refer specifically to a protein product (or other biomolecule) produced by some higher plants, certain animals and pathogenic bacteria, which is highly toxic to other living organisms
Touch plates	Agar plates used for detecting the presence of microbial contaminants on hands. These sterile dishes are often used by operators engaged in aseptic filling

UDF	Unidirectional Flow. New term replacing LAF. UDF is an airflow which has a single flow direction and may or may not contain uniform velocities of airflow. UDF results in quick, directed transport of particles out of the clean zone
ULPA	Ultra Low Penetrating Air filter. An ULPA filter is defined as a filter for ventilation air with the ability to reduce the number of particles in the air of size 0.12 μm and greater, by at least 99.999% as measured with a DOP aerosol
Ultrafine particles	Particles in the size range from approximately 0.01 μm to the upper detection limit of the DPC
Validation	Full and detailed documentation that all processes and procedures are functioning in the manner for which they were designed
Verification	The procedure for determining the compliance of air in a cleanroom or clean zone to an airborne particulate cleanliness class limit or a U descriptor, or both
Viable	Capable of growing and living. The ability of microorganisms to grow and form visible colonies on an appropriate medium
Viricide	An agent that kills viruses
Virus	A group of minute infectious agents that, with certain exceptions, cannot be visualized with a light microscope. Viruses are characterized by their lack of independent metabolism and can replicate only within a living host cell
z-value	The increase in temperature that will improve the destruction rate of microorganisms by a factor of 10. The z-

value is used in relation to sterilization in an autoclave

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