Cleanrooms and environmental monitoring

16

16.1 Introduction

This chapter examines the key aspects relating to the pharmaceutical-manufacturing environment and to matters relating to personnel working in such environments. The chapter examines the design, control, and monitoring of cleanrooms and clean zones. Pharmaceutical manufacturing, both sterile and nonsterile processing, requires that products are developed and manufactured in areas which minimize the potential for contamination through the control of environmental cleanliness and in minimizing the possibility of personnel introducing contamination into the process.

The pharmaceutical-manufacturing environment is based around a series of rooms with specially controlled environments. These are termed cleanrooms. A cleanroom or zone, on one level, is simply a room that is clean. The key aspect, however, is that the level of cleanliness is *controlled*. A cleanroom is a room designed and operated to control particulate levels.

A more specialized meaning is as defined in the international cleanroom standard, ISO 14644-1:

> *A room with control of particulates and set environmental parameters. Construction and use of the room is in a manner to minimise the generation and retention of particles. The classification is set by the cleanliness of the air.*

The regulatory requirements for cleanrooms are detailed by EU GMP or the FDA guidelines. The way in which cleanrooms are qualified and assessed is by a series of ISO standards, such as the ISO 14644 group (which refer to physical parameters, including particle counts); of these, Part 1 (ISO 14644-1) sets the general standard for the classification of air cleanliness and Part 2 (ISO 14644-2) sets out the specifications for testing, are the most important. In addition, ISO 14698 describes some of the standards and testing requirements for biocontamination control.

Cleanrooms can be designed to minimize particulate risk and most cleanrooms operate well, until personnel enter them. People in cleanrooms are the biggest source of contamination [1]. It, therefore, follows that any pharmaceutical process that involves people presents a contamination risk [2].

This chapter discusses the classification and certification of cleanrooms and then proceeds to consider the monitoring steps necessary to demonstrate on-going compliance.

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16.2 Cleanroom contamination

By prescribing a grade or a class to a cleanroom, the areas are then regarded as "controlled" environments. A controlled environment is any area in an aseptic process system for which airborne particulate and microorganism levels are controlled to specific levels to the activities conducted within that environment.

To give this a different perspective, the ambient air outside in a typical urban environment might contain as many as 35,000,000 particles per cubic meter, 0.5 µm and larger in diameter, corresponding to an ISO 14644-1 cleanroom class of 9.

The measurement of airborne particle counts is a key part of environmental control. Particles are measured using optical particle counters, and the regulatory requirement is for two sizes of particle to be counted. These are 0.5 µm (which is close to the size of a microorganism) and 5.0 µm (which is close to the size of a skin cell, which may carry bacteria). These particles are very small and are not visible to the human eye. Generally, the "complete particle" (microorganism in association with the "carrier") is 12 µm diameter or larger. Most microorganisms in cleanroom air are attached to dust or to skin flakes or to water droplets [3].

Thus, the particles measured may be nonviable or viable, but because of the association with microorganisms and the assumption that some particles will be microorganisms designing facilities to minimize the number of particles and then monitoring of particulate levels is an important part of contamination control [4].

These levels of cleanliness are established through the design and construction of the cleanroom, particularly [5]:

- The air entering a cleanroom from outside is filtered to exclude dust, and the air inside is constantly re-circulated through HEPA filters. This is controlled through a HVAC (Heating, Ventilation and Air Conditioning) system. The most important part of this is with air-filtration through a HEPA (High Efficiency Particulate Air) filter, or higher grade ULPA (Ultra Low Penetration Air) filters.
- Staff enter and leave through airlocks (sometimes including an air shower stage), and wear protective clothing such as hats, face masks, gloves, boots, and cover-alls.
- Equipment inside the cleanroom is designed to generate minimal air contamination. There are even specialized mops and buckets. Cleanroom furniture is also designed to produce a low amount of particles and to be easy to clean.
- Common materials such as paper, pencils, and fabrics made from natural fibers are often excluded, however, alternatives are available. Low-level cleanrooms are often not sterile (i.e., free of uncontrolled microbes), and more attention is given to airborne particles. As indicated above, particle levels are usually tested using a Laser particle counter.
- Some cleanrooms are kept at a higher air pressure to adjacent (less clean) areas so that if there are any leaks, air leaks out of the chamber instead of unfiltered air coming in.
- Cleanroom HVAC systems also control the humidity to low levels, such that extra precautions are necessary to prevent electrostatic discharges.

Therefore, cleanrooms are designed to minimize and to control contamination. There are many sources of contamination. The atmosphere contains dusts, microorganisms, condensates, and gases. People, in clean environments, are the greatest contributors to contamination emitting body vapors, dead skin, microorganisms, skin oils,

and so on. Manufacturing processes will produce a range of contaminants. Wherever there is a process which grinds, corrodes, fumes, heats, sprays, turns, etc., particles and fumes are emitted and will contaminate their surroundings. Another key contamination source is water. This is a continuing problem as water is the main ingredient in pharmaceuticals.

16.3 Cleanroom classification

Cleanrooms and zones are typically classified according to their use (the main activity within each room or zone) and confirmed by the cleanliness of the air by the measurement of particles [6]. Cleanrooms are used in several industries including the manufacture of pharmaceuticals and in the electronics industry. For pharmaceutical cleanrooms, air cleanliness is either based on EU GMP guidance for aseptically filled products, and the EU GMP alphabetic notations are adopted (Table 16.1); or by using the International Standard ISO14644 (Table 16.2), where numerical classes are adopted.

Thus, Grade A is the highest grade (that is the "cleanest"), and Grade D is the lowest (that is the least "clean"). With ISO, the lower the number (such as "5") the

Table 16.1 EU GMP the typical room uses and associated grades

Grade	Room use
A	Aseptic preparation and filling (critical zones under unidirectional flow)
B	A room containing a Grade A zone (the background environment for filling) and the area demarcated as the "Aseptic Filling Suite" (including final stage changing rooms)
C	Preparation of solutions to be filtered and production processing; component handling
D	Handling of components after washing; plasma stripping
U[a]	Freezers, computer conduits, store rooms, electrical cupboards, other rooms not in use, etc.

[a]U = unclassified. Unclassified areas are not monitored.

Table 16.2 Comparison of EU GMP and ISO14644 cleanroom states at rest

EU GMP	ISO 14644-1
A	5
B	5
C	7
D	8

"cleaner" the room class and the higher the number (such as "9") the room is considered to be "less clean." ISO 14644 class 5 is the critical zone where sterilized product, components, and product contact equipment are brought together and exposed.

The ISO classes and the EU GMP grades are approximately equivalent (although there are slight differences in the number of particles of a given size permitted). The table below compares the EU GMP grades and the ISO classes in the at rest state:

Cleanrooms have three different "states" of use. These are:

- As built;
- At rest (or static);
- In operation (or dynamic).

"As built" refers to the condition of a newly built cleanroom, with the operational qualification having been completed, at the point it is handed over to the user for performance qualification. There are two approaches to the construction of cleanrooms with regard to maintaining cleanliness. One method is to clean the facility at the end of the construction, often called "final super clean," while the other method, called "clean-build," requires continuous cleaning during construction. "Clean-build" protocols attempt to prevent contamination or capture contaminants at their source during the construction process. This clean construction protocol concept stresses the importance of exercising the discipline to build clean because it was widely believed that ultra-low levels of contamination cannot be reached using normal construction techniques and then by cleaning the facility afterwards.

For "at rest" conditions, there is a difference between European/ISO and US standards. The EU GMP defines the static state as a room without personnel present, following 15–20 min "clean up time," but with equipment operating normally. The US standards indicate that equipment is not running. "In operation" conditions are defined as rooms being used for normal processing activities with personnel present and equipment operating. Although both "static" and "dynamic" states are considered important by European regulators, for the United States the FDA tends only to focus on the dynamic state. This is because excursions within the dynamic state pose the bigger risk to product [7].

16.4 Isolators

Isolator technology is increasing being used in place of cleanrooms for critical activities such as aseptic filling and sterility testing. Isolators are part of a field called "barrier technology." Isolators are superior to cleanrooms in that the contamination risk is reduced through the construction of a barrier between the critical area (sometimes called the "microenvironment") and the outside environment.

Cleanroom and barrier isolator systems have four basic parts: the physical structure, the internal environment, the interaction technology, and the monitoring system. Thus, many of the test parameters described for cleanrooms will apply. It is also normal for isolators to be housed in cleanrooms (certainly those used for aseptic filling); this requires a further understanding of cleanroom disciplines.

Isolators will be positively or negatively pressured depending upon the required application. The main contamination risks from isolators are with the methods for transferring material in and out of the Isolator, especially as this requires personnel manipulations. These risks are minimized by the use of rapid transfer ports and by sanitizing isolators with a sterilant (vapor phase hydrogen peroxide is the most commonly used to decontamination cycle).

16.5 Cleanroom certification

Classification of critical cleanrooms is normally confirmed in the "in operation" state by taking nonviable particulate readings at a defined number of locations for 5.0 and 0.5 µm size particles (some pharmaceutical manufacturers opt to classify in the static state). The following frequencies (Table 16.3) are often adopted (as stated in ISO 14644-2):

The method of classification is based upon a number of locations in a cleanroom being monitored for particle counts (at the 0.5 µm size). Depending upon the grade of the room the sample size should either be one cubic meter per location or the total number of locations in the room should represent a sample size of one cubic meter. The number of room locations is the square root of the room in cubic meters and is defined in ISO14644-2.

16.6 Cleanroom testing

Once a room has been assigned a classification, certain environmental parameters (physical and microbiological) are to be met on a routine basis. For viable monitoring it is normal for the microbiologist to set action levels (and warning levels, which are equivalent to what are sometimes referred to as alert levels) based on an historical analysis of data.

The frequency of the assessment of other parameters (as described below) should be assessed based on a risk management approach. This approach should consider the room use and the risk to the product. Factors to consider may include [8]:

- room activities,
- exposure risk,
- room temperature

Table 16.3 Cleanroom qualification frequencies

Grade	Frequency of classification
A	Six-monthly
B	Six-monthly
C	Annually
D	Annually

- Process stage.
- Duration of process activities.
- Water exposure.

The emphasis should always be upon environmental control rather than simply environmental monitoring. That is, where a risk is identified, the risk should be minimized as part of a strategy of bringing the clean area into tighter control. Where a risk cannot be minimized but continues to exist then carefully targeted monitoring should be undertaken and the data reviewed and examined for trends, by a pharmaceutical microbiologist.

16.6.1 Physical parameters

There are a number of physical parameters which require examination on a regular basis. These parameters generally relate to the operation of HVAC systems and the associated air-handling chapters. Air handler, or air-handling chapter (AHU), relates to the blower, heating and cooling elements, filter racks or chamber, dampers, humidifier, and other central equipments in direct contact with the airflow. The key aspects which require testing are discussed below.

16.6.1.1 Air patterns and air movement

Airflows, for critical activities, need to be studied in order to show that air turbulence does not interfere with critical processes. There are two types of cleanrooms: turbulent flow or unidirectional flow, depending upon the required application. Unidirectional airflow areas are used for higher cleanliness states (such as aseptic filling), and they use far greater quantities of air than turbulent flow areas. Typically all critical rooms and zones within an aseptic filling area relating to batch filling should be assessed. Other critical processes may also be monitored. Airflows are studied using smoke generating devices and should be captured onto video tape or camera, and a report should be generated for each study. For air flow movement, air flow must be from a higher grade area to a lower grade area.

16.6.1.2 Airflows

Grade A zones (undirectional airflow devices in Grade B rooms) have a requirement for controlled air velocity and unidirectional air flow (either horizontal or vertical). These are monitored using an anemometer. The air velocity is designed to be sufficient to remove any relatively large particles before they settle onto surfaces [9].

This monitoring is performed routinely and during re-qualification exercises. The target range is 0.45 m/s (±20%).

16.6.1.3 Air changes

Each cleanroom grade has a set number of air changes per hour. A typical air conditioned office will have something between 2 and 10 air changes per hour in order to give a level of comfort. The number of required air changes in a cleanroom is typically much higher (at least 20, and somewhat higher than this in changing rooms). Air changes are

provided in order to dilute any particles present to an acceptable concentration (thus, air change is a way of expressing the level of air dilution which is occurring). Any contamination produced in the cleanroom is theoretically removed within the required time appropriate to the room grade. Monitoring air changes is necessary because the re-circulation of filtered air is important for maintaining control of the clean area.

16.6.1.4 Clean up times

Connected to air changes is the time taken for a clean area to return to the static condition, appropriate to its grade, in terms of particulates. This is sometimes called the recovery rate. The target time for "cleaning up" is 15–20 min.

16.6.1.5 Positive pressure

Connected to the measurement of air flow is positive pressure. In order to maintain air quality in a cleanroom, the pressure of a given room must be greater relative to a room of a lower grade (ΔP). This is to ensure that air, and hence particulate contamination, does not pass from "dirtier" adjacent areas into the higher grade cleanroom (this can also be observed by smoke studies). Generally, this is 15–20 Pa, although some areas of the same grade will also have differential pressure requirements due to specific activities. The most commonly encountered problems relate to situations when cleanroom doors are opened, and here it can be difficult to maintain pressures.

Note: Pressure differentials (ΔP expressed in Pascals) are the relative pressures from a higher grade area into a lower one. These are guidance values taken from EU GMP Annex 1 Manufacture of sterile medicinal products.

16.6.1.6 HEPA filters

HEPA (High Efficiency Particulate Air) filters are used in cleanrooms in many different industries, including semiconductor, pharmaceutical medical devices, nuclear, and biotechnology. The main function of a HEPA filter is to provide clean air to the cleanroom. HEPA filters are replaceable, extended-media, dry-type filters in rigid frames with set particle collection efficiencies. HEPA filter is constructed with many pleated layers of filter media paper; this design prevents particles from freely passing through the filter as they become trapped and stick onto the filter fibers. The filters are designed to control the number of particles entering a clean area by filtration. In Grade A zones, HEPA filters also function to straighten the airflow as a part of the unidirectional flow. In order to measure the effectiveness of the filters, they are checked for leaks (a DOP test). Leakage is assessed by challenging the filters with a particle generating substance and measuring the efficiency of the filter.

Particles are mainly trapped (they stick to a fiber) by one of the following four mechanisms:

- Straining/sieving. This is defined as when a particle is too large and becomes trapped between two filter fibers.
- Interception, where particles following a line of flow in the air stream come within one radius of a fiber and adhere to it.

- Impaction, where larger particles are unable to avoid fibers by following the curving contours of the air stream and are forced to embed in one of them directly; this increases with diminishing fiber separation and higher air flow velocity.
- Diffusion, an enhancing mechanism is a result of the collision with gas molecules by the smallest particles, especially those below 0.1 µm in diameter, which are thereby impeded and delayed in their path through the filter; this behavior is similar to Brownian motion and raises the probability that a particle will be stopped by either of the two mechanisms above; it becomes dominant at lower air flow velocities.

In Europe, HEPA filter integrity taken from BS EN 1822 Parts 1 and 2. For example, a filter with a 99.997% efficiency is based on the particle sizes 0.3 µm and larger (i.e., theoretically only 3 out of 10,000 particles at 0.3 µm size can penetrate the filter). This is typically assessed through thermally generated dioctylphthalate (DOP) (or specified alternative aerosol) particles, and a maximum clean-filter pressure drop test.

An ULPA filter (theoretically) can remove from the air at least 99.999% of dust, pollen, fungi, bacteria, and any airborne particles with a size of 0.12 µm or larger.

16.6.1.7 Temperature, humidity, lighting, and room design

In terms of the general design of cleanrooms, they should:

- Be built of an airtight structure;
- The internal surfaces should be smooth and suitable for cleaning;
- The internal surface finish should be tough enough to resist chipping or powdering;
- The surfaces should be resistant to the cleaning agents used.

In addition to surfaces, parameters such as temperature and humidity should be controlled through the HVAC system. This is important for operator comfort and as a way of minimizing contamination (e.g., high temperatures which lead to excessive perspiration can reduce the efficiency of the cleanroom suit). HVAC systems use ducts (through ductwork systems) to deliver and remove air. Ducts deliver, most commonly as a part of the supply air, ventilation air. As such, air ducts are one method of ensuring acceptable indoor air quality as well as thermal comfort.

Grade B rooms should have set requirements for temperature and humidity (18 ± 3 °C and 45 ± 15% relative humidity). These are monitored for operator comfort and to avoid a high temperature/humidity situation which may result in the shedding of microorganisms. Other clean areas have a temperature appropriate to the process step (e.g., if the process requires a cold room at 2–8 °C).

Lighting should be adequate, uniform and antiglare, to allow operators to perform process tasks effectively. A range of 400–750 lux is recommended.

Cleanrooms are specially designed rooms. The surfaces are constructed from materials that do not generate particles and are easy to clean.

16.7 Microbiological environmental monitoring

The areas of cleanroom monitoring which typically fall under the responsibility of the microbiology department are viable and nonviable methodologies. This forms the "environmental monitoring program." Environmental monitoring is a program which

evaluates the cleanliness of the manufacturing or process environment; the effectiveness of cleaning and disinfection programs and the operational performance of environmental controls. Environmental monitoring was, arguably, an underdeveloped activity until the 1980s. Current programs are more sophisticated and are focused on contamination control rather than simply detecting contamination events [10].

The emphasis should always be upon environmental control rather than simply environmental monitoring. That is, where a risk is identified, the risk should be minimized as part of a strategy of bringing the clean area into tighter control. Where a risk cannot be minimized but continues to exist then carefully targeted monitoring should be undertaken and the data reviewed, and examined for trends, by a pharmaceutical microbiologist. Furthermore, part of environmental control involves designing facilities in an optimal way so that contamination is minimized, and where environmental monitoring becomes a tool in the assessment of control [11].

However, with no risk able to be reduced completely to zero, there remains an important role for environmental monitoring.

Nonviable monitoring is for air-borne particle counts. These are the same sizes of particles required for the classification (as described above): 0.5 and 5.0 µm. This is undertaken using an optical particle counter. Particle counters are used to determine the air quality by counting and sizing the number of particles in the air.

Viable monitoring is designed to detect levels of bacteria and fungi present in defined locations/areas during a particular stage in the activity of processing and filling a product. Viable monitoring is designed to detect mesophilic microorganisms in the aerobic state. However, some manufacturers may have requirements to examine for other types of microorganisms (such as anaerobes if nitrogen lines are used as a part of the manufacturing process).

Monitoring methods will all use either a general purpose culture medium like tryptone soya agar (TSA), which will be used at a dual incubation regime of 20–25 °C and 30–35 °C or two different culture media are used at two different temperatures, of which one of the media is selective for fungi (e.g., Sabouraud Dextrose agar, SDA). The choice of culture media, incubation times, and temperatures requires validating.

Viable microbiological monitoring is normally performed in the "in operation," as this represents a more realistic assessment of the challenge to the manufacturing process. Dynamic is interpreted as rooms being used for normal processing activities with personnel present.

There are different methods which are used for viable monitoring. These can be grouped into air and surface methods and into primary and secondary methods based on their theoretical efficiencies to recover micro-organisms (Table 16.4):

In addition to the classic methods outlined above, rapid microbiological methods are available. These include spectrophotometric particle counters. Rapid microbiological methods are considered in Chapter 17 [12].

16.7.1 Air sampling methods

For air monitoring, this is undertaken using agar settle plates (placed in the locations of greatest risk) or active (volumetric) air-samplers (to provide a quantitative assessment of the number of microorganisms in the air per volume of air sampled).

Table 16.4 Microbiological viable monitoring methods

	Air	Surface	Personnel
Method #1	Active air Sampler (cfu/m^3)	Contact Plate (cfu/25 cm^2)	Finger plate for Hands (cfu/5 fingers) Contact plate for gowns (cfu/25 cm^2)
Method #2	Settle Plate (cfu/90 mm over "*x*" time period)	Swab (cfu/surface)	

16.7.1.1 Settle plates

A settle plate is an agar plate, placed in a defined location. The exposure time of the settle plate can be varied, although there is probably little value in exposing plates for less than 1 h. For consistency of sampling, for aseptic filling, the EU GMP Guide recommends a 4 h exposure time. This time should not be exceeded without strong justification, and even then there will probably be a challenge from the regulatory authority. For exposure times under 4 h, such as when a shorter activity is being monitored, the result obtained should be extrapolated using the simple equation:

$$\frac{\text{Count}}{\text{Time exposed}\left(\text{min}\right)} \times 240 = \text{cfu} / 4\ \text{h}$$

The risk from any exposure is desiccation. The depth and condition of the agar are the key variables, as is the cleanroom environment. The agar in the plate will dry out faster if the airflow is excessively high or if the air humidity is low. Therefore, the exposure time of settle plates under the conditions of use.

16.7.1.2 Active air samples

Active (or volumetric or bioaerosol) air samplers are a slightly different measure of microorganisms in air than settle plates. As indicated earlier, the settle plate indicates the number of microorganisms that may deposit onto a surface; whereas, the active air-sampler indicates the number of microorganisms present in a given volume of air within the range of the air-sampler. Both of these approaches have merits and any comprehensive program will use both active air samples and settle plates.

Active air-samplers sample a defined quantity of air. The volume of air sampled is normally one cubic meter of air. This allows the data to be quantified as cfu/m^3.

Active air-samplers generally fall into the following different models:

- slit to agar,
- membrane filtration,
- centrifugal samplers.

Where air samplers are required to be used in unidirectional airflow devices, the samplers should be isokinetic in operation as so not to disrupt the air stream [13].

16.7.2 Surface sampling methods

16.7.2.1 Contact plates

Surface contact plates are a common test for surface contamination. Contact plates are Petri-dishes filled with microbiological agar. The plate is filled to a level above the rim of the plate so that the agar surface extends upwards when dry. The plate has a typical diameter of 50–55 mm and a surface area of 25 cm^2.

The raised surface allows the agar to be pressed onto a surface. The design of the contact plate is, therefore, different to the standard Petri-dish, where the agar is contained within the Petri-dish.

The contact plate is a quantifiable method, because the contact between the plate and the surface provides a "mirror image" of the surface. Following incubation, this image transfer provides information relating to the number of microbial colonies and their relative position. The quantification is derived from the recording the number of colony forming units (cfu) per square centimeter. This act of replication gives the contact plate its alternative name: RODAC. This is an acronym for "Replicate Organism Detection and Counting," and the term is more commonly applied in North America.

In Grades A and B cleanrooms, in relation to aseptic processing, contact plates are taken from personnel hands (during processing activities) and from clothing. Personnel gown monitoring should be carried out at the end of a shift. This is because the sampling damages the fabric integrity of the suit through the moisture of the agar plate.

16.7.2.2 Swabs

Swabs are typically made up of sterile cotton tips, although swabs vary in the materials used for the applicator stick (either wood or plastic) and the tip (where materials like cotton, viscose, alginate, and so on are used). Some types of swabs require prewetting using a diluent (such as Ringer's solution, phosphate-buffered saline, sodium hexametaphosphate, sterile water, etc.) before use. Other types of swabs are contained with a transport medium. They are either contained within a transport medium or require prewetting with a suitable recovery medium. Swabbing is performed by rubbing a surface while rotating the swab—so that all parts of the tip are exposed—through a number of strokes (typically between 10 and 25).

Following the sampling, the swab can either be streaked out onto an agar plate (which is the least efficient method) or, where the appropriate tip has been used, dissolved in a diluent and tested either by pour plate or by membrane filtration (of which the latter is the most efficient method). The membrane filter will be either 0.45 or 0.22 µm, and this technique carries a further advantage in that disinfectant residues can be overcome through rinsing the filter.

16.7.3 Key aspects of the monitoring program

The microbiologist should establish the appropriate frequencies and durations for monitoring based on a risk assessment approach [14]. This applies to all cleanrooms

where product is processed. In addition, in relation to other parts of this book, environmental monitoring should extend to the microbiology laboratory [15].

The sampling plan should take into account the cleanliness level required at each site to be sampled. It should consider which types of samples are appropriate that is air samples and/or surface samples. The choice of sample locations has to consider the nature of the work to be carried out in the production process or cleaning process and the impact that cleanroom operators and equipment (both fixed and portable) will have on the biocontamination levels. This requires a detailed understanding of how the cleanroom operates in terms of airflow velocity and direction and how these factors interact with the equipment and people in the cleanroom. Once this has been considered, the number of samples to be taken can be determined.

In terms of sampling frequency, in order to set initial limits for the microbial monitoring, a number of sampling operations are carried out. Initially the locations are sampled with the area at rest to give some base reference information. Thereafter the locations are sampled during the production activity. Assuming the product quality is satisfactory when the sampling was carried out then the monitoring data is used to set limits. Thereafter the test frequency can be reduced to a level that will still demonstrate control of biocontamination. The time of sampling should also take account of testing after a "cleandown," testing at the end of a shift, testing at times of the highest operator activity or high levels of materials in the area. In addition, testing after new installed equipment or routine maintenance work should be considered. If out of limits, microbial levels are found that it would also be standard to increase the sampling frequency until control is demonstrated after any corrective action has been taken.

The environmental monitoring plan should also include sample handling and incubation.

For microbiological monitoring parameters, Table 16.5 below lists those typically applied as maximal values (with appropriate warning and action levels set at some level below):

Where nonviable and viable monitoring levels are exceeded, it is typical to identify to species levels all the contaminants from Grades A and B areas and to have an understanding of the microflora from other areas. The microflora recovered should be compared with the microorganisms from other areas, and a comparison made.

The types of microorganisms can indicate the origin of contamination, The recovery of endospore-forming bacteria and fungi, for example, could indicate a problem with HEPA filters or insufficient pressure differentials between a clean and less clean area; the recovery of Gram-positive cocci is invariably an indication of personnel contamination; whereas the recovery of Gram-negative bacteria can signify water or dampness, or possibly fabric damage. The list below provides examples of some commonly occurring microorganisms and the various aspects of cleanroom operations that they are associated with [16]:

Airborne types

- *Bacillus* spp.
- *Micrococcus* spp.
- *Staphylococcus* spp.
- *Aspergillus* spp.

Table 16.5 **Viable and non viable particulate requirements for cleanrooms**

Parameters	**Grade A**		**Grade B**		**Grade C**		**Grade D**	
Nonviable	***Particle size/m³***		***Particle size/m³***		***Particle size/m³***		***Particle size/m³***	
Particulates Static state	3520 at 0.5 µm	20 at 5.0 µm	3520 at 0.5 µm	29 at 5.0 µm	352,000 at 0.5 µm	2900 at 5.0 µm	3,500,000 at 0.5 µm	29,000 at 5.0 µm
Particulates Dynamic state	3520 at 0.5 µm	20 at 5.0 µm	352,000 at 0.5 µm	2900 at 5.0 µm	3,520,000 at 0.5 µm	29,000 at 5.0 µm	Not defined	Not defined
Air samples (active) Dynamic state Air samples: Settle plates (passive) Dynamic state	cfu/m³ Action = 1 cfu/event Action = 1		cfu/m³ Action = 10 cfu/event Action = 5		cfu/m³ Action = 100 cfu/event Action = 50		cfu/m³ Action = 200 cfu/event Action = 100	
Surface samples at working height: Contact plates Dynamic state	cfu/25 cm² Action = 1		cfu/25 cm² Action = 5		cfu/25 cm² Action = 25		cfu/25 cm² Action = 50	
Surface samples at working height: Swabs Dynamic state	cfu/swab Action = 1		cfu/swab Action = 5		cfu/swab Action = 25		cfu/swab Action = 50	
Surface samples: Floor contact plates/swabs Drain swabs Finger plates Dynamic state Gowning (suit contact plate) Dynamic state	cfu/device Action = 1 N/A cfu/plate (hand) Action = 1 cfu/25 cm² Action = 1		cfu/device Action = 10 N/A cfu/plate (hand) Action = 5 cfu/25 cm² Action = 5		Not defined Not defined cfu/plate (hand) N/A cfu/25 cm² N/A		Not defined Not defined cfu/plate (hand) N/A cfu/25 cm² N/A	

- *Penicillin* spp.
- *Corynebacterium* spp.

Personnel contamination
- *Staphylococcus* spp.
- *Staphylococcus epidermidis*
- *Staphylococcus capitis*
- *Staphylococcus hominis*
- *Propionibacterium* spp.
- *Micrococcus* spp.
- *Trycophyton* spp.
- *Epidermophyton* spp.
- *Micosporon* spp.

Water/water sources
- Gram-negative rods in general
- *Pseudomonas* spp.
- *Alcaligenes* spp.
- *Stenotrophomonas* spp.
- *Burkholderia cepacia*
- *Ralstonia picketti*
- *Serratia* spp.
- *Flavobacterium* spp.

Such data can provide useful information in terms of contamination patterns. Areas from cross comparison may include:

- Cleanrooms or controlled environments,
- Associated manufacturing areas,
- Raw materials or components,
- Personnel, when appropriate,
- Utilities, e.g., water and compressed air,
- Finished product.

Action level excursions should be investigated using established OOS (out of specification or out of limits) procedures. The microbiologist should be at the forefront of such investigations.

Environmental monitoring is normally conducted by microbiologists. However, sampling is better carried out by process personnel in aseptic filling areas in order to minimize the number of personnel present within a cleanroom [17].

16.7.4 Personnel

Personnel working in controlled environments should be correctly trained in cleanroom disciplines and in aseptic technique. The biggest source of contamination in cleanrooms is people, and levels of contamination are the highest where training is poor (such as making rapid movements); gowning is not effective; or where an individual has a high propensity to "shed" skin resident microorganisms. Contamination

shed by people includes a mix of viable and nonviable particulates. The precise pattern is unique to each individual.

Experiments have shown that personnel clothed in new, sterile cleanroom garments slough viable contamination at a rate of roughly one viable particulate to 10,000 nonviable particles [18]. During slow deliberate movements with the best possible clothing, operators will slough particulate and viable organisms. Therefore, the probability of human borne microbial contamination being released in the conventional cleanroom is one over the course of any reasonably long operational shift. The microorganisms typically associated with personnel are those residential or transient to skin such as the *Staphylococci* and the *Micrococci*.

The cleanroom disciplines which require control relate to clothing; entry and exit procedures and movements within the cleanroom. The US Food and Drug Administration (FDA) Centre for Drug Evaluation and Research (CDER) call for both initial and on-going training for cleanroom operators:

> *Appropriate training should be conducted before an individual is permitted to enter the aseptic processing area and perform operations. For example, such training should include aseptic technique, cleanroom behaviour, microbiology, hygiene, gowning, patient safety hazards posed by a non-sterile drug product, and the specific written procedures covering aseptic processing area operations. After initial training, personnel should be updated regularly by an on-going training program.*

Training should not only examine the practical aspects of cleanroom disciplines but manufacturing staff should also have an understanding of basic microbiology and how contamination can be spread in process areas. A feature of personal hygiene will also be important!

16.8 Aseptic technique

A key principle in cleanroom operation is aseptic technique. Aseptic technique is a procedure to minimize contamination, where the performance of the procedure is conducted in a manner that prevents the introduction of contamination.

Aseptic technique is a mental discipline as well as a physical activity. Personnel working in cleanrooms should consider all of the surfaces as “nonsterile” [19]. Within Grades A and B areas, disciplines become tighter. Here, for example, objects, including the operators’ hands, must never be placed between the source of the air and a sterile object. The operators’ hands and arms must always be kept at a level beneath that of open product containers. Sterile components should under no circumstances be touched directly with gloved hands; a sterilized tool should always used for this purpose. Since gloved hands and arms will enter the sterile field, they must never touch walls, floors, doors, or other surfaces. Strenuous lifting and moving of tanks, trolleys, and so on must not be done by operators assigned to work within or near the sterile field, because the more strenuous the activity, the higher the level of particle generation, and at least some of the particulate released by the operator will surely be viable microorganisms.

16.9 Other cleanroom disciplines

As a part of personnel training and cleanroom control, other cleanroom disciplines should be put over to staff. The most fundamental of these relate to movements. In higher grade areas, movements should be slow, controlled and deliberate as to minimize contamination. For example:

- No touching of the face with gloved hand. The glove is to be changed immediately when this occurs.
- No adjustment of hair is to be carried out in the cleanroom. All personnel are required to go to the changing room to tuck their hair under the hair net, after which the glove is to be replaced.
- No torn glove, booties or jumpsuit are to be worn in the cleanroom. They are to be changed immediately when they are found to be torn.
- No removing of items from underneath of the cleanroom jumpsuit.
- No running, fast walking, pushing and shoving, smoking, eating, or drinking in the cleanroom.
- No exposure of forearm bare skin. Jumpsuit and glove must be fully covered while working in the cleanroom.
- No scratching of head, combing of hairs.

Furthermore, disciplines can include such "dos" and "donts" as not allowing the following to be brought into or used in a cleanroom:

- Cosmetic make-up;
- Noncleanroom or regular papers;
- Noncleanroom compatible pen;
- Pencils, erasers, highlighter pen, and correction fluid;
- Photocopied cleanroom paper not contained in cleanroom compatible cover;
- Carton material items, wood, "stera-form," regular cloth, and regular paper materials;
- Rusty tools or equipment;
- When in doubt of other materials which is not stated on the above, please consult the supervisor.

16.9.1 Clothing

Airborne microorganisms, which are almost exclusively bacteria, are normally dispersed into the air around us from the surfaces of our skin cells. Cleanroom garments help to eliminate this source of contamination by acting as a "person filter" to prevent human particulate matter from entering the atmosphere of the cleanroom. Cleanroom clothing is made from fabrics that do not lint or disperse particles and act as a filter against particles dispersed from the person's skin and indoor, or factory, clothing. The type of clothing used in a cleanroom varies according to the type of cleanroom. In cleanrooms where contamination control is very important, personnel wear clothing that completely envelops them to ensure that particles and bacteria are not dispersed into the air such as a coverall, hood, facemask, knee-length boots, and gloves. In cleanrooms where contamination is not as critical, then a smock, cap, and shoe covers may be sufficient.

There are different gowning requirements for different grades of cleanroom. For example:

16.9.2 Grade A areas

Headgear to totally enclose hair and beard; face mask; no jewellery worn; sterilized gloves; protective sterile clothing consisting of one piece suit gathered at wrists with high neck and shedding virtually no particles or fibers and retaining particles shed by body; and trouser bottoms tucked into boots which cover captive shoes.

16.9.3 Grade C areas

Hair and beards covered; no jewellery worn (except for covered wedding rings); protective clothing consisting of one piece suit gathered at wrists with high neck and shedding virtually no particles or fibers; and captive shoes or overshoes worn.

16.10 Cleaning

Part of the control of a cleanroom and a way of minimizing contamination from personnel is established through cleaning procedures [20]. This is often an underplayed aspect of environmental control. Manufacturers spend large sums of money on installing HEPA filters and in establishing controlled HVAC and then proceed to neglect the basic aspects like regular and consistent cleaning. To eliminate potential contamination, all cleanroom surfaces such as rafters, interstitial spaces, duct work, plenum areas, ceiling panels, T-bars, lighting, ionizing grids, return air vents, walls, windows, work stations, equipment surfaces, cabinets, sinks, shelves, furniture, doors, pass-throughs, air locks, floor mats, floors and raised floors, and sub-fabric require periodic cleaning.

Cleaning in a pharmaceutical cleanroom is concerned about minimizing the number of particles and microorganisms (in relation to the grade of the cleanroom). It is important to establish which areas are to be kept clean and at what level. It can be useful to define the critical and general zones within the room and then work out what contaminants and levels are acceptable within these zones, what levels exist now. This can be demonstrated through environmental monitoring. This will give some idea on the frequency of cleaning needed, it maybe hourly, daily, weekly, monthly, and quarterly. Such data can allow the establishment of a documented cleaning program and schedule.

A further important consideration will be the selection of appropriate disinfectants and detergents. These will require validating and regular review against the microflora recovered.

16.11 Conclusion

This chapter has set out to explain the importance of environmental contamination control and has covered:

- Why contamination is a problem;
- Some contamination sources;

- Contamination control;
- Understanding cleanrooms;
- Some aspects of environmental monitoring.

In covering these topics, the chapter has emphasized that environmental, and hence contamination control is of great importance for all areas of manufacturing. This is not only to meet regulatory standards but also to reduce the risk to the product and patient. Here it is important to note that although the standards (and often the level of monitoring) are higher for Grades A and B areas, what happens in other areas contributes to the quality of adjacent areas. For example, if a change area of wash-bay has an operational problem or if staff do not follow changing or cleaning procedures then higher graded area can be put at risk. An appreciation of contamination sources and contamination control is required by all who work within the pharmaceutical sector.

The student will also appreciate that to achieve contamination control requires a multidisciplinary approach. Microbiologists must work with Engineers and Production staff in order to ensure that cleanrooms are constructed, maintained, and monitored to agreed standards that problems are addressed and that risks are evaluated.

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