6

Validating and Monitoring the Cleanroom

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Overview

Many have fallen into the trap of spending huge amounts of money building a cleanroom with little thought and understanding of what is required to get it up and running and the subsequent maintenance of it. It is vital, even at the start, to see that the design of the cleanroom, with regard to the contamination controls required specifically for the product that is to be manufactured within it, is exactly right. Too often not enough time and thought are put into this initial phase with serious consequences with regard to cost of modifying or redesigning the facility and having protocols that cannot be effectively implemented. Validation of the cleanroom takes time, patience, and understanding to complete but is vital to get right the first time. This will ensure that the cleanroom is controlled from the start, that the process within the room is not posing a risk to the environment, that personnel working within the cleanroom are following protocol, and that products manufactured or processes performed within it will be consistently and reproducibly safe and of the highest quality. The two main parts to consider when performing a cleanroom validation are certification including classification and measuring microbial contamination levels. Once validation has been completed, a comprehensive plan should be established for the routine monitoring and maintenance of the cleanroom. This chapter discusses designing, validating, and monitoring of cleanrooms.

Cleanroom Design

A large number of new clients contact the author when they have their cleanrooms built to find out that they may not need a cleanroom of such a high class, that the support rooms may be too small for the number of people going through, that they have no place for waste or finished products to leave the cleanroom except through the gowning room, that they have no material transfer room included, that

they are unsure of what kind of services will be required in the cleanroom that will lead to further drilling and construction at a future date, and so on. In other words, they have a cleanroom built that will now not service the requirements of their current product. Making changes at this stage can become very costly and inconvenient in comparison to building and designing it correctly in the first place.

The process itself should be the main driver of the design of the cleanroom. The process should dictate the level of contamination control required, and hence the class of the cleanroom, not the industry (Hansz 2008). Unlike many facilities, a cleanroom must be designed from the inside out. All efforts to control contamination within a cleanroom are directed at people; therefore the flow and movement of people within the cleanroom must be taken into consideration during the design. In the author's opinion, this is one of the most neglected aspects of cleanroom design. The process within the cleanroom should have a layout that minimizes the movement of personnel as much as possible.

Supporting rooms are vital for a cleanroom to work effectively; i.e., a spacious gowning room is required for cleanroom personnel to enter and leave concurrently and gown without difficulty; the gowning room must allow enough space to store all required apparel. A material transfer room or hatch that incorporates a wipe down area is fundamental. All materials, paperwork, and equipment should enter the cleanroom through the material transfer room or hatch after receiving a thorough clean down. A separate area or transfer hatch that allows the transport of finished products out of the cleanroom may also be incorporated into the design. One of the most forgotten areas during the design stage is how waste is removed from the cleanroom. If no facility has been put in place during the design to allow waste to leave the cleanroom area, more often than not, it is removed through the gowning room. This undesirable practice in turn will affect the process flow and can have serious implications on contamination levels within the gowning area.

The greater the considerations as to the exact contamination controls that are built into the cleanroom design, the more appropriate the specific cleanroom facility design will be for the manufacturing process and operations in question. The appropriate level of quality should be designed and constructed into the facility and systems that support the production process. The development of the cleanroom design, based on the contamination controls required for the process, should necessitate the development of the operational protocols, the first one being the cleanroom validation protocol.

Principles of Cleanroom Validation and Testing

In order to show that the cleanroom environment is in control, it is necessary to demonstrate that the air supplied to the cleanroom is of sufficient quantity to dilute or remove the contamination generated within the cleanroom and that the air supplied to the cleanroom is of a quality that will not add significantly to the contamination levels within the cleanroom. It is important that the air moves in the correct direction from clean to less clean areas and that the air movement within the cleanroom demonstrates that there are no areas within the room with high concentrations of contamination (Whyte 2010). It also important to demonstrate, from a microbial perspective, that operations and manufacturing can be performed within an environment that meets its microbial contamination criteria.

Cleanroom Validation

Within highly regulated environments such as medical device and pharmaceutical industries, there is a requirement to provide an appropriate amount of assurance that critical processes can be performed within controlled conditions in order to produce a final product that is of eminent quality, reliable, and safe for the end user.

Despite popular assumptions, cleanroom validation is, unfortunately, more than just counting particles. It encompasses many different tests that have to be carried out in various cleanroom states in order to show that the cleanroom is fit for its intended use and that the cleanroom meets the required classification.

TABLE 6.1 Different Phases and Occupancy States of Testing Performed during a Cleanroom Validation

Testing Phase Terms	Occupancy State	Test Results
As built testing—phase 1, installation qualification, post-build approval	Testing is performed where the installation is complete with all services connected and functioning but with no production equipment, materials, or personnel present	Proves that the environment was correctly installed and meets its intended design specification
At rest testing—phase 2, operational qualification	Testing is performed when the equipment is installed and operating in a manner agreed upon by the customer and supplier, but with no personnel present	Proves that all parts of the installation operate together to achieve the required conditions. This information can be used as baseline data indicating normal conditions of the cleanroom environment before production commences
Operational testing—phase 3, performance qualification	Testing is performed when the installation is complete and functioning in the specified manner, with the specified number of personnel present and working in the manner agreed upon	Proves that the completed installation achieves the required operational performance with the specified process and a maximum number of personnel working within the environment

Source: Data taken from IS EN ISO 14644-1 classification of air cleanliness.

There are an assortment of standards and guidance documents available to help with this, such as IEST CC006.3 Testing Cleanrooms, IEST CC001.4 HEPA and ULPA Filters, IEST CC012.1, *Considerations in Cleanroom Design*, and the *European Union Guide to Good Manufacturing Practices* (EU GGMP).

However, IS EN ISO 14644 is the prime standard adhered to for validation of cleanroom environments. This standard does not consider specific processes within the cleanroom. Rather, it provides constructional guidance for the start-up and qualification of the cleanroom environment detailing the basic elements needed to ensure continued satisfactory operation and maintenance.

According to the IS EN ISO 14644-2 standard, every time a cleanroom is put into operation initially or changes its intended use, a validation must be performed. The initial setup of a cleanroom requires a validation to be performed over a specified period of time to ensure that the cleanroom is functioning as required over the given period of time. Over this period of time, historical data are collected to ensure that the cleanroom is performing effectively. Changes made to an existing cleanroom require an assessment of how the changes will affect the cleanroom as this in turn will decide how extensive the revalidation will be. Either way, this testing certifies that the cleanroom environment meets the stated standards, protocols, and design criteria.

As mentioned, validation testing is performed when the cleanroom is in different phases or occupancy states. The various terms used for the phases or occupancy states are detailed in Table 6.1.

Therefore, when a cleanroom is certified to a specific class, the room performs to a standard that meets or exceeds the performance of that class under a specific occupancy status.

So How Does One Determine What Tests Need to Be Performed during a Cleanroom Validation?

The majority of companies get an independent testing and certification body to outline an appropriate testing program and perform the required testing. IS EN ISO 14644-1 determines the type and frequency of testing required to conform to the standard. Some tests are mandatory and some are optional. A typical testing program will include the following tests:

Airflow Volume and Velocity Tests

The more clean air supplied to the cleanroom, the cleaner the room will be. A cleanroom must have sufficient clean air supplied to dilute or remove any airborne contamination that may be present. This air

supply to the cleanroom is often reported as air changes per hour. Air change rates within a cleanroom will usually be equal to and above 20/h; however, this measurement should be based on the level of contamination control required within the cleanroom, the number of people present or the level of activity within the room, the size of the room, and the process itself. In unidirectional cleanrooms, air supply velocity is measured. Measuring of the airflow is applicable in all three of the designated occupancy states.

The FDA recommends a velocity of 0.45 m/s ±20% and the EU GGMP suggests a range of 0.36–0.54 m/s. These are guidance values. The air supply volumes and velocities should be decided at the design stage.

HEPA/ULPA Filter Installation Leak Testing

High-efficiency air filters that have been installed within the cleanroom need to be checked to ensure that they are efficiently removing particles from the supply air and will allow an air supply of high quality to enter the cleanroom. Filter systems, therefore, must be tested to ensure there are no leaks or faults in the filters themselves or in their housings.

Air Movement Visualization

Visual airflow patterns are a function of airflow velocity, airflow direction, room design, HVAC layout, air change rates, and equipment layout (Cleanzone Technology). Demonstrating the air movement within the critical areas of the cleanroom, in particular with unidirectional airflow, is important to ensure that airborne contamination has been swept away from personnel and the product, thus minimizing the risk to the finished product.

The FDA Guidance for Industry suggests that

> Proper design and control prevents turbulence and stagnant air in the critical area. Once relevant parameters are established, it is crucial that airflow patterns be evaluated for turbulence or eddy currents that can act as a channel or reservoir for air contaminants. In situ air pattern analysis should be conducted at the critical area to demonstrate unidirectional airflow and sweeping action over and away from the product under dynamic conditions. The studies should be well documented with written conclusions and include evaluation of the impact of aseptic manipulations and equipment design. Videotape or other recording mechanisms have been found to be useful aides in assessing airflow initially as well as facilitating evaluation of subsequent equipment configuration changes.

Airflow patterns can also be observed in turbulently ventilated cleanrooms demonstrating that the air is well mixed within the cleanroom.

Room Recovery

Room recovery tests are generally performed in the "as built" or "at rest" state but can be performed in the "in operation" state also. This test demonstrates the rate at which an area within the cleanroom will recover if it becomes contaminated. It should be noted, however, that if contamination does occur within areas of the cleanroom, production/manufacturing should not recommence after the recovery rate time has lapsed without double checking that particle counts and pressures in the area are within the specification and that the area is clean.

Room Pressurization

Cleanrooms are generally positively pressurized; i.e., there is a higher volume of air entering the cleanroom than the volume extracted. This leads to a buildup of pressure within the cleanroom. The highest pressure should be in the cleanest required area or the room that contains the most critical process. When an opening occurs within the room, there is an outward flow of air from the cleanest area to a less clean area and so on until it reaches the external environment. A pressure differential then exists between the cleanest area and the less clean area minimizing the risk of contaminated air or air from a lower-class area flowing back into the cleanroom.

The IS EN ISO 14644-4 details pressure differentials between adjacent clean zones of different cleanliness levels to lie within the range of 5–20 Pa. The FDA guideline suggests that a positive pressure differential of at least 10–15 Pa should be maintained between adjacent rooms of differing classification.

This differential air pressure test demonstrates that the pressure differences between areas are acceptable and that the air is flowing in the correct direction. This test is applicable in each of the three occupancy states.

Once these tests have been completed with acceptable results, then the concentration of particles and levels of bacterial contamination should be measured to ensure they meet the required standards and protocols.

Airborne Particle Count Test

The airborne particle count test verifies that the cleanroom, personnel, equipment, and process is performing to the intended classification or the clean level. The classification level of the cleanroom is based on the number of particulates present per cubic meter. This particulate level will change dramatically from when the room is first built compared to when the room is in full production due to increased activity from equipment and personnel which are all dispersing particles.

Before testing for the level of particles, a few points to consider are as follows:

Particle Size

The particle size to be measured within the cleanroom should be considered.

Sampling Locations

The minimum number of sample locations taken within the cleanroom is calculated by taking the square root of the area of the cleanroom in square meters. This number should be rounded up to the nearest whole number (IS EN ISO 14644-1).

Air Sampling Volume

A calculation is documented in IS EN ISO 14644-1 for determining the minimum sampling volume to collect at each sampling location.

Statistical Analysis

When the number of locations sampled is more than 1 and less than 10, the mean, standard deviation, and 95% upper confidence limit (UCL) from the average particle concentrations for all locations within the cleanroom must be calculated for each particle size. Calculating the 95% UCL is often found to be problematic. The calculation is avoided by sampling more than 9 locations in the cleanroom or using a particle counter that will automatically display the calculation. It is important to note that samples should be taken within the same time period, i.e., on a given day as opposed to over a week or a month.

If there are ≥10 sampling locations or < 2 sampling locations, no statistical analysis is required. In this case the average of the sampling locations for each particle size is calculated. If only one sampling location is required, a minimum of three single sample volumes at that location is required and the average of the sampling locations for each particle size calculated.

Acceptance Criteria

According to IS EN ISO 14644-1, the cleanroom has met its classification if the average particle concentration at each location or the 95% UCL is below the specified limit. The acceptable particle limits for each classification as detailed in IS EN ISO 14644-1 are shown in Table 6.2.

Cleanroom Classification within Pharmaceutical Facilities

The prime standard adhered to by pharmaceutical industries is the EU GGMP, even though IS EN ISO 14644 still applies for the cleanroom certification. Annexure 1 of the EU GGMP was updated in 2008 to

TABLE 6.2 Selected Airborne Particulate Cleanliness Classes for Cleanrooms and Clean Zones

ISO Classification Number (N)	Maximum Concentration Limits (Particles/m³ of Air) for Particles Equal to and Larger Than the Considered Sizes Shown Below					
	0.1 (μm)	0.2 (μm)	0.3 (μm)	0.5 (μm)	1 (μm)	5 (μm)
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1,000	237	102	35	8	
ISO Class 4	10,000	2,370	1,020	352	83	
ISO Class 5	100,000	23,700	10,200	3,520	832	29
ISO Class 6	1,000,000	237,000	102,000	35,200	8,320	293
ISO Class 7				352,000	83,200	2,930
ISO Class 8				3,520,000	832,000	29,300
ISO Class 9				35,200,000	8,320,000	293,000

Source: IS EN ISO 14644-1: Classification of air cleanliness.

Note: Uncertainties related to the measurement process require that concentration data with no more than three significant figures be used in determining the classification level.

TABLE 6.3 Particle Classifications per Grade

	Maximum Permitted Number of Particles/m³ Equal to or Greater Than the Size Tabulated			
	At Rest		In Operation	
Grade	0.5 μm	5.0 μm	0.5 μm	5.0 μm
A	3,520	20	3,520	20
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Not defined	Not defined

Source: EU guidelines to good manufacturing practices: medicinal products for human and veterinary use. Annex 1 (2008). European Commission's Health and Consumers Directorate-General, © European Communities, 2003.

include a modified IS EN ISO 14644 standard that addresses sterile medicinal products. A table of cleanroom certification values that roughly translate into the IS EN ISO 14644 standard was defined (Table 6.3).

The sample volumes/location should not be less than 1 m³ for Grade A and B areas and is preferable in Grade C areas also. It is advised that a minimum length of sample tubing be used due to the potential loss of large-sized particles (5.0 μm) in the sample tubing.

It should be noted that classification results for sterile medicinal products are very different from those used for operational environmental monitoring and these should be clearly differentiated. A comprehensive report detailing the certification testing and classification of the cleanroom should be documented and supplied by the testing company. A continuous operational environmental monitoring plan should be defined based on the class of the cleanroom and the nature and risks of the product manufactured. Records should be kept of all continuous monitoring results separate to the certification report.

Additional Tests

Additional tests such as measuring temperature, relative humidity, sound, lighting, and vibration levels may be required depending on the process within the cleanroom.

Microbial concentrations within the cleanroom will be ascertained when all the above tests are satisfactory.

Microbial Validation Testing

Even when the cleanroom has been classified and certified that it is working within the required acceptance criteria, a microbial validation, particularly for medical, pharmaceutical, and related applications, should still be performed.

As part of the cleanroom performance validation, the level of microbial contamination should also be measured at the "in operation" state in order to demonstrate compliance and, in some cases, gain historical data to establish alert and action limits. Microbial testing should be performed when there is maximum activity within the cleanroom and when the maximum number of personnel is present. A sampling map detailing types and locations of sampling should be prepared. Depending on the class cleanroom, microbial acceptance criteria may be dictated by the standards or by a process risk assessment. Either way, microbial contamination should be monitored initially to gain an insight into the levels of microbial contamination present within the cleanroom during operations and to demonstrate compliance. The establishment of a comprehensive environmental monitoring program will be discussed further in this chapter.

Validation Report

A complete cleanroom validation report should comprise two parts.

Part 1 is the certification and classification report that is often obtained from an external contractor. This report should detail the occupancy states at which the tests were performed, the acceptance criteria, and the results of tests performed such as air changes/h or air velocity, filter leak tests, airflow visualization, and particle counts. All calculations performed and raw data should be detailed where possible.

Part 2 should take into consideration the microbiological contamination levels within the cleanroom under both static (unmanned) and dynamic (manned) conditions. Alongside the viable monitoring, nonviable particulate levels and pressure differentials should also be measured within the same time frame as these critical parameters can influence the contamination levels of the cleanroom.

The testing performed within each area of the cleanroom, the testing conditions, i.e., static or dynamic conditions, test methods for each test type, sampling map/plan clearly showing the sampling points within the cleanroom, acceptance criteria to be met, and calibration certificates for all equipment required should be attached as part of the final validation report. The cleanroom is now fully validated and will be governed by a routine sampling program to check that it is performing to the standard required with regard to both nonviable and viable contamination levels.

Validation versus Monitoring

Cleanroom certification and validation as discussed previously, involves checking the room for various criteria to ensure that it is built to a specific set of requirements. However, after the room has been certified and validated, it must be monitored periodically, relative to risk, to prove that a clean manufacturing environment can be maintained throughout its life. Monitoring of the cleanroom is important to show that the cleanroom is performing satisfactorily under dynamic conditions, i.e., that all aspects of the construction and supporting equipment are fully operational and performing at the same level as when the room was certified, that the process within the room is not posing a risk to the environment, and that personnel working within the cleanroom are following protocol.

As discussed in the section "Microbial Validation Testing", a comprehensive environmental monitoring program should be in place for the routine monitoring of the cleanroom environment and supporting areas. This program should include the monitoring of airborne viable and nonviable particulates, pressure differentials, as well as surface microbial contaminants on equipment, product contact surfaces, walls and floors, and personnel.

TABLE 6.4 Required and Optional Testing Requirements

Schedule of Tests to Demonstrate Continuing Compliance			
Test Parameter	Class	Maximum Time Interval (months)	Reference Test Procedure
Particle counts Test	≤ISO Class 5	6	IS EN ISO 14644-1
	>ISO Class 5	12	
Pressure differentials	All classes	12	IS EN ISO 14644-3 Annex B.5
Airflow volume or airflow velocity	All classes	12	IS EN ISO 14644-3 Annex B.4
Schedule of Additional and Optional Tests			
Installed filter leak test	All classes	24	IS EN ISO 14644-3 Annex B.6
Containment leakage	All classes	24	IS EN ISO 14644-3 Annex B.14
Recovery	All classes	24	IS EN ISO 14644-3 Annex B.13
Airflow visualization	All classes	24	IS EN ISO 14644-3 Annex B.7

Source: IS EN ISO 14644-2: Specifications for testing and monitoring to prove continued compliance with ISO 14644-1.

Routine Particulate Monitoring

Table 6.4 illustrates the required and optional tests and the maximum time interval for monitoring to demonstrate continuing compliance. Particle concentration testing is the most important analysis that must be carried out to demonstrate that the cleanroom continues to comply with IS EN ISO 14644-1.

In the author's opinion, it is advisable to perform more frequent demonstrations of compliance than those outlined in Table 6.4. If a situation occurs where the cleanroom does not meet its specification or is not in compliance, then the quality of all products, materials manufactured or processes performed within the cleanroom since the last demonstration of compliance are questionable. The more often demonstration of compliance is displayed, the smaller the loss of downtime, product and materials if out-of-specification results are obtained.

All grade cleanroom environments should be routinely monitored while they are in operation. The locations chosen for particulate monitoring should be selected based upon a risk assessment of the area with the certification and classification results in mind.

For Grade A cleanrooms, particle monitoring should be performed for the full duration of critical processing including equipment assembly and setup stages. Exceptions are those justified by contaminants in the process that would damage the particle counter or present a hazard. Examples of exceptions are live organisms or radiological hazards. Grade A cleanrooms should be monitored continuously so that all interventions, transient events, and any system deterioration would be captured and alarms triggered if alert limits are exceeded. It is recommended by the EU GGMP that a similar system be used for Grade B cleanrooms also.

Appropriate alert and action limits should be set relative to the results of particulate and microbiological monitoring. If these limits are exceeded, operating procedures should prescribe corrective action. Monitoring systems must rapidly detect and record any changes that might compromise the cleanroom environment or product/process and alert the relevant personnel of such changes immediately.

Establishing an Environmental Monitoring Program

Before microbial testing is performed, an environmental monitoring plan should be derived. There are many different guidance documents that deal with environmental monitoring, such as the *EU Guide to Good Manufacturing Practice*, Annexure 1, USP 1116, the *FDA Aseptic Processing Guide*, IS EN ISO 14698, but these can be ambiguous and leave a lot of scope for interpretation. There are no commonly

accepted levels of environmental testing probably due to the large and varied number of industries, processes, and subprocesses to which these regulations must apply. The key points of these guidelines should be taken into account, but a commonsense approach is sometimes required for the application to your specific process and facility. In the author's opinion, a good environmental monitoring plan should include the following:

Types of Sampling Performed

The types of sampling that will be performed should be decided. The main sampling types are surface sampling, air sampling, and personnel sampling.

Contact plates are used to sample flat surfaces such as work benches, equipment, floors, and walls. These agar plates have a dome-shaped layer of agar that is pressed gently onto the surface. Organisms on the surface will be transferred onto the agar. This method is considered quantitative as it measures the number of viable organisms on a defined surface. Surfaces that are not flat, smooth, or easy to access can be sampled using a moistened swab. The swabs can be used to inoculate a plate immediately or can be immersed in a diluent before inoculation.

There are two methods for air sampling to choose from: active and passive. Active air sampling is considered to be quantitative as it measures the number of viable organisms in a defined volume of air, i.e., per cubic meter. Several types of active air samplers are available. Passive air sampling is not considered to be quantitative. It is performed by placing an agar plate exposed on the test area for a period of time (no longer than 4 h due to desiccation of the medium that may reduce recovery rates). Viable air sampling gives a snapshot in time of the microbiological status of the cleanroom under dynamic conditions.

Personnel monitoring is important as part of aseptic and GMP training. This type of monitoring is performed by placing some or all pads of the fingers (not the tips of the nails) onto the agar surface. Gloves or finger cots should be changed after this testing and hands washed before recommencing work in the cleanroom. Monitoring of both the garments and hands indicates the level of contamination on these areas before, during, or after working within the cleanroom.

Personnel are one of the greatest sources of contamination within a cleanroom. Poor handwashing can pose a high risk to product or processes within the cleanroom, as hands are used to touch many different materials and surfaces within the cleanroom that could be a potential source of contamination. Therefore, personnel monitoring of hands, gloves or finger cots, and other cleanroom apparel is an obvious tool in assessing contamination risks.

Sampling Locations

The sampling locations within the cleanroom and supporting areas for each sampling type should be decided. A risk assessment of the process in operation within the cleanroom is vital as it will establish the areas within the cleanroom that pose the greatest risk with regard to contamination.

Sampling Map

A map of the cleanroom and supporting rooms should be drawn up detailing the exact sampling locations.

Sampling Frequency

The sampling frequency for each sampling type within the different areas should be decided. In high-class cleanrooms, the frequency of monitoring may be continuous, i.e., during each batch or critical operations. In lower-class cleanrooms, monitoring may take place monthly or quarterly depending on the associated risk.

Interpretation of Results

In some cases, companies do not have their own in-house microbiologist for the environmental monitoring of the cleanrooms. In such instances, personnel who are responsible for this monitoring may

not fully understand the importance of performing appropriate microbial testing, the meaning of the results, and the impact of the results on the final product or process. Personnel looking after microbial testing must be trained, fully understand the testing performed, be able to interpret the results, and know the impact the results may have on the finished product.

Organisms that can sometimes be detected within cleanrooms are *Staphylococcus aureus* as it is a commensal of the human skin and therefore can enter the cleanroom via people. Bacillus species are spore-forming bacteria that can be very difficult to eradicate from the cleanroom once they enter. This organism can enter cleanrooms on materials/items transferred into the cleanroom that have not been cleaned effectively or by torn shoe covers. Depending on the quantity of spore-forming bacteria present in the cleanroom, this could seriously compromise the sterility assurance level of the end product or process and could shut down production within facilities. Other organisms such as gram negative bacteria, for example, *Pseudomonas* spp., *Escherichia coli* spp., and *Salmonella* spp., must under no circumstances enter the cleanroom environment as they produce endotoxins that cannot be removed by sterilization techniques. Such organisms could very seriously compromise the health of a patient leading to even fatality. The biggest source of these organisms is water. Figure 6.1 shows *Pseudomonas aeruginosa* bacteria growing on a surface.

There seems to be an attitude present in some lower-class cleanrooms that products going for sterilization will ultimately be free from all contamination and that the sterilization process will kill "everything." Be assured that this is not the case at all. Sterilization, as discussed, will not inactivate or eradicate endotoxins that can be fatal to the end user.

Also, if a product is not clean before it goes to the sterilization process, then it can still have a negative impact on the host. The host is not sterile, so any contaminants such as oils or residues on the product can provide "food" for the host bacteria leading to toxicity or infection of the host. Contaminants can interact with the host immune system and can interfere with device interaction within the host. It is, therefore, important to physically and effectively remove undesirable surface material from these products.

With sterilization or disinfection, the issue is rendering the contaminant nonviable. With cleaning, the issue is physically removing contaminants from the surface without changing that surface in a way that interferes with product performance (Broad and Kanegsberg 2007).

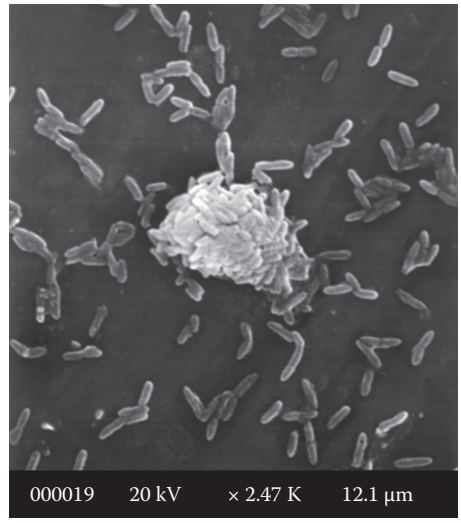

FIGURE 6.1 *Pseudomonas aeruginosa* biofilm forming on a surface. (From O'Donoghue, K., Physical and biological characterisation of biofilms and bioaerosols, PhD thesis, National University of Ireland, Galway, 2004.)

Establishing Alert and Action Limits

A method for establishing alert and action limits should be detailed. Cleanrooms that are in compliance with the *EU Guide to Good Manufacturing Practice*, Annexure 1, have recommended limits stated for microbial contamination within a Grade A to Grade D cleanroom (reference Table 6.5). However, cleanrooms that do not adhere to Annexure 1 have very little guidance as to the microbial levels that should be met.

In this case, microbial acceptance criteria must be obtained based on the requirements and contamination risk factors associated with the process. From the author's experience working with different cleanroom industries, there are two ways this information can be obtained. The first method is by monitoring microbial contamination levels over a period of consecutive days in the dynamic/occupied state. The second method is by monitoring microbial contamination levels routinely over a period of months. Data gathered from both these methods can be used to establish temporary alert and action limits until further historical data have been obtained to set more permanent limits. Where possible, limits should be as strict as those in the standard.

Some cleanrooms will monitor the microbial contamination levels in the static state first in order to establish a baseline status showing the contamination levels in the room without people or the process in operation.

When validating the microbial contamination levels, whether to observe if they are in compliance with the standard or to establish historical data, other parameters should be monitored at the same time. For example, particulate levels and pressures should also be taken into consideration at the time of microbial monitoring as these may have a direct impact on the level of contamination within the room. For example, particulate levels that are over the action limit and a decrease in positive pressure could be an underlying reason for an increase in contamination levels.

Actions to Be Taken for Out-of-Spec Results

Comprehensive procedures should be in place detailing actions to be taken if results are over the alert or action limits. Typical microflora should be identified in order to determine the risk posed by the out-of-spec result and help identify the root cause. In the author's opinion, if results are above alert and action levels, colony morphology and colony identification should be performed, respectively. The data obtained from both counts and organism identification lead to a greater overall understanding of the microbial presence within the cleanroom environment and can be used to identify high-risk areas as well as to eliminate potential routes and sources of contamination.

Trending and Documenting of Results

The aim of an environmental monitoring program is to detect any changes in the numbers and types of organisms present within the cleanroom outside of the "norm." Results should be trended on a continuous basis highlighting any results that are over alert or action limits. A library of organisms that

TABLE 6.5 Recommended Limits for Microbial Contamination

Grade	Air Sample (cfu/m^3)	Settle Plates Diameter 90 mm (cfu/4 h)	Contact Plates Diameter 55 mm (cfu/Plate)	Glove Print Five Fingers (cfu/Glove)
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	—
D	200	100	50	—

Source: EU guidelines to good manufacturing practices: medicinal products for human and veterinary use. Annex 1 (2008). European Commission's Health and Consumers Directorate-General, © European Communities, 2003.

Note: These are average values and settle plates may be exposed for less than 4 h.

have been identified within the cleanroom should be established and maintained. This library is a good source of information with regard to the contamination found within the cleanroom. Such a library increases awareness of the types of organisms entering the cleanroom, the preventive action that can be implemented to reduce these organisms from entering, or decreasing the quantities of these types of organisms present, and understanding the impacts of this type of contamination in the environment and on the end product.

It is worth spending the time defining a good environmental monitoring program. Microbiological results and trends provide vital information on the quality of the cleanroom environment, minimize the risk of release of a potentially contaminated product or process, and prevent future contamination by detecting adverse trends. It also serves as a reminder to cleanroom personnel of the importance of their actions, behavior, and good manufacturing practices within the cleanroom environment.

Often, microbiology testing is observed as a technically specific task that is not understood by personnel trained in other departments. Therefore, it is often not seen as a priority. This testing is paramount for all products entering the human body as well as for assessing how the cleanroom environment itself is performing.

It is important that the seriousness of microbial testing is understood and that the results of iterative communication with experts in this area is received by pertinent technicians and management. It is critical to interpret the results obtained correctly and understand the impacts if the results do not meet those expected.

Summary

Cleanrooms are costly to build and maintain. Therefore, they must be customized to produce or manufacture a specific product/service. Cleanrooms are used primarily for the production of products that are subject to special requirements that have been established to minimize risks of particulate, microbial, or pyrogen contamination. Quality assurance is imperative and this type of manufacture must strictly follow carefully established and validated methods of preparation and procedure within an environment that has itself met the appropriate validation criteria. Sole reliance on terminal sterility should not be taken for granted.

When building or extending a cleanroom, it is paramount to spend time on the design to get it right from the beginning and ensure all aspects of the cleanroom are addressed. A poorly designed cleanroom will lead to increased costs as many changes will be required to customize the cleanroom to the process or bring the cleanroom up to specification. The most common mistake personnel make is starting the cleanroom validation process without the appropriate knowledge and expectations. This can result in further associated costs, an increase in timelines, a cleanroom that may not be working to its required classification, and a higher degree of required maintenance.

Thorough procedures and policies are required for the routine monitoring of contamination levels within the cleanroom. These procedures require strict adherence. In order to achieve a high-standard cleanroom with regard to controlling contamination on a day-to-day basis, all personnel, regardless of frequency of entry or job classification, need to be given a basic understanding and fundamental awareness of contamination control. Personnel must understand how contamination can enter the cleanroom through the specific processes in place within the facility, how in-house processes can be improved, and how contamination can be reduced to a minimal level within the facility. Personnel must gain an understanding of why there are such strict controls in place within this working environment as opposed to any other working environment. This understanding and awareness can sometimes be difficult to achieve because within cleanrooms we are dealing with particles and organisms that we cannot see. Very often, the saying "out of sight, out of mind" comes into play. From the author's experience in this field, the key to controlling a large amount of contamination from poor personnel activities and practices is to develop knowledgeable, aware, and empowered employees.

Consistent, quality education to achieve this understanding and awareness, along with support and commitment from management down through all levels of staff can yield many benefits including a substantial decrease in product rejects, a corresponding increase in profitability, and an output of high-quality products/services.

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