Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 5 Issue 2 February 2022

Review Article

Importance of Environmental Monitoring in Clean Room

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Received: December 31, 2021

Published: January 21, 2022

Abstract

The aseptic area or clean room in pharmaceutical industries is where sterile products are produced. Where the final sterile product is not subjected to terminal sterilization. For this sterile product to meet the requirements of sterility and absence of pyrogenics, the environment must be controlled, meet cleaning standards and undergo qualification. For this, Normative Instruction Number 35 of August 21, 2019 and ISO 14644-1 must be followed. In this case, for a safe production with regard to contamination by microorganisms, it is necessary to carry out environmental monitoring. These areas are called Clean Rooms and are classified into classes 5, 6, 7 and 8. Each degree has a need for environmental monitoring in addition to having control of air flow, pressure, temperature, humidity, noise, vibration and lighting.

Keywords: Clean Room; Steriles; Microorganisms; Environmental Monitoring

Introduction

For the manufacture of sterile products in the pharmaceutical industry it is necessary that the work environment receives due care. According to ISO 14644-1:2019 [1] the definition of a clean room is a room in which the concentration of airborne particles is controlled, and which is constructed and used in such a way as to minimize the introduction, generation and retention of particles within the living room. This shows us that this environment needs to be controlled and follow the strict specific parameters of current legislation.

ISO 14644-1:2019 [1] provides the proper specifications that the clean area must have for the production of sterile products. The prevention of microbiological contamination, microbial cross-contamination or any other source, is an important essential consideration to be made when designing a project to build an HVAC system. Important parameters related to HVAC systems that can affect the quality of pharmaceutical products during the production or storage stages, such as temperature, humidity, pressure differentials and air renewal and cleaning, they must be properly designed, controlled and monitored. These requirements need to be frequently monitored to ensure the quality of the aseptic process. This monitoring is done through sampling of viable particles, microbiological environmental monitoring of the area and the operators involved in the process, in addition to the certification of operators and areas.

According to the American Pharmacopoeia (USP 44/NF 39) [2] the terms aseptic and sterile are not synonymous. Sterile means the complete absence of viable microorganisms or organisms with the potential to reproduce. In the purest microbiological sense, an aseptic process is one that avoids contamination by excluding microorganisms.

Taking into account all this information from the legislation highlighted in this article, the project (wall, doors, antechambers, air treatment units, pressure) and viable and non-feasible environmental monitoring need to strictly follow GMP minimum requirements in addition to ISO 14644- 1:2019 [1] and Normative Instruction No. 35 (ANVISA 2019) [3] in order to meet the specification of the sterile product.

Microbial environmental monitoring in classified areas

Microbiological contamination in today's aseptic processing, be it an insulator, RABS or conventional cleanroom, has become increasingly rare. However, a monitoring program must be able to detect a change in the validated control state in a facility and provide information for implementing appropriate countermeasures.

Clean rooms are classified, according to table 1 below, by the ABNT ISO 14644-1:2019 [1] standard according to the number and size of particles allowed by air volume, in addition to defining all structures (floor, wall, ceiling, antechambers, doors), air treatment systems, services and utilities. In Article 4 of NORMATIVE INSTRUCTION Number 35 of ANVISA, it also informs that clean areas must be kept in an appropriate standard of cleaning and receive air that has passed through filters of appropriate efficiency in order to meet and maintain the microbiological conditions favorable to aseptic processing and meet the product's sterility and apyrogen specification.

Clean rooms must maintain particulate air through the use of a HEPA or ULPA filter, so the airflow system directs filtered air downward in a constant, unidirectional flow.

ISO 14644-1 [1] stipulates the total allowable particle count for a clean environment to meet defined air quality ratings.

ISO Class	Particles ≥ 0,5 mm/m^3
ISO 5	3520
ISO 6	35,200
ISO 7	352,000
ISO 8	3.520,000

Table 1: Airborne Total Particulate Cleanliness Classes. Author: USP 44/NF 39 [2].

For a Class 5 environment with operators the airflow should be predominantly unidirectional (vertical or horizontal), particularly when products, product containers and closures are exposed. In addition to having an environmental monitoring of viable and non-viable particles. Qualification of these areas, an assessment of air movement in a clean room, visually studying the airflow through smoke studies or other suitable means is necessary to ensure that the airflow is correct.

Per American Pharmacopoeia USP 44/NF 39 [2] careful observation and cleanroom mapping during the qualification phase can provide useful information about personnel movement and placement. This observation can also provide important information about the most frequently performed manipulations and interventions.

According to the 6th edition of the Brazilian Pharmacopoeia [4], when an aseptic process is developed and installed, it is necessary to qualify the microbiological state of the process, performing at least three consecutive average fills. The problems in the development of the medium fill program to be considered include medium filling procedures; medium selection; filling volume; incubation time and temperature; inspection of filled units; interpretation of results and possible corrective actions required.

The air treatment system used in the pharmaceutical industry is divided into external air intake, air treatment unit (ATU), insufflation in the clean area, air recirculation and the treatment of the air that will be discarded.

The pressure differential is an important parameter to maintain the different degrees of cleanliness between the cleaned areas. In clean areas where the product is bottled, pressures must be positive in relation to adjacent areas that have a lower degree of cleanliness. In this way, it is possible to control the entry of contaminants.

Per American Pharmacopoeia USP 44/NF 39 [2] aseptic processing environments are much more critical in terms of patient risk than controlled environments used for other manufacturing operations. Therefore, the importance of an entire control through environmental monitoring of viable and non-viable, certification of operators and qualification of areas.

In this case, Normative Instruction Number 35 (Anvisa 2019) [3] and American Pharmacopoeia USP 44/NF 39 [2] recommend the air and surface specifications for each class of the clean area. according to table 2 below.

Environmental monitoring of non-viable particles is of paramount importance as they are considered vehicles for viable particles. Since microorganisms are often associated with 10 to 20 µm particles. Therefore, the monitoring of these particles aims to evaluate the efficiency of the filtration and air exchange system in the clean area.

Room Classification	Active Air Sample (%)	Settle Plate (9 cm) 4 h Exposure (%)	Contact Plate or Swab (%)	Glove or Garment (%)
Isolator/Closed RABS (ISO 5 or better)	<0,1	<0,1	<0,1	<0,1
ISO 5	<1	<1	<1	<1
ISO 6	<3	<3	<3	<3
ISO 7	<5	<5	<5	<5
ISO 8	<10	<10	<10	<10

Table 2: Suggested Initial Contamination Recovery Rates in Aseptic Environments.

All operators are aseptically gowned in these environments (with the exception of background environments for isolators). These recommendations do not apply to production areas for nonsterile products or other classified environments in which fully aseptic gowns are not donned.

Author: USP 44/NF 39 [2].

The objective of the microbial monitoring program is to assess the effectiveness of cleaning and sanitizing practices of areas and personnel that could have an impact on the biological load. The American Pharmacopoeia USP 44/NF 39 [2] tells us that microbiological monitoring of a cleanroom is technically a semi-quantitative exercise, as a truly quantitative assessment of the environment is not possible, given the limitations of the sampling equipment.

Environmental monitoring is unable to provide us with direct quantitative information due to the lack of accuracy of enumeration methods regarding the restricted sample volumes that can be effectively analyzed.

In fact, routine environmental monitoring provides enough information to demonstrate that the environment is operating in an adequate state, in addition to detecting changes in the control of the environment if the rate of recovery of the contamination of microorganisms increases significantly.

For more effective environmental monitoring, it is important to determine the points through the layout, materials, equipment, people present, in addition to a risk analysis. This assessment of risks associated with manufacturing environments should be done over a significant period of time. And contamination recovery rates should be used to track ongoing performance and refine the microbiological control program and thereby drive improvement. Because only in this way will ideal operating conditions be achieved and contamination recovery rate levels will become relatively stable within a normal range of variability.

For real monitoring sampling must take place when materials are in the area, processing activities are in progress and a full complement of personnel is working in the aseptic processing environment.

Operators involved in the aseptic process need to receive adequate training before starting activities in these areas as they play an essential role in the control of contamination. Proper training, discipline, and supervision are critical to contamination control. This training is equally important for personnel responsible for the microbial environmental monitoring program because contamination of the clean work area can inadvertently occur during microbial sampling. This training should include instruction in the basic principles of aseptic technique and emphasize the relationship of manufacturing and handling procedures to potential sources of product contamination. In addition to being formalized and documented for all personnel who have access to the clean area.

Microbial contamination in an environment where there are operators at some level is unavoidable. As such an expectation of zero contamination during all aseptic processing operations is impossible. Even the most careful design and operation of the cleanroom environment will not eliminate the release of microorganisms if human operators are present.

Interventions must always be minimized, to avoid possible contamination, including those necessary for monitoring activities, but if necessary, they must be conducted with aseptic technique that approaches perfection as much as possible.

To enter and operate within a clean room or even carry out environmental monitoring in addition to training, it is necessary to be in adequate health conditions; sick people cannot have access to clean area. The American Pharmacopoeia USP 44/NF 39 [2] tells us that the only significant sources of microbial contamination in aseptic environments are people. As operators disperse contamination and the ultimate goal of aseptic processing is to reduce end-user risk, only healthy individuals should have access to controlled environments. Sick individuals should not be allowed to enter an aseptic processing environment, even one that employs advanced aseptic technologies such as isolators, blow/fill/seal or closed RABS. In general, fewer personnel involved in aseptic processing and monitoring, along with a reduction in interventions, reduces the risk of microbial contamination.

For conventional clean areas it is also necessary to wear a suitable uniform,as no part of the body can be exposed. Thus, training in dress and certification of the operators involved is necessary.

The frequency of environmental monitoring must follow the recommendation of American Pharmacopoeia USP 44/NF 39 [2] in table 3 below.

Sampling Area/Location	Frequency of Sampling
Clean room/RABS	
Critical zone (ISO 5 or better)	
Active air sampling	Each operational shift
Surface monitoring	At the end of the operation
Aseptic area adjacent critical zone	
All sampling	Each operational shift
Other nonadjacent aseptic areas	
All sampling	Once per day
Isolators	
Critical zone (ISO 5 or better)	
Active air sampling	Once per day
Surface monitoring	At the end of the campaign
Non aseptic areas surrounding the isolator	
All sampling	Once per month

Table 3: Suggested Frequency of Sampling for Aseptic Processing Areas.
Author: USP 44/NF 39 [2].

Walls and floors should be sampled as sampling in these locations can provide information about the effectiveness of the sanitation program. However, this sampling can be done less frequently and with the operators; in accordance with good conduct practices in these areas, they must never touch the floor and walls, and there must not be mechanical transmission of contamination from these surfaces to critical areas where the product is exposed. However according to American Pharmacopoeia USP 44/NF 39 [2] it has been found that surface monitoring recovers <50% even when used with relatively high inoculum levels in standardized coupons. In real production environments, where organisms are stressed to varying degrees, recovery rates may be even lower.

It is noteworthy that in this way a critical eye must be taken on environmental monitoring in general, so when contamination recovery rates increase from an established norm, an operational and process investigation must be carried out. As investigations need to be different depending on the type and processing of the product manufactured in the clean room, RABS or insulator.

The investigation needs to include a review of area maintenance documentation, reviewing area sanitation documentation, whether there has been an occurrence of different than expected events in the area, changes in ambient temperature and relative humidity, and the training status of personnel. After a thorough investigation, actions must be taken to correct or eliminate the most likely causes of contamination.

Often due to the relative rarity of contamination events in modern facilities, investigations prove inconclusive. When corrective actions are taken, they may include reinforcement of personnel training to emphasize acceptable clothing and aseptic techniques and microbial control of the environment, additional sanitization or with a different sanitizing agent, and identification of the microorganism to know its possible origin. This entire course of investigation, action and conclusion must be documented.

For an assessment of environmental monitoring, it is necessary to take a critical look at the whole as the growth and recovery of microorganisms are not exact. In this way they can account for the frequency with which contamination is detected, rather than the absolute numbers of CFU detected in a single sample. Since a CFU is not a direct enumeration of the microorganisms present, but rather a measure of contamination that may have originated from a cluster of organisms.

Conclusion

When there is contamination in multiple locations in an environment within a single sampling period it may indicate an increased risk for the product or even an inadequate sampling technique, therefore a more critical assessment must be carried out, a careful review of this environmental monitoring before any conclusion of the investigation.

American Pharmacopoeia USP 44/NF 39 [2] brings us a typical range of 1-5 CFU but it is important that during an investigation these recoveries are used and even identified. Because if a high number is recovered during an environmentalmonitoring, the growth rates must be reviewed for at least two weeks before the incident.

Final Considerations

An environmental monitoring program alone does not prove the absence of microbial contamination, even when there is no viable recovery. This just shows us that the growth has not been discovered and not that the environment is free from contamination. The real value of a microbiological environmental monitoring program is its ability to confirm consistent, high-quality environmental conditions at all times. In addition to being accompanied by a critical eye from well-trained/certified microbiologists, managers, supervisors and operators. It is also necessary to carry out simulations of medium fill, risk analysis of environmental and process monitoring to reduce the risk of contamination from human sources and thus minimize interventions. In addition to always having a continuous and complete supervision of the entire process in order to guarantee control of the operational process.

Environmental monitoring is one of several key elements necessary that can assure those responsible for a process that a production system is in a consistent and validated state of control. However, it alone does not guarantee anything and its results should not be used to approve a batch.

Therefore, for a microbiological environmental monitoring program to be effective, there must be the engagement of all people (operators, managers, supervisors and microbiologists) involved in the entire aseptic process [5-7].

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