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Research Article

Morphological & Identification of Different Microorganisms from Classified Area in an Environmental Monitoring

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Abstract

The pharmaceutical manufacturing unit is a facility where staff and supplies can be used repeatedly to complete various activities. Results from environmental monitoring reveal details about the room's physical arrangement, the operation of the HVAC system, gowning customs, employee hygiene, equipment, and cleaning procedures. Environmental monitoring is a key part of any pharmaceutical, biotechnology-produced, or medical device-controlled process to demonstrate that the microbial particulate level of all clean-room air and work surfaces is at acceptable levels. It is also regulation-driven. Microorganisms are used to classify particulate particle levels, microbial diversity, and ecosystem health.

Keywords

Environmental Monitoring, Biochemical Reaction, Microbial Contamination, Sterilization

1. Introduction

Environmental Monitoring (E/M) is a program designed to show that the areas of significant relevance include both viable (living microorganisms) and non-viable particles in critical areas. [1]

Environmental Monitoring is a critical process utilized to determine the cleanliness of controlled environments within the pharmaceutical and biotechnology industries. [2] These services are usually provided by pharmaceutical consulting firms who also inspect the manufacturing facility to make sure everything is safe and contaminated free products. [3]

Environmental monitoring is a key pro-active tool for quality assurance programs for the manufacture and management of sterile and non-sterile products and support sectors. [2]

In the pharmaceutical and drug industry, maintaining the safety and efficacy of medications is paramount. [4] Quality control is essential in upholding high standards for pharmaceutical products, and environmental monitoring is a key

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aspect of quality control. [5] Through vigilant monitoring of the environment where drugs are processed and manufactured, companies can pinpoint areas where contamination can be introduced and apply corrective measures before they affect the safety and quality of the products.[6]

According to the ISO Standards 14644-1 clean room define.

A cleanroom whose concentration of airborne particles is regulated, and which is designed and operated in a way to avoid. [7] The introduction, generation, and retention of particles within the room and other pertinent parameters, e.g. temperature, humidity, and pressure, as required.

The cleanroom environmental monitoring system is an important piece of equipment in ensuring that the environment in the cleanroom is kept to the required standards. [8] These systems are employed in the real-time measurement of important parameters in the cleanroom, ensuring regulatory compliance in line with ISO standards and other applicable regulations.

The passive air sampling technique involves counting the number of microorganisms that fall to the bottom of a culture plate due to gravity. Air Sampler is a microbiological equipment that actively collects airborne microorganisms on Culture Media such as Soya Casein Digest Medium. [9] Therefore, it is also referred to as Microbial Air Monitoring System.

Microbial air sampling is carried out in cleanrooms and clean zones to quantify the airborne microbial levels and for authorized pharmaceutical and healthcare use, [10] the airborne concentrations limits are defined in the guidelines of the regulatory authority ¦ The results of the sampling are used to ensure that the required operational cleanliness levels have been achieved and are also likely to be used to justify the release of any product that has been manufactured.

2. Materials and Methods

2.1. Chemicals and Reagents

SCDA- Soyabean Casein Digest Agar *SDA*- Sabouraud Dextrose Agar *TSB*- Typtone Soya Broth.

FTM-Fluid Thioglycollate Medium, Sterile 70% IPA Isopropyl Alcohol, Contact and RODAC Plates (90 mm, Paraffin Filn, Sterile Gloves, Lab Coats, Blue Cotton Stain and Gram Stain like Crystal vilot, Gram Iodine, De-colurized, Safranin, Glass Slide, Slide Cover. Make sure that daily Disinfection of deep decontamination Procedures are part of the evaluation of your facility and process.

2.1.1. Microorganisms Like

Bacillus Subtilis (ATCC6633), Staphylococcus Aureus (ATCC6538), Clostridium Sporogenes (ATCC19404), Pseudomonus Aeruginosa (ATCC9027), Candida Albicans (ATCC10231), Aspergillus Brasiliensis (ATCC16404).

2.1.2. Instrumentation

Air Sampler Make, pH Meter Make, Autoclave Make, Biosafety Cabinet, Laminar Airflow Unit Make, Microscope, Analytical Balance, Incubators (20-25 ℃ & 30-35 ℃).

2.1.3. Purpose of Environmental Monitoring

Environmental monitoring within the pharmaceutical sector is the systematic measurement of information regarding environmental conditions that have the potential to impact product quality. [11] Monitoring includes air quality, temperature, humidity, and microbial contamination. [12]

Below are the purpose of environmental monitoring:

1) Determines the microbiological and particulate matter content of clean room surfaces and air;
2) Identifies the causes of excessive microbiological and particulate matter levels, such as poor cleaning, issues with staff or equipment, and more.
3) The cautions on conditions that exceed the USFDA/WHO/ISO14644-1/EU-cGMP standard limit.

2.1.4. What is Monitored

World Bank

According to the World Bank, monitoring refers to:

A regular process monitoring what is occurring in a project. [13] It is the systematic gathering of information on stipulated indicators to give management and key stakeholders of an on-going development intervention signals of the level of progress and attainment of goals, and progress in expenditure of allocated resources.

What is in need of Monitored?

1) Non-viable particulate air monitoring that is not feasible
2) Sustainable microbiological for viable air monitoring
3) Surface observation (facilities and equipment)
4) "Personnel monitoring guardians and clothing"
5) Humidity and temperature, Pressure differentials (Ap).

2.2. Methods of Environmental Monitoring Procedure

Environmental monitoring includes monitoring of the clean rooms for Physical and Microbiological Parameters.

2.2.1. Physical Parameters

A specially designated analyst for core area, inoculation area, cooling zone, exit corridor, and change rooms I, II, and III monitors parameters such as temperature, relative humidity, and differential pressure. The readings shall be recorded in accordance with Annex I and II. [14]

Table 1 shows the temperature, relative humidity, and differential pressure requirements and frequency for the specified area or room. In going to make sure the area is within the acceptance limits.

Table 1. Physical Environmental Monitoring.

Serial NO.	Area Room	Frequency	Acceptance criteria		Different Pressure (Pascal).
			Temp.	R.H.	
1	Change room I (L0014)	Twice in day	23±2 ℃	NMT 60%	NLT-15 Pascal
2	Change room II (L0014 A)	Twice in day	23±2 ℃	NMT 60%	NLT-15 Pascal
3	Change room III (L0014 B)	Twice in day	23±2 ℃	NMT 60%	NLT-15 Pascal
4	Core Area (L0014 C)	Twice in day	23±2 ℃	NMT 60%	NLT-15 Pascal
5	Inoculation Area (L0014 D)	Twice in day	23±2 ℃	NMT 60%	NLT-15 Pascal
6	Cooling zone (L0014 E)	Twice in day	23±2 ℃	NMT 60%	NLT-15 Pascal
7	Exit corridor (L0014 F)	Twice in day	23±2 ℃	NMT 60%	NLT-15 Pascal

2.2.2. Microbial Environmental

Monitoring: The Active sampling and passive sampling are proposed to monitor the microbial population in the air. [15] Both are useful, but active sampling techniques are becoming essential for environmental monitoring, particularly in the medical device and pharmaceutical sectors.

Figure 1. Microbial Isolate Plate by www.sgs.com.

Figure 2. Microbiological Tests & Analysis by https://www.istockphoto.com.

2.3. What Is a Microbiological Air Sampling

Microbial air contamination monitoring is a critical process in special facilities that have particular air cleanliness requirements. [16] Air sampling is a controlled procedure where, by following these easy steps, we can get an indication of how good quality of air is the microbial contamination free.

2.3.1. Passive Air Sampling Monitoring

Passive sampling with settle plates is a traditional method for sampling for microorganisms. [17] Simply put, these plates are set out in a work area deemed high-risk through a thorough risk assessment.

The plates should be out no longer than 4 hours as this can cause potential dehydration of the sampling media or create a film encapsulating the agar and preventing proper microbial growth.

Procedures:

The label of the plates with the date of exposure and location, Transfer the soybean casein digest agar plates to an aseptic area by using a pass box. Where they are, where they are.

Labeling of Plates:

Name of Media: …….

Plate preparation Date: ……

Area: ……

Date of Exposure: ……

Sampled By: ……

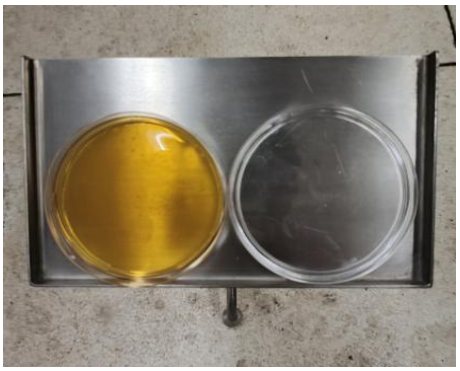

Figure 3. *Petri Dish Plate Stand in Passive Air Sampling Environmental Monitoring.*

The plates should be placed at working height in the area shown in Table 3 and exposed for 30 minutes to 4 hours.

2.3.2. Active Air Sampling Monitoring

Active air samples take about 5-10 minutes to take active sampling compared to settle plate testing. [17] While the equipment is simple to use, Trace Analytics offers detailed instructions and videos to help you through the sampling process.

Procedures

Active air sampling is the process of actively collecting air from an environment using an air sampler. Transfer the Soybean Casein Digest Agar plates to the area that is aseptic. Write down the date and location of the air sample on the plates.

Labeling of Plates:

Name of Media: …….

Plate preparation Date: ……

Area: ……

Date of Exposure: ……

Sampled By: ……

The plates and air sampler should be placed in the correct location. Seal the plates, insert them into the air sampler, and turn it on. Its speed and timing must be set to suck in 1,000 liters, or m3, of air simultaneously.

Figure 4. *Air sampler on the plates by https://www.istockphoto.com.*

2.3.3. Surface Monitoring

The personnel testing is used to sample, identify, and detect various microorganisms in order to evaluate how well disinfection measures work in critical and non-critical production zones.

Surface Monitoring using RODAC or Contact Plate

The ground floor is monitored using road plates. Triptic soy (TSA) or Soybean Casein Digest Agar are both products of triptic soy. This method is to be used to monitor the surfaces of walls, floors and equipment, which should be in a diameter of 55 mm in a contact plate falling in class 10,000 or grade C...

Figure 5. *RODAC and Contact Plates Monitoring by https://www.istockphoto.com.*

2.3.4. Swab Method

Swab technology is utilized to detect Salmonella, Listeria, and E. coli on environmental surfaces.

Procedures

This procedure has to be applied for surface monitoring of floors, walls, and equipment which is of Class 10,000 or Grade C. Swabs should be sterilized, or they can be sterilized for 20 minutes at 121 ℃.

The sterilized swabs must be added to the sterile 0.9% saline solution. Store them in 0.9% saline solution tubes. Each tube should be marked with the sampling site and date. Place a swab sample in a sterilized Petri plate containing 1 mL of saline solution. Use the swab gently over the surface to be observed.

Figure 6. *Swab Sure surface methods by https://www.istockphoto.com.*

2.3.5. Personal Monitoring

The biggest source of contamination in clean areas is humans. They take millions of tiny particles with them wherever they go. Gowns are the most effective way of protecting the clean room environment from people.

Contact Plates:

Figure 7. *Contact plate by istock photo ID: 2107742825.*

These are special Petri dishes that hold sterilized growth medium that has been prepared so that the surface of the medium extends past the edge of the plate. Any other flat surface being sampled is compressed against the contact plate. All live bacteria that are on the surface will stick to the surface of the agar and multiply. This is an example of how many living things are on a surface.

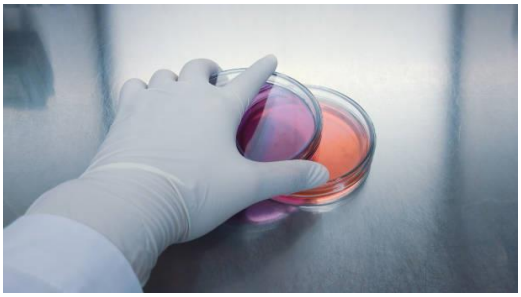

Figure 8. *Personal Monitoring by https://www.istockphoto.com.*

Procedures

Test the gloved thumb and fingers before starting work. After opening the media petriplate lid, place your fingers on the agar surface to create the glove impression of LHFD and RHFD.

The individuals who are engaged in class 10,000 environments have their clothing and gloves monitored using the contact plate method.

2.4. Observation

After sampling is done, cover petri plate lid (Soybean Casein Digest Agar) in sampling area and seal with paraffin film. To incubate, take them to it. Incubate the plates for 48 hours at 30 to 35 degrees Celsius. Then they are transferred to 20 to 25 degrees Celsius for another 72 hours. After 48 and 120 hours, respectively, monitor the platelets. Negative control (Unexposed plates) should remain free of growth.

3. Results

Microbiological environmental monitoring of results is the used to validate the efficacy of the controls of operation in lowering microbial contamination of satisfactory levels. [18]

Figure 9. *Colony counts after culture growth of Environment Monitoring Results by https://www.shutterstock.com.*

Table 2. *EU CGMP/WHO/ISO Clean Room Standard for permitted microbial parti.*

Grade	RECOMMENDED LIMITS FOR MICROBIAL CONTAMINATION			
	Air Sample cfu/m3	**Settle Plate (diam. 90 m), cfu/2 hrs**	**Contact Plates (dia. 55), cfu/plate**	**Glove print, 5 fingers, cfu/glove**
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	_

Grade	RECOMMENDED LIMITS FOR MICROBIAL CONTAMINATION			
	Air Sample cfu/m3	Settle Plate (diam. 90 m), cfu/2 hrs	Contact Plates (dia. 55), cfu/plate	Glove print, 5 fingers, cfu/glove
D	200	100	50	_

Table 3. *Passive air sampling results cleanroom Classified D area.*

Location Name	Method used	Colony count			Pr (in case AAS)
		Bacteria CFU	Fungal CFU	Total CFU	
Auto clave area	PAS (≤100)	8	9	17	-
Incubator area		22	11	33	-
Door site area		29	11	40	-
Middle area		7	9	16	-
Upper Table area		11	10	21	-

Table 4. *Active air sampling results clean room Classified D area.*

Location Name	Method used	Colony count			Pr (in case AAS)
		Bacteria CFU	Fungal CFU	Total CFU	
Auto clave area	AAS (≤200)	3	5	8	8
Incubator area		21	9	30	31
Door site area		19	6	25	26
Middle area		16	8	24	25
Upper Table area		22	11	31	32

Table 5. *Surface Monitoring results clean room Classified D area.*

Location Name	Method used	Colony count			Pr (in case AAS)
		Bacteria CFU	Fungal CFU	Total CFU	
Auto clave area	AAS (≤200)	35	15	50	53
Incubator area		23	19	43	45
Door site area		14	7	21	-
Middle area	PAS (≤100	18	9	27	-
Upper Table area		23	13	36	-

Table 6. Personnel Monitoring results clean room Classified D area.

Location Name	Method used	Colony count			Pr (in case AAS)
		Bacteria CFU	Fungal CFU	Total CFU	
Auto clave area	AAS (≤200)	43	25	68	74
Incubator area		55	32	87	98
Door site area		29	8	37	-
Middle area	PAS (≤100	18	12	30	-
Upper Table area		25	18	43	-

4. Discussion

In the discussion of microbiological environmental monitoring in the laboratory, the results should provide details of trends, [19] observation of data and analysis from action or warning limits and identification of microbial contaminants and relate to the effectiveness of the equipment in preventing contamination and ensuring product safety and quality. [20] This discussion also includes an analysis of how the identified microbes may pose a risk, an assessment of the effectiveness of hygiene measures and confirmation that regulatory guidance, such as that of the FDA or the World Health Organisation, is being followed. [7]

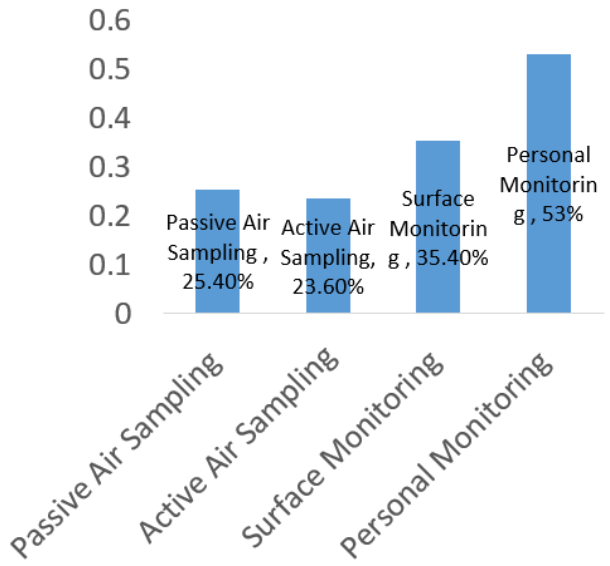

Figure 10. Microbial growth of observation of data analysis.

5. Conclusion

In addition, environmental monitoring and microorganism tests play an important role in patient safety and in ensuring efficacy of drugs and biology, which prevent their contamination with germs. [21] Microbiological testing does not provide complete or full assurance that there is no microbial contamination. [22] However, such testing provides a robust environmental monitoring program and high levels of microbial protection of medicines using appropriate manufacturing processes. [23]

Abbreviations

E/M	Environmental Monitoring
HVAC	Heating Ventilation Air Conditioning
FDA	Food And Drugs Administration
ISO	International Standard Organization
GMPs	Good Manufacturing Practices
NA	Nutrient Agar
SDA	Saboruad Dextrose Agar
HEPA	High Efficiency Particulate Air
CFU	Colony Forming Unit
SCDA	Soyabean Casein Digest Agar
LAF	Laminar Air Flow
TSA	Triptic Soya Agar
SOP	Standard Operating Procedure
AAS	Acative Air Samples
PAS	Passive Air Samples
GPT	Growth Promotion Test

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Conflicts of Interest

The authors declare no conflicts of interest.

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