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Environmental Monitoring in Pharmaceuticals

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Abstract

Microbiology play an important and key role in pharmaceutical industry particularly injectable (dry powder injection, tablet and capsule, liquid and lozenge's). At all the stages during the manufacturing of a dry powder injection, tablet and capsule, liquid and lozenge's, almost precaution are to be taken to prevent external contamination of particulate matter, impurities and microbiological contamination. Microbial environment monitoring is done to check the bio-burden of the aseptic area of controlled environment. The purpose of this is to understand the various issues that relate to aseptic processing of bulk drug substance or finished products (sterile), dosage forms and in certain cases and to establishments, maintenance and control of the microbiological quantity of the controlled environment.

***Keywords:** Microbiological contamination; Environment Monitoring; Aseptic processing; Sterilization; Injection*

Abbreviations: BET: Bacterial Endotoxin Test; CFU: Colony Forming Unit; CSE: Control Standard Endotoxin; DW: Distilled Water; EU: Endotoxin Unit; Gm +ve: Gram negative bacteria; Gm+ve: Gram positive bacteria; GTP: General Test Procedure; IP: India Pharmacopoeia; IU: International Unit; LAL: Limulus Amoebocyte Lysate; Lambda: Lambda sensitivity; LAL reagent in EU/ml law LAL reagent water; MLT: Microbial Limit Test; MVD : Maximum Valid Dilution; NAM: Nutrient Agar Medium; NPC: Negative Product Control; NWC: Negative Water Control; Ph. Eur: European Pharmacopoeia; PPC: Positive Product Control; PPM: Pour Plate Method; PWC: Positive Water Control; RW: Raw Water; SM: Selective Medium; SOP: Standard Operating Procedure; SPC: Sterilized Potable Count; TBC: Total Bacterial Count; TFC: Total Fungal Count; TMC: Total Microbial Count; USP: United States Pharmacopeia; WFI: Water For Injection; WOA: Water of Analysis

Introduction

Microbiological evaluation of clean rooms and other controlled environments: The purpose of this information is to review the various issues that relate to aseptic processing of bulk drug substances, dosage forms and in certain cases, medical devices; and to the establishment, maintenance and control of the microbiological quality of controlled environments. This chapter includes discussions on:

- The classification of a clean room based on particulate count limits.
- Microbiological evaluation programs for controlled environments.
- Training of personnel.
- Critical factors in design and implementation of a microbiological evaluation program.
- Envelopment of a sampling plan.
- Establishment of microbiological alert and action levels.
- Methodologies and instrumentation used for microbiological sampling.
- Media and diluents used.
- Identification of microbial isolates.
- Operational evaluation *via* media fills.

A glossary of terms: A large proportion of sterile products are manufactured by aseptic processing. Because aseptic processing relies on the exclusion of microorganisms from the process stream and the prevention of microorganisms from entering open containers during filling product bio-burden as well as microbial bio-burden of the manufacturing environment are important

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factors relating to the level of sterility assurance of these products [1].

Establishment of clean room classifications: The design and construction of clean rooms and controlled environments are covered in federal standard 209E. This standard of air cleanliness is defined by the absolute concentration of airborne particles.

Methods used for the assignment of air classification of controlled environments and for monitoring of airborne particulates are included. The federal document only applies to airborne particulates within a controlled environment and is not intended to characterize the viable or nonviable nature of the particles. The criticality of the number of nonviable particulates in the electronic industry makes the application of federal standard 209E a necessity, while the pharmaceutical industry has a greater concern for viable particulates (*i.e.* microorganisms) rather than total particulates as specified federal standard 209E. A definite concern for counts of total particulates in inject able products exists in the pharmaceutical industry [2-4]. Federal standard 209E, as applied in the pharmaceutical industry is based on limits of all particles with sizes equal to or larger than 0.5 um (Table 1). It is generally accepted that if fewer particulates are present in an operational clean room or other controlled environment, the microbial count under operational conditions will be less, provided there are no changes in airflow, temperature and humidity. Clean rooms are maintained under a state of operational control on the basis of dynamic (operational) data [5].

Cleanroom

Monitoring of total particulate count in controlled environments, even with the use of electronic instrumentation on a continuous basis, does not provide information on the microbiological content of the environment (Table 1) [6].

TABLE 1. **Airborne particulate cleanliness classes.**

Particle equal to and larger than 0.5 um				
S. No	**Grade**	**Class**	$/m^3$	$/ft^3$
1	A	100	3530	100
2	B	1000	35300	1000
3	C	10000	353000	10000
4	D	100000	3530000	100000

The basic limitation of particulate counters is that they measure particles of 0.5 um or larger. While airborne microorganisms are not free floating or single cells, they frequently associate with particles of 10 um to 20 um. Particulate counts as well as microbial counts within controlled environments vary with the sampling location and the activities being conducted during sampling. Monitoring the environment for nonviable particulates and microorganisms is an important control function because they both are important in achieving product compendium requirements for particulate matter [7-9].

Microbial monitoring programs for controlled environments should assess the effectiveness of cleaning and sanitization practices by and of personnel that could have an impact on the bio-burden of the controlled environment. Microbial monitoring, regardless of how sophisticated the system may be, will not and need not identify and quantities all microbial contaminants present in these controlled environments [10,11]. Environmental microbial monitoring and analysis of data by qualifies personnel will permit the status of control to be maintained in and analysis of data by qualified personnel will permit the status of control to be maintained in clean rooms and other controlled environments.

Establishment of sampling plan and sites (Table 2).

TABLE 2. **Sampling area and frequency of sampling.**

Sampling area	**Frequency of sampling**
Class 100 or better room designations	Each operating shift
Supporting areas immediately adjacent to class 100 (e.g. class 10,000)	Each operating shift
Other support areas (class 100,000)	Twice/week
Potential product/container contact areas	Twice/week
Other support areas to aseptic processing areas but non-product contact (class 100,00 or lower)	Once/week

Microbial considerations and action levels for controlled environments

Classification of clean rooms and other controlled environments is based on federal standard 209E based on total particulate counts for these environments. The pharmaceutical and medical devices industries have generally adopted the classification of class 100, class 10,000 and class 100,000 especially in terms of construction specification for the facilities.

There have been reports and concerns about differences in these values obtained using different sampling systems, media variability and incubation temperatures. It should be recognized that and through on system is absolute, it can help in detecting changes and thus trends, in environmental quality. Each manufacture s data must be evaluated as part of an overall monitoring program (Tables 3-6).

TABLE 3. **Microbial considerations and action levels for controlled environments for settle plate.**

S. no	Class	Grade	CFU/4 hrs.
1	100	A	Less than 1
2	1000	B	Less than 5
3	10000	C	Less than 50
4	100000	D	Less than 100

TABLE 4. **Microbial considerations and action levels for controlled environments for air sampling.**

S. no	Class	Grade	CFU/m^3	CFU/ft^3
1	100	A	Less than 3	Less than 0.1
2	10000	C	Less than 20	Less than 0.25
3	100000	D	Less than 100	Less than 2.5

TABLE 5. **Microbial considerations and action levels for controlled environments for surface swab by contact plate.**

S. no	Class	Grade	CFU/ 24 cm^2-30 cm^2
1	100	A	3 including floor
2	10000	C	5, 10 for floor

TABLE 6. **Microbial considerations and action levels for controlled environments for parsonnel monitoring.**

S.no	Class	Grade	Cfu per contact plate	
			Gloves	Personnel clothing and grab
1	100	A	3	5
2	10000	C	10	20

Culture media and diluents used for sampling or quantitation of microorganisms

The type of medium, liquid or solid, that is used for sampling or quantization of microorganisms in controlled environments will depend on the procedure and equipment used. A commonly used all-purpose medium is soybean casein digest agar when a solid medium is needed. Other media, liquid or solid are listed below.

Liquid media: Tryptone saline, peptone water, buffered saline, buffered gelatin, enriched buffered gelatin soybean casein medium.

Solid media: Soybean casein digest agar, nutrient agar, tryptone glucose extract agar, lecithin agar brain heart infusion agar, brain heart infusion contact plate agar liquid and solid media are sterilized using a validated process.

These media are commercially available in dehydrated from. They are also available in ready to use from when disinfectants or antibiotics are used in the controlled area, consideration should be given to using media with appropriate inactivating agents. Alternative media to those listed can be used provided they are validated that are validated for the purpose intended.

Materials and Methods

Aim: Environment monitoring.
Objective: To estimate the number of viable aerobic microorganisms present in environment.

Sampling materials: Cotton swab with a sterile transport media solution.

Alternative sampling system: Sterile sponge with detachable handle.

- Check surface samplers.
- RODAC plates (with locking lid).
- Use media containing lecithin and twin neutralizer's sterile 70% alcohol spray bottle or wipes.

Testing equipment and materials

- Biological Safety Cabinet (BSC) with HHEPA filtration.
- Laminar Flow Hood (LFH) HEPA filtration.
- 10% bleach or appropriate disinfectant/parricide.
- Sterile 70% ethanol (ETOG) or Isopropyl Alcohol (IPA).
- Sterile sleeves.
- Sterile gloves.
- Lab coat.
- Incubator set 32.5°C ± 2. 5°C
- Incubator set 22.5°C ± 2. 5°C
- Sabouraud dextrose broth.
- Sabouraud dextrose agar.
- Sampling preparation.

Sterile disposable lab coat or gown

- Safety goggles.
- Hair net, mask and/or beard cove.
- Sterile gloves.
- Shoe covers.

Materials required:

- Soybean casein digests agar media.
- Petriplates.
- Sterile RODAC plates.
- Sterile cotton swab.
- Normal saline.
- SS container.
- SS stand.

Equipment required:

- Double door autoclave.
- Centrifugal air sample.
- Particle counter.
- BOD incubator 22.5°C-2.5°C
- BOD incubator 32.5°C-2. 5°C
- Laminar air flow.

- Colony counter.

EM sampling procedures: Disinfect gloved hand with a suitable sanitizing agent *i.e.* sterile 70% alcohol. Repeat this step between each EM sample.

Allow gloves to air dry so no alcohol is dripping from gloves: Some swab/sponge sampling packages include a secondary set of sterile gloves. In these instances, the secondary glove can be aseptically used on top of the primary gloves to expedite the sampling process. The secondary pair of gloves will need to be disposed of aseptically after use. If the primary gloves touch the secondary gloves outer surface, then a suitable sanitizing agent must be used on the primary gloves. Allow primary gloves to air dry after sanitizing.

When sampling a class 100 scenario: Allow LFH\BSC (Biological Safety Cabinet) to run approximately 10 minutes before initiating sampling. Wipe down all outer sampling containers with a suitable sanitizing agent before placement in the LFH\BSC. Do not open sampling materials outside the LFH\BSC.

Environment monitoring

Objective: To estimate the number of viable aerobic microorganisms present in environment.

Definition: Monitoring of viable contamination with in clean environments.

Principle: Microbial environment monitoring is done to check the bio-burden of the aseptic area of controlled environment. The purpose of this is to understand the various issues that relate to aseptic processing of bulk drug substance or finished products (sterile), does arms and in certain cases and to establishments, maintenance and control of the microbiological quantity if the controlled environment.

The improper application of microbiological sampling and analysis may cause significant variability and the potential for in advert ant contamination.

Large processing product bio-burden as well as microbial bio-burden of manufacturing environment is important factors relating to the level of sterility assurance of these products.

Following methods are performed for examine the environment free from contamination:

Methods of EM: By settle plate, by active air sampler, by swab, by finger dab, by contact plate.

Personal monitoring: This is done by two methods as follows: By contact plate, by finger dab.

By settle plate methods

Plate preparation: Prepare SCDA medium. Aseptically transfer the pre incubated plate into the manufacturing room and mentions plate code and exposure time on the plate, expose as per layout. For class 100 expose the plate for 30 min and in other for area for 2 hrs. After completion of exposure time close the plates in the same manner. Incubate the plate at 30°C-35°C for 48 hrs for bacterial count and after 48 hrs transfer the plate in BOD incubator at 20°C-25°C for 72 hrs for fungal count. After completion of each incubation period observe the result for bacterial and fungal count respectively. Fungus should be absent (Table 7).

TABLE 7. **Showing limits of bacterial count in settle plate method.**

Class	Alert	Action	Limit
100 A	<1 CFU/plate	<1 CFU/plate	<1 CFU/plate
1000 B	<2 CFU/plate	<3 CFU/plate	<5 CFU/plate
10,000 C	<25 CFU/plate	<35 CFU/plate	<50 CFU/plate
1,00000 D	<50 CFU/plate	<70 CFU/plate	<100 CFU/plate

By active air sampler

Plate preparation: Prepare SCDA medium. Aseptically transfer the per incubated plates into the concern area and mention plate

code sampling time on the plates, take the sample 1000 liter of air ($1m^3$) as per layout. After sampling close the lid of the plates aseptically. Incubate the plates at 30.5°C for 48 hrs. Bacterial count and after 48 hrs, transfer the plates into at 20°C-25°C for 72 hrs for fungal (Table 8).

TABLE 8. **Showing limits of bacterial count in active air sampling.**

Class	Alert (CFU/cu.m^3)	Action (CFU/cu.m^3)	Limits (CFU/cu.m^3)
100 A	<1 CFU cu.m^3	<1 CFU cu.m^3	<1 CFU cu.m^3
100 B	5 CFU cu.m^3	7 CFU cu.m^3	10 CFU cu.m^3
10000 C	50 CFU cu.m^3	70 CFU cu.m^3	100 CFU cu.m^3
100000 D	100 CFU cu.m^3	150 CFU cu.m^3	500 CFU cu.m^3

By swab: This method of environment monitoring in sterile manufacturing area is used for detection of any bacterial and fungal count attached to some surface.

Requirements: Sterile swab sticks, pre-incubated Petri plates containing SCDA, normal saline water solution.

Procedure:

- First prepare the SCDA media plates and put in the bacteriological incubator at 32.5°C-2.5°C for 48 hrs.
- After 48 hrs put these plates under LAF.
- Now prepare NS (0.9 gm of NaCl in 1000 ml of WFI) and put 3 ml of it in to tubes of sterile swab.
- Place the tubes in the test tube stand and put the stand in to S.S. container.
- Bring the S.S. container to the sterile area.
- Sample the 5 m 2 desired surface areas with the help of swab.
- Now place the swab again in the tubes and rotate clockwise and anticlockwise and put tubes in S.S. container.
- Bring the container in microbiology lab.
- Take 1 ml of NS from tube of sterile swab and in the SCDA plates and rotate clockwise and anti-clockwise.
- Incubate the plates in the bacteriological incubator at 32.5°C-2.5°C for 48 hrs further incubate the plates in BOD incubator at 22.5°C-2.5°C for 72 hrs.
- After 5 days record the bacterial and fungal count.

By contact plate method: Surface monitoring by contact plate.

Plate preparation: Prepare DE-engle neutralizing agar medium. Aseptically transfer the pre-incubated plate into the concern area mention plate code and sampling time on the plates, open the lid of the plate and press smoothly on the surface which has to be monitored. After sampling close the lid of the plate. Incubate the plate at 30°C-35°C for 48 hours for bacterial count and after 48 hours transfer the plate at 2025°C for 72 hours for fungal count. After completion of each incubation period observe the plate for bacterial and fungal count respectively (Table 9).

Table 9. **Showing limits of bacterial count in surface monitoring by contact method.**

Class	Alert CFU/swab	Action CFU/swab	Limit CFU/swab
100 A	<1 CFU/swab	<1 CFU/swab	<1 CFU/swab
1000 B	2 CFU/swab	3 CFU/swab	5 CFU/swab
10.000 C	15 CFU/swab	20CFU/swab	25 CFU/swab
100000 D	25 CFU/swab	35 CFU/swab	50 CFU/swab

Personal monitoring by contact plate method

Plate preparation: Prepare DE-Engley neutralizing agar medium. Aseptically transfer the pre-incubated plate into the concern area and mention plate code and sampling time on the plates open the lid of the plate and press smoothly on the surface (forehead, chest, armpits) after sampling close the lid of the plate. Incubate he plate at 30°C-35°C for 48 hours for bacterial count and after 48 hours transfer the plate in BOD incubator at 20°C-25°C for 72 hrs. After completion of each incubation period observe the result for bacterial and fungal count respectively (Table 10).

TABLE 10. **Showing bacterial count in personal monitoring by contact method.**

Class	Alert CFU/plate	Action CFU/plate	Limit CFU/plate
100 A	<1 CFU/plate	<1 CFU/plate	<1 CFU/plate
1000 B	3 CFU/plate	4 CFU/plate	5 CFU/plate
10.000 C	15 CFU/plate	20 CFU/plate	25 CFU/plate

In personal monitoring count of all the parts (forehead, arm pit and chest) is nil.

Personal monitoring by finger dab method

Plate preparation: Prepare SCDA medium. Aseptically transfer the pre-incubated plate into the concern area and mention name of the person and the location (standing position), aseptically open the lid of the plate and take the finger impression hand in separate plate. After completion of the sampling close the lid of the plates. Incubate the plate at 30°C to 35°C for 48 hrs for bacterial count and after 48 hrs transfer the plates in BOD incubator at 20°C to 25°C for hrs for fungal count. After incubation period observed the result for total microbial count (Table 11).

TABLE 11. **Showing bacterial count in personal monitoring by finger dab method.**

Microorganism	Class 100 A (CFU/5 fing.			Class 1000 B (CFU/5 fing.)		
	Alert	Action	Limit	Alert	Action	Limit
Bacteria	<1	<1	<1	<3	<4	<5

The count is nil in finger dab method.

Results

Results of the works carried out are represented below in following tables which are as follow.

Environment monitoring

In environment monitoring the various parameters were adopted to test the presence of microorganisms on the fingers, working table, laminar air flow, shoe cover etc. It was found that, a few microorganisms were associated with the changing room or working table, but these viable counts are found under the limit (Table 12).

Result: It was found that viable counts were found under the limit.

TABLE 12. **This table shows the method of testing, TBC, TFC and limit**.

S. no.	Method of testing	TBC	TFC	Limit
1	Plate expose D grade	65 CFU	Nil	NMT 100 cfu
2	Plate expose C grade	31 CFU	Nil	NMT 50 cfu
3	Plate expose B grade	02 CFU	Nil	NMT 05 cfu
4	Plate expose A grade	Nil	Nil	NMT 01 cfu
5	Active air sampling D grade	70 cfu/m^3	Nil	NMT 500 cfu/m^3
6	Active air sampling B grade	20 cfu/m^3	Nil	NMT 100 cfu/m^3
7	Active air sampling C grade	1 cfu/m^3	Nil	NMT 10 cfu/m^3
8	Active air sampling A grade	Nil	Nil	NMT cfu/m^3
9	Surface swab D grade	19 cfu/25 cm^3	Nil	50 cfu/25 cm^3
10	Surface swab B grade	10 cfu/25 cm^3	Nil	
11	Surface swab C grade	1 cfu/25 cm^3	Nil	5 cfu/25 cm^3
12	Surface swab A grade	Nil	Nil	1 cfu/25 cm^3
13	Personnel monitoring forehead	Nil	Nil	3 CFU/plate

14	Personnel monitoring arm pit	Nil	Nil	3 CFU/plate
15	Personnel monitoring chest	Nil	Nil	3 CFU/plate
16	Personnel monitoring finger dab	Nil	Nil	5 CFU/plate

Discussion

Environment monitoring was mainly done to check the bio-burden of the sterile area. Sometime the micro flora in the sterile area will cause contamination to the product and also cause the contamination in the microbiological lab. So it is necessary to detect the microflora of the environment. It was done by many methods like settle plate method, air sampler and personal monitoring by using RODAC plates. Personal monitoring was done to check the personal hygiene of the work working in the sterile area because worker can also contaminate the products. All the results of the environment monitoring were found satisfactory if the micro flora is increased in the sterile area then fumigation of sterile area and also performed in other 70% IPA was done to kill the microorganisms. These types of test are also performed in other pharmaceutical companies like Dr. Reddy and Ranbaxy, Fresenius Kabi oncology and much other pharmaceutical company. For the quality control of their pharmaceutical products if the quality of the product did not checked properly it may cause harmful effects in human beings. So these tests have great importance in the pharmaceutical companies.

Conclusion

From the observation it was concluded that the bio-burden limit of the aseptic area is properly in control, because the washing procedure as well as the precaution during purification process in the aseptic area is properly maintained. So according the USP guideline the aseptic area is perfect for the purification of formulation drug in sterile condition.

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