International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume: 3 | Issue: 3 | Mar-Apr 2019 Available Online: www.ijtsrd.com e-ISSN: 2456 - 6470

A Brief Overview on Active Air Sampling Procedure for Environment Monitoring

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How to cite this paper: Juhi Rastogi | Faiz Hashmi "A Brief Overview on Active Air Sampling Procedure for Environment Monitoring" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-3 | Issue-3 , April 2019, pp.732-736, URL: http://www.ijtsrd.com/papers/ijtsrd22987.pdf

IJTSRD22987

ABSTRACT

In this paper, we are going to discuss the 'Active Air Sampling procedure for EM'. EM stands for Environment Monitoring. Environment monitoring is performed in the pharmaceutical manufacturing plants to monitor the contamination of viable and non-viable particle count. Viable particle count can be observed through the '*Settle Plate method, Active Air Sampling, Surface Monitoring (contact plate & swab test), and Personnel Monitoring method*'. Non-viable particles are dust particles and other non-living particles.

Active Air sampling is performed by microbiologist in the production & manufacturing area using the equipment known as '*Air Sampler*'. A media plate of SCDA (*Soybean-Casein Digest Agar*) prepared under sterile condition by the microbiologist. The media plate is then allowed to adjust under the 'Air Sampler hood' and then it is used for sampling purpose. Air sampler captures 1000L air as per validated time in a 1cubic meter of volume and therefore air sampling is thus performed in the middle of the surrounding area. The sampled plate is then incubated, and after then the required incubation is provided, and the plate is thus analyzed to determine whether our manufacturing area meets the level of expected counts or it crosses the required limit; and, on this basis, the reporting is thus generated on regular basis.

KEYWORDS: *Environment Monitoring, Clean Room, Grades and their respective limits, Checkpoints of Media, Air Sampler and its operation, Incubation of the sampled plates, Trend chart analysis*

INTRODUCTION

Active Air Sampling for Environment Monitoring is performed in the pharmaceutical manufacturing plants. The purpose here is to monitor the contamination present in the environment which ultimately causes contaminate in the medicines i.e. being manufactured in specified areas. The contaminants can be non-viable particles and viable particles as well.

Active Air sampling is done by microbiologist in the production & manufacturing area using the equipment known as '*Air Sampler*'. A media plate of SCDA (*Soybean-Casein Digest Agar*) which is prepared under the sterile condition by the microbiologist. The media plate is then allowed to adjust under the 'Air Sampler hood' and then it is used for sampling purpose. Air sampler captures 1000L air per 10 minutes in a 1cubic meter of volume and therefore air sampling is thus performed in the middle of the surrounding area. The sampled plate is then incubated and after the required incubation, the plate is thus analyzed to determine whether our manufacturing area meets the level of expected counts or is it crosses the required limit; and, on this basis the reporting is thus generated on a regular basis, weekly basis, monthly basis or twice a month.

ENVIRONMENT MONITORING

Environment Monitoring is the monitoring of surroundings in the production and manufacturing areas. EM is performed by various procedures, those are:

- **Settle Plate Method**:

The 90mm Petri plates are being prepared with 20ml-25ml of SCDA (*Soybean casein digest agar*), after the pre-incubation of 24 to 48 hours the Petri plates are allowed to be exposed for the 4 hours in the manufacturing area and then it is incubated at 20°C-25°C for 72 hours and observation is being recorded. Later the same plate is then incubated at 30°C-35°C for 48 hours and later the observation is being recorded.

- **Active Air Sampling Method**:

The 90mm Petri plates are being prepared with 20ml-25ml of SCDA (*Soybean casein digest agar*), after the pre-incubation of 24 hours the Petri plates are allowed for sampling and the Active Air Sampling procedure is to be started and to be continued up to 10 minutes or as per the time validated by each industry and then it is incubated at 20°C-25°C for 72 hours and observation is being recorded. Later the same plate is then incubated at 30°C-35°C for 48 hours and later the observation is being recorded.

➢ **Surface Monitoring methods**:

The surface monitoring procedure is performed to monitor the contaminants which stick to the surface of the working machine, laminar air flows, conveyor belts, door surface, floor surface, window glass wall surface. Then it is incubated at 20°C-25°C for 72 hours and observation is being recorded. Later the same plate is then incubated at 30°C-35°C for 48 hours and later the observation is being recorded.

Surface Monitoring is performed by the following two ways:

- **Contact Plate Method**:
  The 55mm Petri plates are being prepared with 10ml - 12ml of DNA (*De-Engley Neutralizing Agar*), after the pre-incubation of 24 – 48 hours, the Petri plates are allowed to get in contact with the working machine, laminar air flows, conveyor belts, door surface, floor surface, window glass wall surface, etc. Then it is incubated at 20°C-25°C for 72 hours and observation is being recorded. Later the same plate is then incubated at 30°C-35°C for 48 hours and later the observation is being recorded.

- **Swab Test Method**:
  Normal saline is used for this purpose. Swab sticks are dipped in normal saline in the swab collection tubes and this configuration is used for surface monitoring by swab test. All those locations where sample collection by the contact plate is not possible, swab test are then performed for all those locations, like a hopper, LAF corners, door handle wall corners, etc.

  The collected samples are then is being solidified by SCDA (Soybean *casein digest agar*) via pour plate techniques. Then it is incubated at 20°C-25°C for 72 hours and observation is being recorded. Later the same plate is then incubated at 30°C-35°C for 48-hour sand later the observation is being recorded.

➢ **Personnel Monitoring Method**:

The 55mm Petri plates are being prepared with 10ml-12ml of DNA (*De-Engley Neutralizing Agar*), after the pre-incubation of 24 – 48 hours the Petri plates are allowed to get in contact with the personnel working in the manufacturing area. The contact plate is being taken from various locations like forehead, chest, armpits, elbows, booties, and fingers. Then it is incubated at 20°C-25°C for 72 hours and observation is being recorded. Later the same plate is then incubated at 30°C-35°C for 48 hours and later the observation is being recorded.

CLEAN ROOM AND ITS CLASSIFICATION

A cleanroom is a facility ordinarily utilized as a part of specialized industrial production or scientific research, including the manufacture of pharmaceutical items and microprocessors. Cleanrooms are designed to maintain extremely low levels of particulates, such as dust, airborne organisms, or vaporized particles. Cleanrooms typically have a cleanliness level quantified by the number of particles per cubic meter at a predetermined molecule measure. The atmosphere outdoor air in a typical urban area contains 35,000,000 particles for each cubic meter in the size range 0.5 µm and bigger in measurement, equivalent to an ISO 9 cleanroom, while by comparison an ISO 1 cleanroom permits no particles in that size range and just 12 particles for each cubic meter of 0.3 µm and smaller.

Table 01: The Area Classification of Inject-able Plants in general

Room name	Grade
Change Room-I of filling area	Grade D (ISO -8)
Change Room-I of Manufacturing area	Grade D (ISO -8)
Change Room-I of Sampling area	Grade D (ISO -8)
Change Room-II of sampling area	Grade C (ISO -7)
Change Room-II of dispensing area	Grade C (ISO -7)
Change Room-II of Manufacturing room	Grade C (ISO -7)
Change Room-II of washing room	Grade C (ISO -7)
Change Room-II of preparation room	Grade C (ISO -7)
Staging room	Grade B (ISO -6)
Cooling Zone	Grade B (ISO -6)
Filtration Room	Grade B (ISO -6)
Corridor area of filling	Grade B (ISO -6)
Blending room	Grade B (ISO -6)
Mobile LAF	Grade A (ISO -5)
Filtration LAF	Grade A (ISO -5)
Garment Cabinets	Grade A (ISO -5)
Passbox	Grade A (ISO -5)
RLAF	Grade A (ISO -5)

GRADES AND THEIR RESPECTIVE LIMITS

Table 02: Limits According to EU guidelines

Grade	Active Air Sampling	Settle Plate (90mm), cfu/4hrs	Contact Plate (55mm), cfu/plate	Glove print 5 fingers, cfu/glove
A	<1	<1	<1	<1
B	10	5	5	5
C	100	50	25	n.a.
D	200	100	50	n.a.
According to EU guidelines				

Table 03: Limits According to FDA guidance (2004)

Clean area classification (0.5µm particles/ft3)	Active Air Sampling, cfu/m3	Settle Plate (90mm), cfu/4hrs	Glove Print 5 fingers, cfu/glove
100	ISO 5	1	1
1000	ISO 6	7	3
10000	ISO 7	10	5
100000	ISO 8	100	50
According to FDA guidance (2004)			

Table 04: ISO 14644-1 Cleanroom Standards

Class	maximum particles/m³						FED STD 209E equivalent
	>=0.1 µm	>=0.2 µm	>=0.3 µm	>=0.5 µm	>=1 µm	>=5 µm	
ISO 1	10	2					
ISO 2	100	24	10	4			
ISO 3	1,000	237	102	35	8		Class 1
ISO 4	10,000	2,370	1,020	352	83		Class 10
ISO 5	100,000	23,700	10,200	3,520	832	29	Class 100
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293	Class 1,000
ISO 7				352,000	83,200	2,930	Class 10,000
ISO 8				3,520,000	832,000	29,300	Class 100,000
ISO 9				35,200,000	8,320,000	293,000	Room Air

Table 05: BS 5295 Cleanroom Standards

	maximum particles/m³				
Class	>=0.5 µm	>=1 µm	>=5 µm	>=10 µm	>=25 µm
Class 1	3,000		0	0	0
Class 2	300,000		2,000	30	
Class 3		1,000,000	20,000	4,000	300
Class 4			20,000	40,000	4,000

MEDIA AND ITS PREPARATION

Checkpoints of the media:

- Name of Media
- Media Lot No/Batch No.
- Media Mfg. Date and Expiry Date
- Receipt of Certificate of Analysis
- Is Physical condition OK
- Is Label claim matched with INDENT and CoA
- Color
- Lumps
- pH (before and after sterilization)
- solubility
- GPT/GIT

Preparation of media

The purified water are used for the media preparation. The quantity of dehydrated media required for the volume of media to be prepared is to be calculated. The calculated quantity of media on a calibrated weighing balance is to be weighed and then is transferred into the conical flask/ Bottle/ Tubes containing a small part of required water quantity. The remaining quantity of water is added into the conical flask/ Bottle/ Tubes. The media is then dissolved by swirling the flask or boil/heat the media (as per manufacturer recommendation if required) on a water bath or hot plate to dissolve completely. The pH of the unsterilized media is then checked and recorded the verified pH of the media. If the pH varies, it is then adjusted by the addition of 0.1N Hydrochloric acid or 0.1N Sodium hydroxide before sterilization so as to pH remains under limit after sterilization. If the pH of media is satisfactory then set the cotton plug in the flask / bottle / tube mouth. Before sterilization, chemical indicator shall be placed in the autoclave. The test tubes/Flask /Bottles shall be labeled with 3M tape with the name of the media lot No. The media is then sterilized in steam sterilizer by operating the autoclave standard process 1 (121°C) & standard process 2 (115°C) for 20 minutes. For Heat Labile Media boil the media up to 80ºC up to dissolved. The sterilized media is then unloaded from autoclave in the Cool Zone area. Allow the media to cool according the room temperature. Check the pH of sterilized media by taking a broth media tube.

Preparation of Plates

SCDA (1% glycerol added) media is used for active air sampling. The media shall be prepared and autoclaved respectively. Approximately 15-20 ml of liquefied SCDA (1% glycerol added) media is being under LAF and cooled up to 40^{0}C-45^{0}C in all the Petri plates required as per the exposure location. The Petri plates is then covered and the poured media is allowed to solidify. After the solidification, all media containing Petri plates are incubated for the purpose of pre-incubation in the incubator at 32.5^{0}C± 2.5 ^{0}C for 24 - 48 hours in an inverted position for detecting any contamination during plate pouring operations. After

the pre-incubation, examine all Petri plates for microbial growth. Petri plates without contamination and having no air bubbles shall be selected for active air sampling.

Result Calculation

Calculate the result as per the formula is given here.

$$X - \frac{Pr * 1000}{V}$$

Where;

X = CFU/m^3

V = Volume of air sampler

r = CFU counted on 90 mm plate

Pr = Probable count obtained by positive hole correction against r value.

AIR SAMPLER AND ITS OPERATION

Preparation of Air Sampler and its Accessories

The Sterilized/ Depyrogenate hood and labeled Petri plates are to be kept in the hatch box as per requirement. The Air Sampler and hatch box are being carried into the aseptic area by mopping with 70% IPA.

Working of Air Sampler:

The pre-incubated sanitized labeled SCDA (1% glycerol added) plate is being placed in the feeder cone circular clamp assembly in position. The air sampler is then placed in the area where air sampling is being done. The "ON" switch is then turned on which is provided on the back side of the air sampler which shows power "ON" by glowing lamp. The following procedure is then followed for the sampling purpose:

- Initial display shall be observed.
- Press "START ON" the control panel to obtain 1000 Ltr air
- Instrument shall be automatically OFF after sucking 1000 Ltr of air.
- Then open the SS hood and remove the plate aseptically.
- For next location, sanitize the stainless steel Hood and carry within air Sampler trolley.

Cover the plate with their upper lid then incubate for first 72 hours at 22.5 ^{0}C ± 2.5 °C and further 48 hours at 32.5^{0}C ± 2.5 °C for fungal and bacterial growth respectively.

OBSERVATIONS

Table 06: TAMC < Action limit or Alert limit signifies that the area is pass.

Grade-B | Method: Active Air Sampling		
Date	**Observation after 72hrs.**	**Observation after 48hrs.**
1/3/2019	<1	1
2/3/2019	<1	2
3/3/2019	<1	1
4/3/2019	<1	2
5/3/2019	<1	2
6/3/2019	<1	1
7/3/2019	<1	2

Table 07: TAMC > Action limit or Alert limit signifies that the area is fail.

Grade-B | Method: Active Air Sampling		
Date	Observation after 72hrs.	Observation after 48hrs.
1/3/2019	<1	2
2/3/2019	<1	3
3/3/2019	<1	4
4/3/2019	<1	3
5/3/2019	<1	2
6/3/2019	<1	3
7/3/2019	<1	3

TREND CHART ANALYTICAL DATA

Table 08: TAMC < Action limit or Alert limit signifies that the area is pass.

Demonstration of One week Trend Chart for Active Air Sampling in Grade-B Area							
Date	1/3/2019	2/3/2019	3/3/2019	4/3/2019	5/3/2019	6/3/2019	7/3/2019
TAMC	1	2	1	2	2	1	2
Limits	10	10	10	10	10	10	10
Action Limits	8	8	8	8	8	8	8
Alert Limits	6	6	6	6	6	6	6
Minimum	1						
Maximum	2						
Average	1.571428571						

Chart 01: TAMC < Action limit or Alert limit signifies that the area is pass.

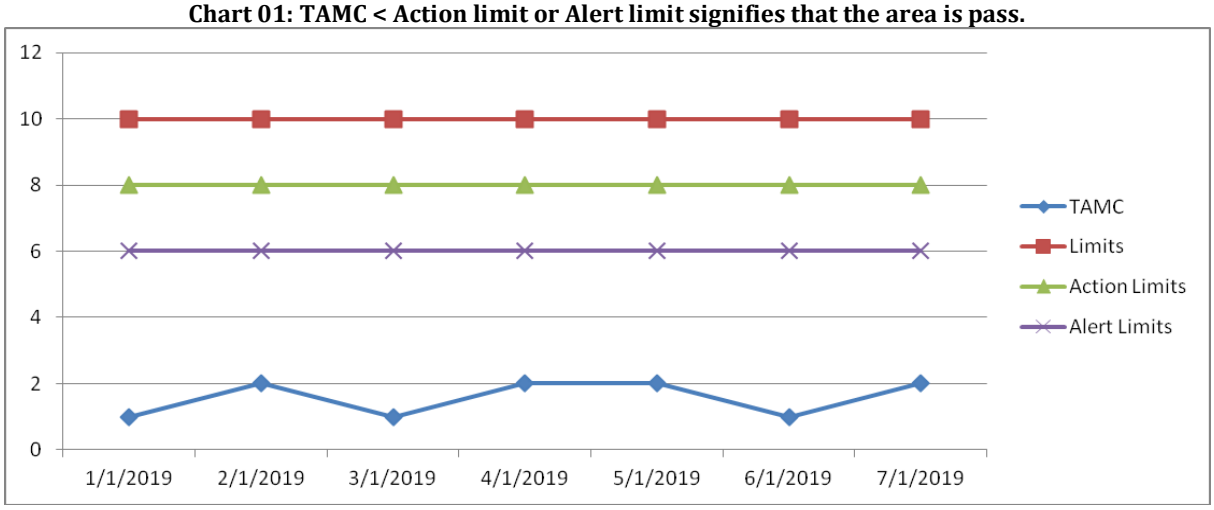
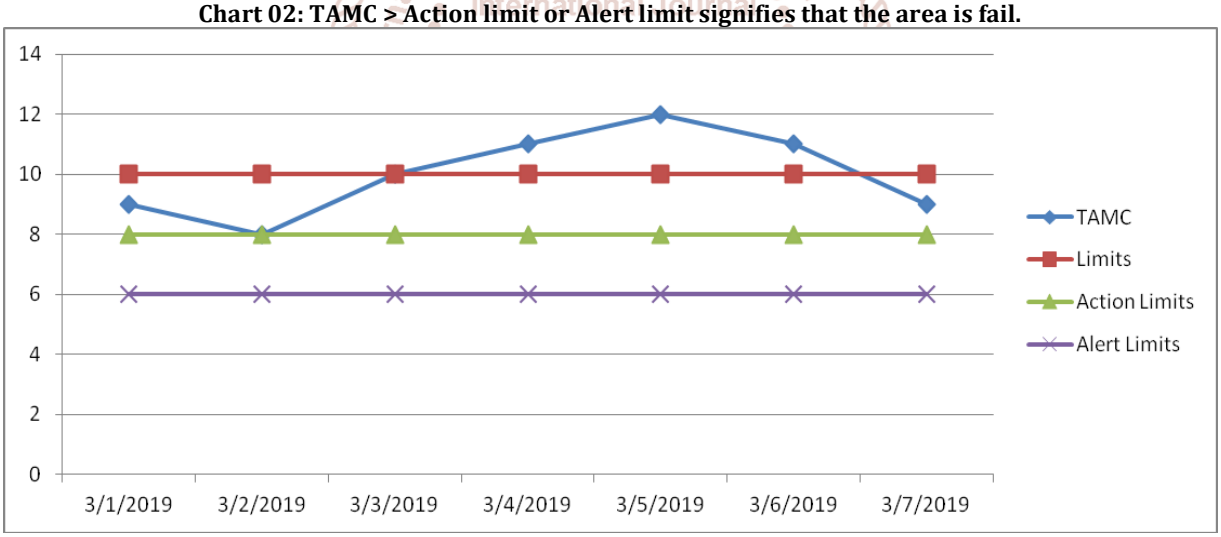


Table 09: TAMC > Action limit or Alert limit signifies that the area is fail.

Demonstration of One week Trend Chart for Active Air Sampling in Grade-B Area							
Date	1/3/2019	2/3/2019	3/3/2019	4/3/2019	5/3/2019	6/3/2019	7/3/2019
TAMC	9	8	10	11	12	11	9
Limits	10	10	10	10	10	10	10
Action Limits	8	8	8	8	8	8	8
Alert Limits	6	6	6	6	6	6	6
Minimum	2						
Maximum	12						
Average	10						

Chart 02: TAMC > Action limit or Alert limit signifies that the area is fail.



REFERENCES

[1] USP, "USP Microbiological Control and Monitoring of Aseptic Processing Environments," USP 35 vol. 1 2012a, 2012: pp. 697-707.

[2] Guidance for Industry – Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice, U.S. Food and Drug Administration, 2004

[3] "Manufacture of Sterile Medicinal Products" In EudraLex – The Rules Governing Medicinal Products in the European Union, Volume 4 EU Guidelines to Good Manufacturing Practice – Medicinal Products for Human and Veterinary Use – Annex 1: Manufacture of Sterile Medicinal Products, European Commission, 2008

[4] Dalmaso, G., and Denoya, C. "Microbial Control and Monitoring in Aseptic Processing Cleanrooms" Controlled Environments (2015) http://www.cemag.us/articles/2015/01/microbial-control-and-monitoring-asepticprocessing-cleanrooms

[5] ISO International Standard 14644 Part 1, International Organization for Standardization, May 1999

[6] https://en.wikipedia.org/wiki/Cleanroom