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Book Review

Compounding Safe Sterile Preparations According to USP Chapter <797>: How to Get Started with an In-House Microbial Air Sampling Plan

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Abstract

How to get started with an in-house microbial air sampling plan.

The microbial air monitoring in aseptic activities is an important process to guarantee the quality of medicine for the patients' health. The protocol to apply these tests should be a fundamental part of the education of the involved personnel.

The steps for the implementation of an active microbial air sampling plan are: A. Proper training on aseptic practices. Competencies for hand hygiene, garbing and aseptic techniques should be a minimal requirement for the involved staff. B. Risk assessment. Identification of the potential sources of contamination. C. Standard Operating Procedure. A SOP document should be prepared to cover all the steps of the air sampling plan. D. Sampling plan. The sampling should be performed when staff and material are in the area while activities are ongoing. E. Sampling location. Caution must be taken to ensure the sampling process doesn't contaminate the operation. F. Material. Adopt the model of air sampler suitable for the correct operation. G. Growth media. Adopt the agar media as indicated by the regulatory authorities. H. volume of air. It is suggested a volume of 1000 litres of air. I. Protocol and result interpretation must be part of the sampling plan.

Keywords: *Safe Sterile Preparations; USP Chapter <797>; In-House Microbial Air Sampling Plan*

Glossary

Active air sampler, aspirating chamber, aspirating head, at rest, classified area, CFU, contamination, bacteria, culture plate, disinfection, incubation, microorganism, Petri dish, risk assessment, standard, sterilisation, unidirectional airflow, viable.

Introduction

The microbial air monitoring in aseptic activities of hospitals and pharma industries is an important process to guarantee the quality of the medicines and the patients' health.

The protocol to apply this test should be a fundamental part of the education of the involved personnel.

The steps to follow for implementation of an active microbial air sampling plan

A. Proper training on aseptic practices

B. Risk assessment

C. SOP

D. Sampling plan

E. Sampling location

F. Material

G. Microbial air sampler

H. Volume of air

I. Protocol

J. Result interpretation

K. Conclusion

L. USP Guidelines

Proper training on aseptic practices

All the personnel must be trained for the correct aseptic practices. Staff must be trained on how to properly handle, label, report and incubate the media devices. Competencies for hand hygiene, garbing and aseptic techniques should be a minimal requirement for staff involved in an environmental monitoring to ensure the correct collection while minimizing the risk of false positive and false negative results.

- Always wipe hands and work area with 70% ethanol.
- It is recommended to wear gloves, better if sterile. This will prevent any foreign contaminants in contact with the customers and samples during testing. If gloves are not used, it is necessary to wash hands before and after testing.
- Wipe the outside of the containers, flasks, plates, and dishes with 70% ethanol before use.
- Always cap the bottles and flasks after use and seal multi-well plates with tape or place them in resalable bags to prevent microorganisms and airborne contaminants from gaining entry.
- Never uncover a sterile flask, bottle, Petri dish, etc. until you are ready to use it and never leave it open to the environment. Place the cover as soon as you are finished.
- If you remove a cap or cover and have to put it down on the work surface, place the cap with opening facing down on a cleaned/disinfected surface.
- Use only sterile glassware and other equipment.
- Be careful not to talk, sing, or whistle while performing sterile procedures.
- Perform experiments as rapidly as possible to minimize contamination.

Risk assessment

- Identification of the potential sources or routes of contamination (e.g.: the manual activity of operator, the correct packing of all introduced material, the efficiency and cleaning of HEPA filter, etc.).

- Preparation of a daily, weekly, monthly microbiological monitoring schedule to maintain under control the potential sources of contamination.
- Establishment of "alert and action levels" and resulting corrective actions if the levels are exceeded.
- Verification and analysis of the trend of microbiological sampling results to monitor the upper and lower limit and the slopes that can give indication about the future. The adverse trend should be considered more significative than the individual results.
- Management of appropriate documentation.
- Regular education and training of the involved personnel about the contamination control protocol.
- Review of the microbial results by a microbiologist to identify possible quantitative and qualitative shift of micro-organisms.

Standard operating procedure (SOP)

A SOP document should be prepared to cover all the steps of the air sampling plan. The SOP should include:

- Title
- Object
- Purpose
- Glossary
- References
- Responsibility
- Biological safety
- Material
- Protocol
- No-conformity
- Name and function of responsible
- Date of Revision
- IQ OQ PQ Guidance documents go hand in hand with establishing sampling SOPs.

Sampling plan

Microbial sampling should be performed when staff and material are in the areas while processing activities are ongoing with the full complement of people involved in the environment.

The manual operations under a unidirectional airflow must follow a strict movement: on the left side the clean material to be used, on the centre the handle manipulation, on the right side the dirty material.

Sampling location

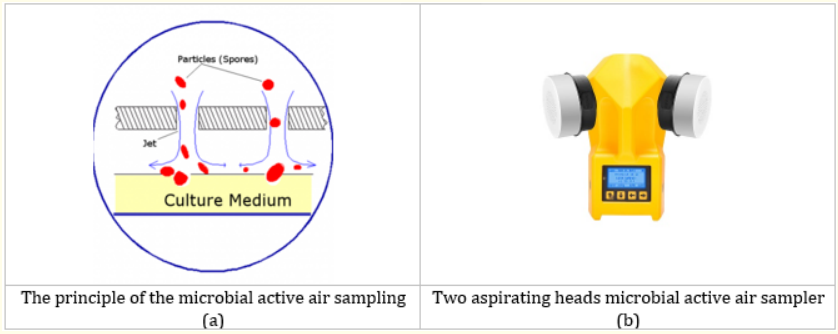
Microbial air sampling, part of Protocol, should occur when personnel and materials are in the area while processing activities are ongoing, with the full complement of personnel working in the environment. Caution must be taken to ensure the sampling process doesn't contaminate or impede defined operations.

A sampling map should be prepared for a visual guide to staff for obtaining consistency in result trending.

Material

Viable microbial air sampler

USP <797> states that the preferred method of volumetric air sampling is inertial impaction, an active monitoring process that physically draws a specified volume of air into a sampling "head" by a fan; air is accelerated through a perforated cover (e.g. sieve sampler). Once the air enters the chamber and meets the media of the culture plate, the air tangentially changes direction, and any suspended viable airborne particles impacts on the media. The revised USP <797> document states the required volume of air is 1.000 litres. The culture plate is then incubated to verify the presence of micro-organisms growth (CFU).



The principle of the microbial active air sampling (a)

Two aspirating heads microbial active air sampler (b)

Figure 1: *Example of single stage inertial impaction concept (a) and a dual sampling heads on active microbial air sampling with single impaction stage.*

Growth media

- USP states that the Trypticase Soy Agar (TSA) and Malt Extract Agar (MEA) are appropriate for conducting viable air sampling.
- Once the samples have been labelled and collected from the air sampler, they must be incubated at the proper temperature.
- The actual USP<797> indications require incubation of TSA at 30-35°C for 48-72 hours and 26-30°C for 5-7 days for MEA.
- The new proposed version states incubation of TSA at 30-35°C for no less than 48 hours followed by 20-25°C for no less than 5 days, which is considered adequate for both bacterial and fungi growth.

Microbial air sampler

An example of active microbial air sampler is the TRIO.BAS microbial air sampler. It is a high-performance instrument based on the principle of the Andersen impaction air sampler which aspirates air through a perforated plate. The resulting airflow is directed onto a standard Petri dish with nutrient agar. After the collection cycle, the Petri dish is incubated and then colonies counted.

Volume of air

The volume of aspirated air during sampling is 1.000 litres.

Protocol (Example)

Example case using a TRIO.BAS microbial air sampler with two aspirating heads with an airflow of 100 liters/minute per head.

Case 1: 1.000 liters of air sampled in 2 fractions (A, B) per each sampling head (1, 2). Sampling airflow rate equal to 100 liter per minute. Therefore, each fraction run is 500 liters.

Total working shift 2 hours, total cumulative sampling time 20 minutes

Starting "At rest" at 09:00 a.m. / "At end" at 11:20 a.m.

Case 2: 1,000 liters of air sampled in 3 fractions (A, B, C) per each sampling head (1, 2). Sampling airflow rate equal to 100 liters per minute. Therefore, each fraction run is 333 liters.

Total working shift 3 hours, cumulative sampling time 20 minutes

Starting "At rest" at 09:00:00 a.m. / "At end" at 12:20:30 p.m.

Case 3: 1,000 liters of sampled air in 3 fractions (A, B, C) per each sampling head (1, 2): sampling airflow rate equal to 100 liters per minute. Therefore, each fraction run is 333 liters.

Total working shift 4 hours, cumulative sampling time 20 minutes

Starting "At rest" at 09:00:00 a.m. / "At end" at 01:20: p.m.

Case #1: 2:20 hours Work Shift						
Head	**Fraction**	**Airflow Rate**	**Start Time**	**End time**	**Sampled volume**	**Total Sample Fraction**
[-]	**[-]**	**[l/minute]**	**[hh:mm]**	**[hh:mm]**	**[liters]**	**[%]**
1	A	100	09:00	09:05	500	50%
1	B	100	10:05	10:10	500	100%
2	A	100	10:10	10:15	500	50%
2	B	100	11:15	11:20	500	100%

Table 1*: Case 1 based on 2:20 hours work shift with a dual head microbial air sampler. Two sampling fractions of 5 minutes per sampling head, 1m³ of sampled air per head.*

Case #2: 3:21 hours Work Shift						
Head	**Fraction**	**Airflow Rate**	**Start Time**	**End time**	**Sampled volume**	**Total Sample Fraction**
[-]	**[-]**	**[l/minute]**	**[hh:mm:ss]**	**[hh:mm:ss]**	**[liters]**	**[%]**
1	A	100	09:00:00	09:03:30	333	33%
1	B	100	09:48:00	09:51:30	333	66%
1	C	100	10:37:00	10:40:30	333	100
2	A	100	10:40:30	10:44:00	333	33%
2	B	100	11:29:00	11:32:30	333	66%
2	C	100	12:17:00	12:20:30	333	100

Table 2: *Case 2 based on 3:21 hours work shift with a dual head microbial air sampler. Three sampling fractions of 3.30 minutes per sampling head, 1m³ of sampled air per head.*

Case #3: 4:21 hours Work Shift						
Head	**Fraction**	**Airflow Rate**	**Start Time**	**End time**	**Sampled volume**	**Total Sample Fraction**
[-]	**[-]**	**[l/minute]**	**[hh:mm:ss]**	**[hh:mm:ss]**	**[liters]**	**[%]**
1	A	100	09:00:00	09:03:30	333	33%
1	B	100	10:03:00	10:06:30	333	66%
1	C	100	11:07:00	11:10:30	333	100
2	A	100	11:10:30	11:14:00	333	33%
2	B	100	12:14:00	12:17:30	333	66%
2	C	100	13:17:00	13:20:30	333	100

Table 3: *Case 3 based on 4:21 hours work shift with a dual head microbial air sampler. Three sampling fractions of 3.30 minutes per sampling head, 1m³ of sampled air per head.*

Result collection, recording and evaluation

- All testing processes, results, and any corrective actions must be reported to demonstrate compliance.
- Data should be trended to determine the environment's state of control.
- Historical data should highlight any excursion points or emerging patterns of concern as shown below in figure 2.

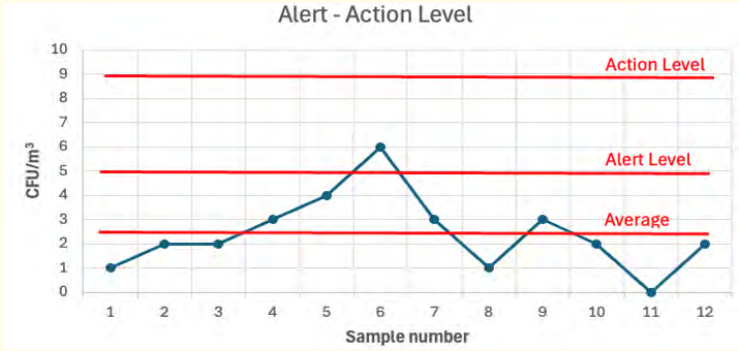


Figure 2: *Example of alert and action levels. Limits should be set for each sample site and individual sample result should be evaluated against the action and alert levels.*

Conclusion

The microbial environmental air monitoring, with surface monitoring included (carried out with RODAC plates) is a key performing tool to demonstrate a state of control of personnel and processes within the compounding locations.

USP Guidelines

USP Guidelines state that the contamination monitoring is performed during the normal working day activity of each shift. The current indications are under revision [1]:

Currently viable air sampling	Proposed changes revision for viable air sampling
Risk-based with initial sampling and at least every 6 months thereafter	Every 6 months for categories 1 and 2 and at least monthly for category 3. • Test at least 1.000 liters of air from each location sampled • Test must be performed in all classified areas during dynamic operating conditions to confirm that the required environmental quality is maintained

Table

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