RESEARCH

Selection and use of an active microbial air sampler

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ABSTRACT

The fields interested in the microbial test of the air are pharma, cleanroom, food, dairy, agro, beverage, hospital, clinical, bio-safety, space, defence, research, HVAC, microbiological laboratory, environment.

Several models of microbial air samplers are worldwide available.

Active air samplers have the purpose to collect viable particles onto a microbiological culture medium in a given volume of air for their enumeration.

Active air sampling can also be obtained by filtration on the membrane and consequent transfer of the membrane in an agar surface.

This paper has the purpose of helping to find the more appropriate instrument and it's correct us.

Key Words: *Active air sampler; Collection efficiency; Contamination; Culture plate; d50; Non-viable particle; Passive air sampler; Petri dish; Stokes number viable particle*

INTRODUCTION

The fields interested in the microbial test of the air are pharma, cleanroom, food, dairy, agro, beverage, hospital, clinical, bio-safety, space, defence, research, HVAC, and microbiological laboratory. Different reasons for this interest pharmaceuticals for the production of medicine in sterile environments; food, dairy, agro, and beverage companies to have under control the production; hospitals and clinics to monitor the operating theater and high-risk patient wards; environmental authorities to check the bio-safety; army to protect the soldiers in case of biological attack; research laboratory for experiments; HVAC to check the disinfection of the air in the building; disinfectant producers to check the activity of their product; space for the safety of astronauts; waste and water treatment plants to protect operators and town. Several models of microbial air samplers are worldwide available. Active air samplers have the purpose of collecting viable particles onto a microbiological culture medium in a given volume of air for their enumeration. Active air sampling can also be obtained by filtration on the membrane and consequent transfer of the membrane in an agar surface. Today are present on the market other systems based on fluorescence scan give the result in the shortest time in comparison with traditional microbiology but are not able to detect all types of viable particles. Several factors like moisture, temperature, electrostatic charge, light, air movements, and uneven distribution in the air of micro-organisms influence their distribution. This paper has the purpose of helping to find the more appropriate instrument.

Active and passive air sampling

The microorganisms present in the air were traditionally collected by simple gravity on the agar surface of a Petri dish; this method is called "passive sampling" or "settle plate sampling". The "active sampling" collects the microorganisms on the agar surface of a Petri dish in a given volume of aspirated air (typically a volume of 1 cubic meter = 1000 litres is considered). Active sampling is also used for membrane filtration. In the cleanroom of the pharmaceutical companies, both methods are used. Another important consideration is the "portability" and "stationary use" of the instrument because are two different conditions.

Active air sampling

Different parameters should be considered to evaluate the performances of an active air sampler:

1. Easy use
2. Easy cleaning and disinfection
3. Low weight (for portable instrument)
4. Robustness
5. Validation and Calibration
6. Price

Types of culture plates

Disposable 90 mm Petri dish or 55 mm Contact plate are used.

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Physical collection efficiency

The most viable particles present in the air are typically between 5-15 microns and increase to 15-18 microns for naturally occurring airborne particles such as skin flakes which contain bacteria. The air sampler must be able to precipitate particle sizes of at least 2 microns. This is called the "d50" or Stokes number.

Microbial collection efficiency

The microbial collection efficiency must be certified by an independent authority. The Annex G of EN 17141:2022 document gives guidance on verifying these performances and indicates two methods for this efficiency: the experimental method with aerosol chamber and a simplified laboratory method.

Types of active air samplers

The active air samplers can be classified as:

1. Portable unit to be used in different environments and positions at different times (Figure 1).

They are battery operated with charging by direct connection to the main by cable or by induction. These models are the most used.

2. Stationary unit to be used in a fixed position. They are used for Isolators and RABS (Figure 2).

They are battery or direct main operated.

Figure 1) *The yellow family is equal to portable units*

Figure 2) *The stainless steel family equals stationary units*

3. Direct impact on agar surface unit and subsequent incubation (Figure 3).

The impact on the agar surface is produced through small holes of an aspirating head or through a slit (Figure 4).

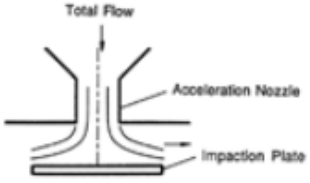

Figure 3) *Direct impact on agar surface*

Figure 4) *Impact holes on agar surface*

4. Filtration through a gelatin membrane to capture the microorganisms.

Subsequent aseptic transfer of the membrane on the agar surface of a culture medium before incubation.

Microbial air sampler principle

The aspirating chamber head contains uniformly spaced orifices or a slit. The base of the aspirating chamber accommodates a culture Petri dish with a nutrient agar. The air is aspirated by a vacuum produced by a turbine motor and allowed to impact the agar surface. The air is exhausted beyond the culture dish. The principle of the filtration system is more complex: after the exposure time, the gelatin filter is transferred and plated on the agar surface of a Petri dish.

Material to be used for the microbial test

The air sampler should be completed with agar culture media plates

1. TSA for Total Bacterial Count,
2. SDA for Total Yeast and Molds count,
3. Disinfectant spray like sterile isopropyl alcohol 72%,
4. Wipes for surface cleaning and disinfection,
5. Sterile aspirating head.

Protocol for the microbial test

A dedicated Standard Operative Procedure (SOP) should indicate to the operator all the necessary actions:

1. Availability of the document for test reporting,
2. Preparation of the air sampler (cleaning, disinfection),
3. Preparation of the culture plate sampling points,
4. Programming of the sampler air sampling,
5. Transfer of the culture plates to the laboratory.

It is essential to clearly specify all the actions related to the personal movements of the operator, ASEPT plating and removing the culture plate from the aspiration chamber, transfer of the plate to the laboratory (time and temperature). If the bacterial count is high, it is necessary to apply a corrective statistical table that is reported in the instruction manual of the sampler.

Calibration

The air sampler should be calibrated at regular intervals (generally every year). This fact should be considered with attention to find the calibration company that has the official approval for this test and can guarantee this control in a few working days.

Result Recording

The results of the reading of culture plates are reported as the number of CFU (Colony Forming Unit) referred at a volume of 1000 litres of air (1 cubic meter). If the plates are photographed, the pictures are included in the report to show them to the regulatory authorities in case of control. An example of such an application is the CFU Photo Camera (Orum International) (Figure 5 and 6).

Figure 5) *Photo camera for culture plate*

Figure 6) *Record of the result with the plate picture*

Microbial air sampling in cleanroom and associated controlled environments

The protocol for pharmaceutical companies that produce medicine in sterile conditions is indicated in several official documents of the FDA, ISO 14698-1, EN17141. A specific request of the EN17141 document is the continuous monitoring of the air in Grades A and B of the cleanroom. The continuous monitoring requests a dedicated air sampler that limits the dehydration of the nutrient agar during the aspiration of the air.

Microbial passive air sampling

The official documents for pharmaceutical companies request to adopt not only "active sampling" but also "passive sampling". The table or floor support for the culture plate should be adopted (Figures 7 and 8).

Figure 7) *Table Support for Passive Sampling*

Figure 8) *Floor Support for Passive Sampling*

Accessories for E.M. in cleanroom

Simple but effective E.M. accessories include (Figure 7):

1. Settle plate stands for a defined sample point reducing the risk of a spoilt sample;
2. Culture plates carriers to aid safe handling, transfer and correct use of incubator space;
3. Sterile aspiration head that removes the need for a validated sterilization process that is substituted with a certified sterile product, reducing the risk of false positive;
4. Table and floor tripods for the positioning of air samplers higher than work surface;
5. S/s cart on wheels for safety during movement staff;
6. Bags for culture plates, swabs, etc. during transfer to the laboratory.

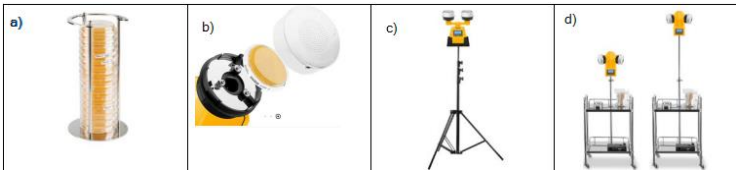

Figure 7) *a) Culture plate carriers b) Sterile aspiration head c) Tripods d) Stainless steel cart*

Characteristics and performances to take into consideration in a microbial air sampler selection

1. Basic performances (Air flow rate, culture plate)
2. Handling, cleaning, disinfection,
3. Weight
4. Number of aspirating chambers (1, 2, 3)
5. Portable or stationary model
6. Battery charging by cable or induction
7. Bluetooth incorporated
8. Sotiware with a photo camera for "CFU" recording
9. Explosion-proof
10. IP65 water and dust protection
11. Annual calibration
12. Continuous microbial monitoring
13. Availability of sterile aspirating head
14. IQ, OQ, PQ Documentation

CONCLUSION

The aim of this article was not to indicate a particular type of air sampler model but to highlight the important practical factors that should be considered. Application notes are available for the different applications in different fields for more dedicated and specific suggestions.

-Cleanroom	-Hospital
A.N. n. 125 Care, maintenance of air samplers	A.N. n. 121 Evaluation of microbial contamination in hospital, clinic, healthcare premises
A.N. n. 111 Applied microbiology in cleanroom	A.N. n. 66 Air sampling in hospital
A.N. n. 105 Microbial air sampler positioning in critical points	A.N. n.17 Nosocomial infections: Aspergillosis
A.N. n. 65 Air sampling in isolator	-Agro
A.N. 88 Pharma environmental microbiology	A.N. n. 117 Airborne bacteria in poultry premises
A.N. n.84 OOS according USP 1116	-Food and Dairy
-HVAC	A.N. n. 67 Air sampling in food premises
A.N. n. 123 Indoor biosafety sampling from building according NIOSH	A.N. n. 73 Air sampler disinfection
A.N. n. 86 Indoor air filtration	A.N. n. 23 Food glossary according EFSA
	A.N. n. 04 Staff training for food production

REFERENCES

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