Comparing Microbiological Air Monitoring Techniques for Critical Environments

Abstract

This paper discusses the use of active air and passive air settle plate monitoring for routine and continuous pharmaceutical manufacturing. Scientific literature supporting settle plates and active air sampler monitoring effectiveness is evaluated to support the transition from passive to active air monitoring.

Introduction

Contamination is monitored continuously with the latest in cleanroom technologies. However, traditional growth-based solutions, limited to 4-hour sampling periods, are still the most common method used for microbial monitoring. To provide evidence that a continuous sampling of cleanroom air is performed, pharmaceutical manufacturers widely use settle plates even if scientific and regulatory experts agree that they are a non-quantitative and non-validatable method. Compare this to active microbial air samplers, validated to run for a prolonged period in continuous mode to sample one cubic meter of air.

FIGURE 1 Example of active microbial air device: **MiniCapt Mobile® Microbial Air Sampler**

Method Efficiency

ISO 14698:2003's Annex B describes a technique for determining collection efficiency of microbial air samplers, broken into two separate types:

Physical efficiency is the ability of the sample to collect various sizes of particles.

Biological efficiency is the efficiency of the sample in collecting microbe-carrying particles.

Physical efficiency is the same for inanimate particles, particles carrying a microorganism or particles that are microorganisms. Biological efficiency is expected to be lower than physical efficiency because it depends on the survival of the collected microorganisms and the growth medium. Annex B is mainly concerned with physical efficiency.

In a 2005 publication, a highly accredited author concluded settle plates were a "fundamental method of measuring the number of microbe-carrying particles that will deposit onto a given area in a given time. There is therefore no need to determine its collection efficiency" [1]. In 2016, the European Journal of Parenteral & Pharmaceutical Science in partnership with Whyte and T. Eaton reassessed and suggested improvements to EU cGMP's Annex 1, specifically for how airborne concentration and settle plate counts of MCPs contribute to the grade of a pharmaceutical cleanroom [2]. Using more accurate deposition velocities, the EU cGMP maximum concentrations can be revised to provide more accurate settle plate counts.

ISO 14698:2003's Annex A specifies the selection of the microbial air sampling device to be dependent on the purpose of sampling. In addition, the device should have an impact velocity (speed of the air hitting the culture medium) that is a compromise between:

1. A high enough velocity to allow the entrapment of viable particles down to approximately 1 µm, and
2. A low enough velocity to ensure viability of particles by avoiding mechanical damage or the break-up of clumps of bacteria or micromycetes.

The ISO standard's recommendation has generally been a sampler at or near 50 percent physical recovery at 1 µm (a D50 of 1 µm). From a microbiological stance, 1 µm is the size of most common species of individual bacteria. Fungal particles are usually 2 to 5 µm, and *Bacillus anthracis* spores have a size range from 0.65 to 2.0 µm.

The Importance of Design

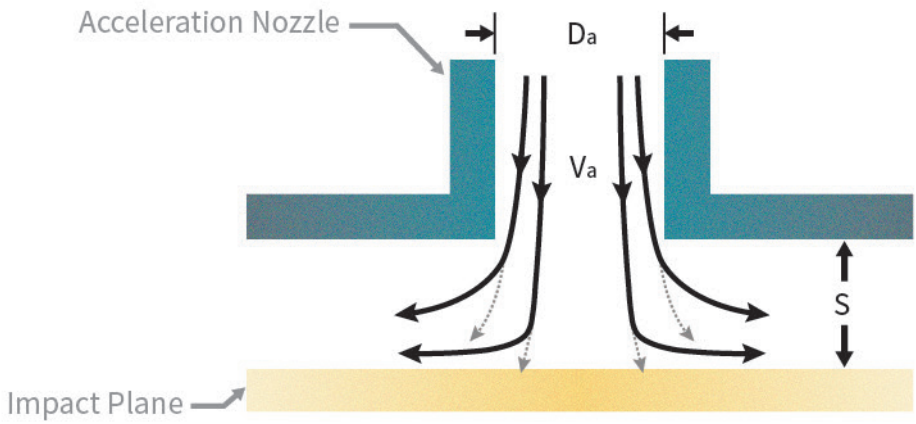

FIGURE 2 Airflow model of an active air sampler

If a stream of gas undergoes a sharp change in direction, the particles it transports will tend to continue in their original direction. Particles having different dimensions and densities will follow different trajectories and may be collected separately. When a jet of air is accelerated through a nozzle, the particles it transports are carried at the same speed as the fluid and follow its flow line. If the fluid flow lines rapidly change direction at the nozzle output, the particle trajectories will appreciably depart from the airflow lines, depending on the inertia associated with the particles. In other words, the particles will tend to run in a straight line and if they find a surface in their path, they can adhere to it.

Active air impactors are designed to sample particles in the air or other gas through a collision with a solid surface. The impactor's geometry is optimized to allow laminar flow into the nozzle (e.g., Re < 2300), with a velocity as high as possible and a D50 as low as possible.

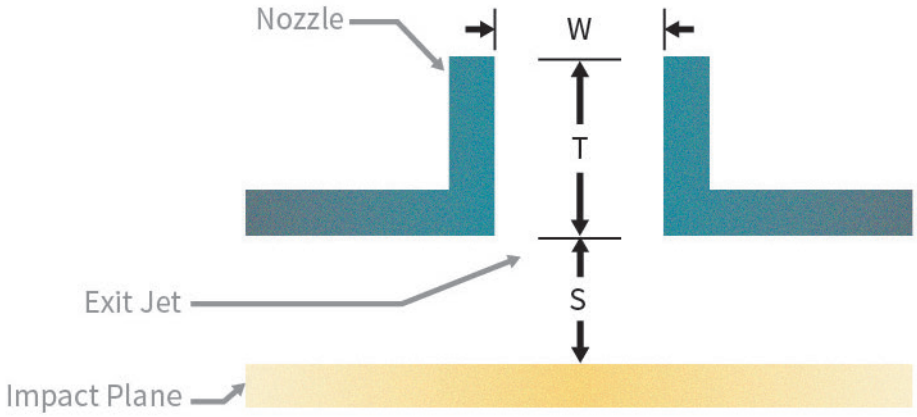

FIGURE 3 Active air sampler geometry

Comparing Monitoring Methods

Due to modern cleanroom clothing features, the microbial-carrying particles detected in aseptic areas with operators are mainly in the size range of 0.5 µm to 5 µm. For these reasons, continuous microbial active air sampling in Grade A is recommended, replacing the combination of settle plates and single or intermittently used active air sampling activities. The following table summarizes the differences between techniques.

TABLE 1 Technique comparison

COMPONENT	SETTLE PLATES	CONTINUOUS MICROBIAL ACTIVE AIR SAMPLING
Continuous monitoring	Both can be used to monitor all phases of production.	
Measuring the concentration of microorganisms in the air	Measures the number of microorganisms settling from the air onto a known surface area within a known time in a turbulent environment.	Measures the total number of microorganisms in a quantifiable volume of air.
Quantitative method	Not a quantitative method. Results are often the number of CFUs per settle plate, with the size of the plate and the time exposed often not reported.	A quantitative method. The results can be analyzed in terms of time and air quality.
Detect low concentrations of microorganisms	Does not detect low concentrations of microorganisms and provides very low sensitivity in Grade A due to high airflow.	Detects low concentrations of microorganisms.
Position in the filling machine	Both can be placed closer than traditional volumetric samplers to critical areas where the product is exposed to air. Both can be provided in sterile form and are smaller than traditional volumetric samplers.	
Comparison of microbiological and particulate data	The correlation of data is not definable due to the two methods' differences.	Correlation of data is possible because airflow speed of the two systems is similar.
Validation	Not validated.	Validated according to ISO 14698-1.

Table 1 highlights that active air sampling is an improved approach compared to both settle plates and traditional volumetric air samplers in Grade A continuous monitoring applications.

Monitoring in Different Cleanroom Areas

When patient safety is key, the qualification of pharmaceutical cleanrooms is a necessary step. Particularly, microbiological qualification verifies the cleanliness level of rooms where medicines are manufactured. Following qualification, companies must design a monitoring plan that demonstrates air quality is in accordance with specifications established during qualification. Through monitoring, microbiological contamination can be controlled and minimized.

Grade A (ISO 5) areas include the product and materials in contact with the product, including the surrounding environment (i.e., air). For this reason, they are considered extremely critical and subject to continuous monitoring with high air frequencies during all production phases. Grade B (ISO 7) areas are used to protect Grade A areas and include the presence of operators in a variable number depending on the production process. Here, the purpose of microbiological monitoring is to verify the level of microbiological contamination is within specifications. The microbial trend of these areas must always be constant or slightly decreasing.

Settle plates are not recommended in Grade A areas because they do not detect low concentrations of microorganisms and offer low sensitivity with their high airflow rate. Settle plates are only acceptable in Grade B, C, and D areas where less turbulent air movement allows for microbe-carrying particles to be deposited at a higher rate.

Continuous microbiological monitoring of Grade A air is already required by cGMP and implemented for total particle monitoring. It provides key information on the amount and size of total particles present in the air at a given sampling point. It is therefore extremely important to have a strategy for both particle and biological monitoring that utilize validated methodologies quantifying both the microorganisms and particles present in a sampling area. Doing so will aid in determining potential correlation between events and provide the data necessary for root cause investigations.

Conclusion

The settle plate method is a non-validated, non-quantifiable method that does not take into account a microorganism's recovery rate. This method should not be used in Grade A (ISO 5) where high air speeds are common and air changes are frequent. These conditions make it difficult or impossible for microorganisms and particles containing microorganisms to settle. Settle plates have more viability in static environments.

Continuous microbiological monitoring of air in critical areas should be performed with validated methodologies with a recovery rate as high as possible. Regulations will keep pushing standards higher, and this strategy promotes better process knowledge while substantially increasing sterility assurance of the released product.

Authors

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Gilberto Dalmaso has over 25 years' experience in pharmaceutical microbiology and sterility assurance, primarily with GlaxoSmithKline (GSK). In 2003, his laboratory gained the distinction of obtaining the world's first rapid microbial PAT approval from the US FDA. Today, Gilberto is the Global Life Science Scientific Officer for Particle Measuring Systems, serves on the European PDA Committee, is a reporter to numerous symposia on the microbiology and Pharma in Europe, Asia and United States, and is an ISO 9001 and HACCP quality system auditor.

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Anna Campanella, PhD, is the Global Sterility Assurance Advisor for Particle Measuring Systems. In this role, she uses her industry experience to collaborate and consult with pharmaceutical companies to develop and implement science-based strategies, principles of monitoring, and controlling and improving the chemical, physical, and microbiological state of various production processes. Anna has a diverse background in the Pharmaceutical field including a PhD in Molecular Medicine, expertise in QA&QC processes, validation of chemical and microbiological methods, validation of sterile production processes and experience in microbiological aspects of aseptic production processes.

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Paola Lazzeri has experience in supporting pharmaceutical companies in contamination control including cleaning and disinfection strategies. Her experience with pharmaceutical manufacturers started in 2005 in a cleanroom contamination control systems dealer company.

Today, Paola is the GMP specialist of the Sterility Assurance Team for Particle Measuring Systems SRL. In this role, she collaborates and consults with pharmaceutical companies to develop and implement principles to monitor and control microbiological contamination by improving science-based cleaning and disinfection strategies.

Bibliography

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