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LOW FLOW IS THE WAY TO GO FOR CONTINUOUS MICROBIAL AIR SAMPLING

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Overview

Since EU GMP Annex1 was updated in 2022, one of the major changes in Environmental Monitoring is for continuous microbial monitoring during the Aseptic Process in Grade A/B environments. This change has had an impact on current Environmental Monitoring programs that many Big Pharma companies adhere to. Most of those EM programs used active air samplers to do pre and post monitoring of the aseptic process.

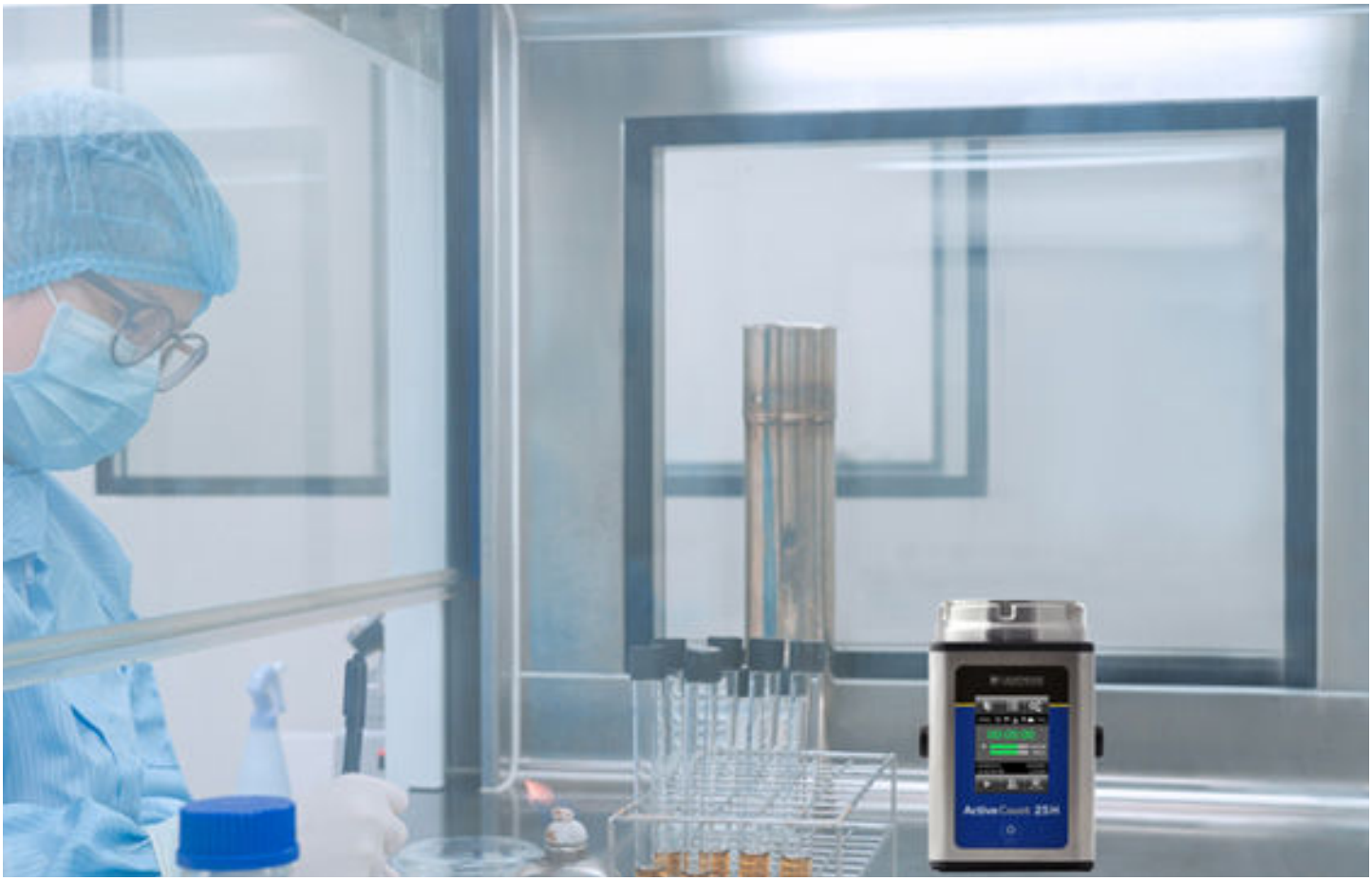

Fig.1 Biological Safety Cabinet testing using active air sampler with low flowrate.

The challenges in going from a pre and post monitoring program need to be understood correctly in order to implement a microbial monitoring program that is effective and mitigates risks of contamination to the product. Understanding air sampler technology, instrument specifications and applications as well as limitations is a starting point. Knowledge is key and it is always recommended to educate yourself on these technologies and how to successfully implement them.

So what challenges are faced in adopting a continuous microbial monitoring program?

Most companies already use microbial air samplers in their EM programs. As mentioned they are typically used to take a 1 cubic meter volume sample prior to aseptic processing operations whether it is in a BSC, LAF cabinet, Isolator, RABs system or sterile filling line this practice has been consistent for the last few decades.

One of the most important factors to consider is the flowrate of the air sampler. With Annex 1 CFU data is based on a sample of one cubic meter of air sampled. Below shows the table from the recent 2022 Annex 1, GMP Standard - Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use.

Table 6: Maximum action limits for viable particle contamination

Grade	Air sample CFU /m^3	Settle plates (diam. 90 mm) CFU /4 hours[a]	Contact plates (diam. 55mm), CFU / plate[b]	Glove print, Including 5 fingers on both hands CFU / glove
A	No growth[c]			
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

[a] - Settle plates should be exposed in grade A and B areas for the duration of operations (including equipment set-up) and changed as required after a maximum of 4 hours (exposure time should be based on validation including recovery studies and it should not have any negative effect on the suitability of the media used).

- For grade C and D areas, exposure time (with a maximum of 4 hours) and frequency should be based on QRM.

- Individual settle plates may be exposed for less than 4 hours.

[b] Contact plate limits apply to equipment, room and gown surfaces within the grade A and grade B areas. Routine gown monitoring is not normally required for grade C and D areas, depending on their function.

[c] It should be noted that for grade A, any growth should result in an investigation.

Note 1: It should be noted that the types of monitoring methods listed in the table above are examples and other methods can be used provided they meet the intent of providing information across the whole of the critical process where product may be contaminated (e.g. aseptic line set-up, aseptic processing, filling and lyophilizer loading).

Note 2: Limits are applied using CFU throughout the document. If different or new technologies are used that present results in a manner different from CFU, the manufacturer should scientifically justify the limits applied and where possible correlate them to CFU.

Fig.2 Maximum action limits for viable particle contamination (Source: Annex 1 -Manufacture of Sterile Medicinal Products, Aug 2022)

Therefore, the flowrate of the air sampling device plays a critical role. Many companies opted to purchase air samplers with fast flowrates. Some of these devices will sample a cubic meter air volume in 10 minutes with flowrates of 100L/min. These higher flowrate air samplers were ideal for pre and post sampling as outlined in EU GMP Annex 1 2003. Now with the 2022 update for continuous microbial monitoring these high flowrate models would not be practical for use because of the high flowrate. Lower flowrate air samplers will work best in continuous microbial monitoring. Let's consider an 8hr shift where an aseptic medical injectable product is processed and filled into a vial on a filling machine. The image below indicates the use of different flowrate air samplers. The cost of the media plates and labor costs are on the conservative side.

How air sampler flowrates affect your costs and time consumption

Fig.3 Impact of using a lower flowrate microbial air sampler in labor and cost.

Looking at the breakdown above it is pretty obvious that "low flow is the way to go" in continuous microbial monitoring in Grade A/B environments. Another major factor is the media changes. A 10L/min flowrate will only require 5 media dish changes throughout an 8hr production run compared to 48 media plate changes if using a 100L/min flowrate air sampler. This will reduce operator interventions into the critical zones with longer sample intervals the continuous air sampling objective is easily achieved.

How do you meet Annex 1:2022 compliance for continuous microbial sampling?

Annex 1 requires several data sets to be captured during monitoring of the critical zone in the aseptic environment where aseptic products are processed. These sampling modes are settle plates using 90mm diameter plates, active air sampling, contact plates using 55mm diameter plates and glove print method on a 90mm plate or 55mm plate. The latter two methods are tested pre, during operations and post processing activities to ensure that the operator and the surfaces in the critical zone capture data during the aseptic process. Also note that Annex 1 provides these methods as examples but the caveat is outlined in Note 1 from Section 9 covering the "Maximum action limits for viable particle contamination"

Note 1 states: "It should be noted that the types of monitoring methods listed in the table 6 are examples and other methods can be used provided they meet the intent of providing information across the whole of the critical process where product may be contaminated (e.g. aseptic line set-up, aseptic processing, filling and lyophilizer loading)".

Fig.4 Recommended method to monitor Grade A BSC gathering multiple data sets in the critical aseptic zone.

In the above recommendation I call this trifecta traditional microbial monitoring method. The remote particle counter is easily installed inside the BSC eliminating long tubing runs and tubing bends and effectively meets ISO/TR 14644-21 which requires sampling systems to be setup to eliminate particle losses in the data gathering system. This technical report was released in Nov 2023 and if the sample tubing and bends are over a set limit (refer to ISO/TR 14644-21 for details) then the sampling system must be completely validated for particle losses. This is something you want to avoid and therefore the recommendation is to place the sample probe directly onto the particle counter inlet and the particle counter over the critical zone.

The objective of the particle counter is not to monitor the process but to ensure the process receives clean air from the HEPA filter above the process. Airborne Particle Counters act as the alarm system which will notify users that the HEPA filter has failed or if there are excess particles in the environment. The particle counter counts and sizes all particles whether viable or non-viable. The role of the settle plate is to monitor the process. Larger particles drop out of the environment faster than smaller particles with lower inertia. The settle plate in this position is there to ensure that there is no viable contamination coming from the operator. The operator should be aseptically gowned up and if they are correctly then they should really only generate non-viable particles but as we know gloves and gowns sometimes move in opposite directions and skin around the wrists is unintentionally exposed to the critical zone. If the particle counter alarms based on excess particles then the operator must pause and check their gloves and gowns around the wrists and check gloves for any holes or damage. The probability of a viable event is extremely low if the operator is aseptically gowned up.

The active air sampler pulls a sample of air out of the critical zone environment using a vacuum to pull the sample. These instruments can be controlled by monitoring software and by the local operator to start and stop the sample and load a new agar plate when the sample is completed. The automated monitoring system will alert the operator to unload and load new agar plates during the aseptic processing duration.

Categories of aseptic processing and risk designation

Aseptic processing can be identified into the following Risk categories;

(1) High-Risk Aseptic Processing includes manual cleaning and disinfection, cleaning of components, bulk solution preparations, manual activity around the processing, sterilization of components and equipment, aseptic filling and aseptic sealing. Aseptic filling lines are a prime example of high risk aseptic processing as well as manual aseptic manipulations in BSC or LAF cabinets.

Fig.5

Aseptic Filling Line with manned operators – High Risk

(2) Medium Risk Aseptic Processing refers to systems where human interventions are protected by sealed gloved systems like isolators or RABs systems. Operators conduct process manipulations outside of the core aseptic areas.

Fig.6 Aseptic Isolator with sealed gloved ports– Medium Risk

(3) Low Risk Aseptic Processing refers to automatic Robotic Systems and operations that are conducted under isolator or barriers where the aseptic core is free of any human presence or activities. These systems effectively have removed cleanroom operators from the process by utilizing robotics to conduct the aseptic activities.

Fig.7 RIVA IV automated compounding system– Low Risk

How do I develop a robust continuous microbial monitoring program?

With the release of Annex1 and the need for continuous microbial monitoring many aseptic product manufacturers are asking the same questions. The best way to move forward is to pause and to reflect on the process. Review your monitoring plans, your sampling methods, and the sampling equipment. Risk Assessments are highly recommended, and it would be worth conducting one. Assemble a team with subject matter experts across the different departments and engage with creditable consultants to assist in the process

In the Risk Assessment the main criteria are to look at the whole process and here are some main areas to focus on.

- Determine if your process is a High, Medium, or Low risk process.
- Look at cleaning and disinfection activities, data, and review SOPs.
- Review gownup SOPs and data and type of gowns used (Low shedding gowns?)
- Can existing air samplers be retrofitted for low flow sample heads.
- Review how to minimise human interventions in the process.
- Review cleanroom certification data on particle concentrations
- Is the HVAC Air Change Rates cleaning up the aseptic area sufficient?
- What are the particle recovery rates like do should air changes be increased?
- Review operator cleanroom behaviour and enforce correct behaviour.
- Review real-time automated particle monitoring systems.
- Check that the sampling systems comply to ISO/TR 14644-21
- Understand air sampler technology and the critical d50 factor.
- Select the right monitoring equipment and strategies based on knowledge.
- Conduct robust performance qualifications of alert and action alarms.
- Engage in subject matter experts to assist in the Risk Assessment.
- Review ISO 14698 and EN17141 standards.

Why are air samplers so important and the selection of the right one?

If you type into google active air sampler you will get pages upon pages of information regarding many active air samplers. Engage with vendors that provide knowledge and can assist you in the technical review of their products. Not all air samplers are equal and some on the market are not even capable of capturing particles below 10µm as the d50 is at 10µm. The d50 is effectively the resolution of the air sampler. So what should you look for in an active air sampler? The following features should be reviewed when selecting an active air sampler.

- Footprint of the unit, size matters especially if you need it inside the critical zone
- Wipe down – how effective can the unit be disinfected are there knobs, switches that offer particle traps or areas of cleaning solution ingress.
- How well sealed is the air sampler, this is important when wiping down.
- Does the air sampler have a d50 of 1µm as per ISO 14698?
- Touchscreen interfaces offer a better interface and are easier to use.

- Security features enable unauthorized access to make unwanted configuration changes.
- Has the air sampler the ability to sample compressed gases (ISO 8573) with ease?
- What are the Biological Efficiency and Physical Efficiency properties of the air sampler.
- Has the air sampler validated by an independent and accredited facility?
- Is the exhaust HEPA filtered? You do not want desiccated agar media exhausted over surfaces.

Understand air sampler technology can help with the right selection.

What is the d50?

Essentially it is the resolution of the air sampler capability of the smallest particle size the system can capture.

ISO 14698 states that air samplers should have the ability (physical efficiency to capture particles of 1µm

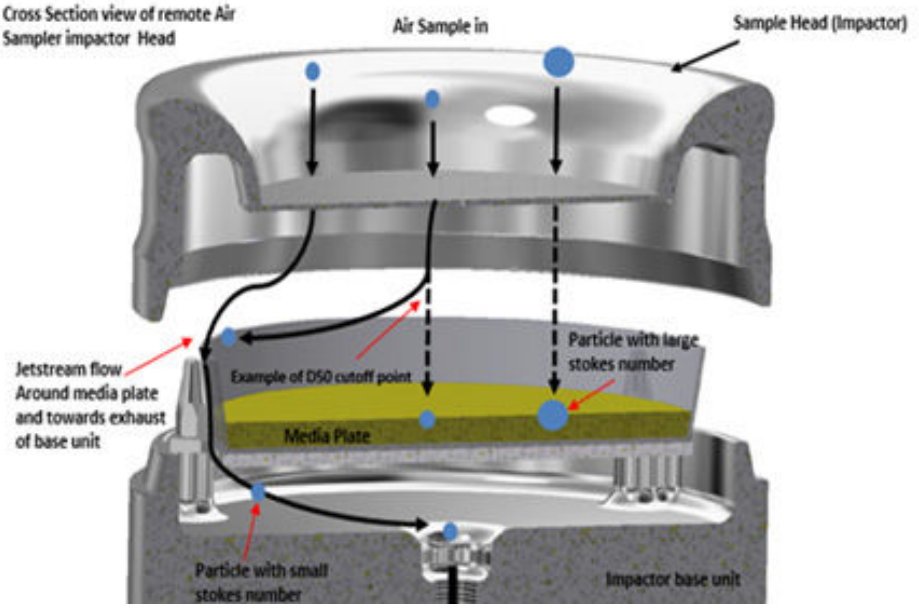

Fig.8 Overview of the d50 of an air sampler sample head

Which type should I use: Remote or Portable?

Fig. 9 Overview of two types of active air samplers 1.Portable 2.Remote

Summary

This tech paper discusses the implications of the 2022 update to EU GMP Annex 1, focusing on continuous microbial monitoring during the Aseptic Process in Grade A/B environments. Key points include:

1. Change in Environmental Monitoring (EM) Practices: The update has shifted the focus from pre and post monitoring of the aseptic process using active air samplers to continuous microbial monitoring. This requires a thorough understanding of air sampler technology, instrument specifications, applications, and limitations.

2. Challenges in Continuous Microbial Monitoring: Many companies already use microbial air samplers in their EM programs, typically for sampling a 1 cubic meter volume of air. The update necessitates a change in the approach, particularly regarding the flowrate of air samplers.

3. Importance of Flowrate in Air Samplers: The 2022 Annex 1 emphasizes that CFU data is based on a sample of one cubic meter of air. Lower flowrate air samplers are more suitable for continuous monitoring, reducing the frequency of media plate changes and minimizing operator interventions.

4. Compliance with Annex 1:2022: The update requires capturing several data sets during monitoring, including using settle plates, active air sampling, contact plates, and glove print methods. The methods should cover the entire critical process to avoid contamination.

5. Risk Categories in Aseptic Processing: Aseptic processing is categorized into high, medium, and low risk, based on the level of manual intervention and the use of protective systems like isolators or robotic systems.

6. Developing a Robust Monitoring Program: To comply with Annex 1, companies should review their monitoring plans, sampling methods, and equipment. Risk assessments and consultations with subject matter experts are recommended.

7. Importance and Selection of Air Samplers: The selection of the right air sampler is crucial. Factors to consider include the unit's footprint, ease of disinfection, sealing, resolution (d50), and features like touchscreen interfaces and security as well as HEPA filtered exhausts.

In summary, the 2022 update to EU GMP Annex 1 has significant implications for continuous microbial monitoring in aseptic processing. It necessitates a reevaluation of current practices, particularly in the selection and use of air samplers, to ensure compliance and effective monitoring.