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Air Monitoring in Cleanroom Environments by Gelatin Filters according to EN 17141 and ISO 14698

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Abstract

Controlled environments are classified based on the concentrations of airborne particles, with prescribed limits defined for non-viable and viable particles. Therefore, biocontamination control strategies are a key requirement of recent standards and guidelines, such as the EN 17141 and the revision of the EU GMP Annex 1. The EN 17141, published in 2020, has been adopted in the European Union and United Kingdom, replacing the ISO 14698. The Annex E chapter of EN 17141 details guidance on culture-based microbiological monitoring methods and sampler verification. This Application Note focuses on viable microbial monitoring and demonstrates that microbial air monitoring by Gelatin Membrane Filtration meets the requirements of the EN 17141 and the ISO 14698, which remains the standard outside the EU and the United Kingdom.

Introduction

Sterile and biopharmaceutical manufacturing necessitates the implementation of biocontamination control strategies. These are achieved through the careful design of processes to avoid contamination, implementation of monitoring systems to detect contamination, and remedial strategies to swiftly address contamination incidents. Failure to adopt such strategies often has serious consequences.

There are multiple sources of contamination in a manufacturing process, and the risk of contamination increases with every routine intervention, such as stopper refills, and the change of air monitoring plates. Thus, microbial environmental monitoring programs, which includes volumetric air sampling, are crucial in determining the efficiency of biocontamination prevention measures.

Air contaminants can be measured by active sampling, which requires active drawing of a defined volume of air by a specialized device. This facilitates accurate measurement of microbes in colony forming units (CFU) per cubic meter of air (m^3).

Sampler efficiency can be categorized into physical efficiency and biological efficiency. Physical efficiency is a ratio of the number of microorganisms collected by the sampler to the total number present in the sampled volume of air. The biological efficiency is the ratio of the number of viable microorganisms recovered from the air sample to the number that was expected to be collected. This measures the stress caused during sampling.

Within the EN 17141, additional requirements for verifying the suitability of air samplers have been established. Annex E provides information relating to culture media-based air sampling methods and verification requirements. It requires that the physical and biological sampling efficiencies of the air sampling method are established.

The EN 17141 requires that a d_{50} value be determined to qualify the physical efficiency of the sampler. The d_{50} is the particle size at which 50% of the particles are collected, while the rest evade impaction. Particles that are larger than the d_{50} size have a higher probability of recovery and vice versa.

The standard specifies that the mean equivalent diameters of microbe-carrying particles (MCP) forming CFU are generally larger than 1 µm and a d_{50} value of less than 2 µm is considered suitable. It adds that the d_{50} value can be determined for impaction samplers with multiple holes (sieve) or with rectangular slots (slit) and is necessary only for their comparison.

Since the Gelatin Membrane Filtration (GMF) method is filtration-based, a d_{50} value is not applicable. Instead, a retention rate is specified for filter membranes.

Our results demonstrate that the GMF method provides retention rates of > 97,996% for *Bacillus subtilis* spores at different particle sizes.

For the biological sampling efficiency in both standards, the membrane filtration method is defined as the reference method.

The GMF method was also challenged with environmental air to prove the sampling performance for a wider range of airborne microbes.

We demonstrate, that even after 8 h of continuous sampling, microbial recovery was unperturbed and that there were no adverse effects caused by long-term sampling.

Gelatin Filters have a nominal pore size of 3 µm, which is indirectly determined by the air flow rate. However, Gelatin Filters have the retentive capacity of a depth filter (e.g., HEPA H14 filter) and facilitate near complete retention of viruses due to the sieving and diffusion effect. In sum, Gelatin Filters are purpose built for reliable, continuous, intervention-free microbial air monitoring. Our results demonstrate that a requalification of Sartorius microbial air monitoring instruments and consumables are not required and that the GMF method is compliant with both the EN 17141 and ISO 14698 requirements.

Physical Sampling Efficiency

Spores of *Bacillus subtilis var niger* spores (1.2 µm x 0.8 µm) is a robust microorganism that withstands the stress imposed by aerosolization.

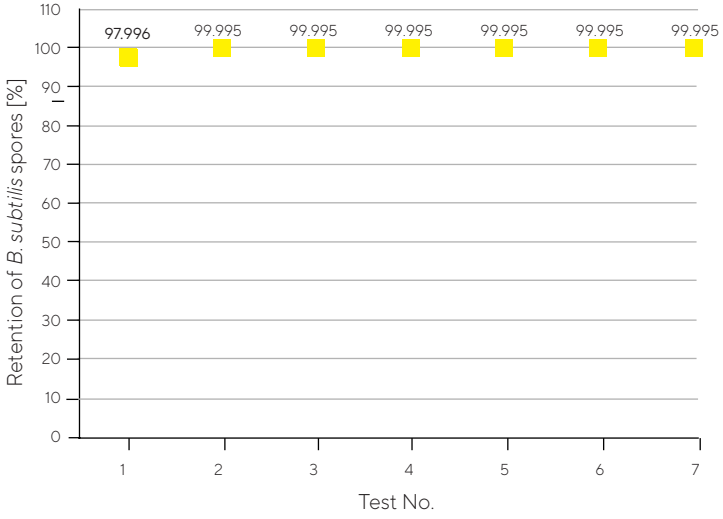
We generated microbial aerosols of particles of different sizes containing viable *B. subtilis* spores in a controlled environmental chamber by a spinning top aerosol generator. We injected suspensions (about 10^6 CFU per ml) of the spores in 0-7% (w/v) solutions of potassium iodide (KI) in 80% ethanol into the aerosol generator. The size of the particles was regulated by the speed of the spinning top and the KI concentration in the suspension.

Samplers placed equidistant from the aerosol generator collected samples simultaneously.

Retention Rate

We determined the retention rate of the Gelatin Membrane Filter by a bacterial challenge test (BCT) using aerosolized *Bacillus subtilis* spores. In the BC test, we used 1.2*10^5 CFU/cm^2 *B. subtilis* spores to challenge a Gelatin Filter and then tested the filtered air for *B. subtilis* contamination.

Figure 1: *Retention rate of Gelatin Membrane Filters challenged with 1.2*10^5 CFU/cm^2 B. subtilis spores.*

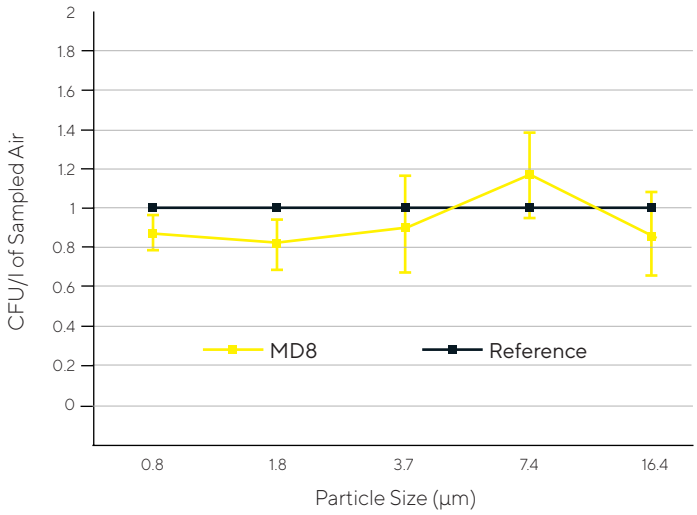


The Gelatin Filters provide highest retention rates of > 97.996% for *B. subtilis* spores (Fig. 1).

Physical Sampling Efficiency

The results demonstrated that there were no significant differences in collection efficiencies between the particles of 0.8 µm and 16 µm (Fig. 2).

Figure 2: *Physical sampling efficiency of Gelatin Membrane Filters challenged with 1.2*10^5 CFU/cm^2 B. subtilis spores at different particle sizes compared to Casella slit sampler.*



Biological Sampling Efficiency

There is a common misconception that the desiccation caused by the air being drawn through all membrane filters can adversely affect the viability of vegetative microorganisms. Our results demonstrated that Gelatin Membrane Filters can reliably collect viable airborne particles with no loss of recovery.

Gelatin Filters, unlike conventional membrane filters, are hygroscopic and absorb moisture from the environment. This residual moisture creates a protective capsid and shields microorganisms that may be susceptible to dehydration stress.

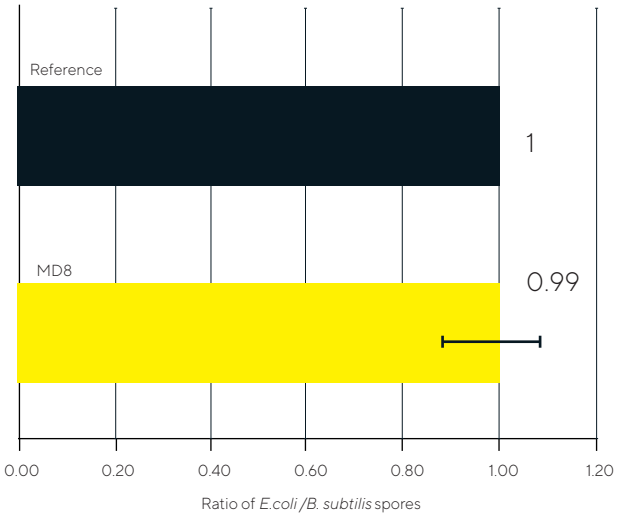
We determined the biological efficiency of the GMF method by sampling from aerosols containing vegetative bacteria and by comparing to the recovery of microorganisms in the natural air of laboratories [1].

Escherichia coli and *Bacillus subtilis*

Aerosols containing a mixture of *B. subtilis var niger* spores (1.2 µm x 0.8 µm) and gram-negative *E. coli* (2 µm x 1 µm) were generated in an environmental chamber by spraying.

Our results demonstrated that there were no significant differences in the ratio of *E. coli* to *B. subtilis* spores compared to the Casella slit sampler (Fig. 3). This confirms that GMF-based air sampling does not adversely affect the viability of vegetative microorganisms.

Figure 3. *Biological sampling efficiency of the Gelatin Membrane Filters for a suspension of E. coli/B. subtilis spores compared to Casella slit sampler.*

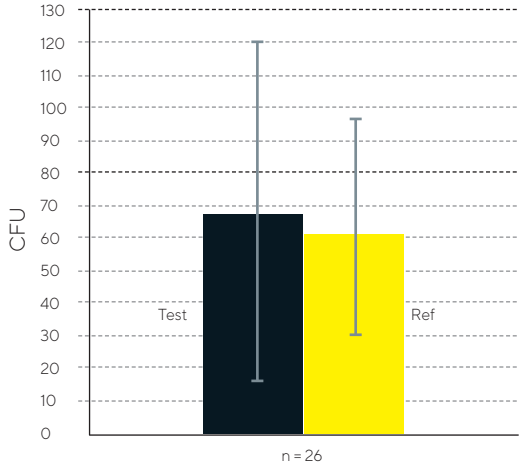


The complete report of the tests described can be reviewed during an audit.

Continuous Microbiological Air Monitoring (8 h)

We demonstrated that following 8 hours of continuous air sampling from environmental air, there was no significant change in the mean CFU count compared to a control. Further, there was no significant change in the microbial range compared to a sampling period of only 30 minutes (Excerpt from our second Application Note: Fig. 4 and 5).

Figure 4. *Microbial recovery on Gelatin Membrane Filters after 8 h of sampling (Test) compared to Gelatin Filters placed on nutrient medium directly after 30 min. of sampling (Ref).*



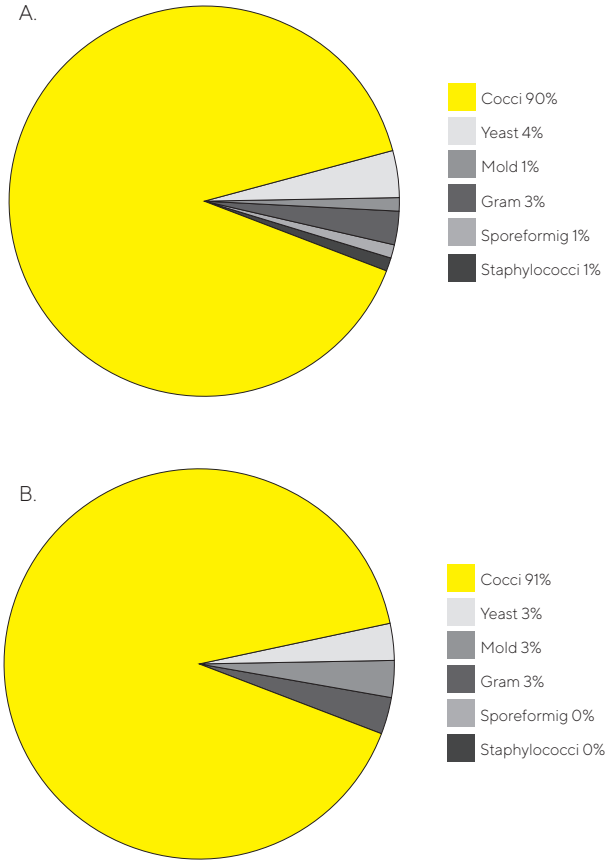


Figure 5. *Comparison of the range of species recovered on Gelatin Filters after 8 h of sampling (A) and Gelatin Filters placed on nutrient medium directly after 30 min. of sampling (B).*

Virus Detection

Although beyond the scope of this Application Note, it is worth mentioning that the unique Gelatin Membrane Filters have been successfully used for over three decades to sample viruses from air samples. The Gelatin Membrane Filters have been used to sample for the Middle East Respiratory Syndrome coronavirus (MERS-CoV) during the MERS outbreak in 2013 [5] and the Severe Acute Respiratory Syndrome coronavirus (SARS-CoV-2) during the recent pandemic [7]. Studies have demonstrated that the physical efficiency of the Gelatin Membrane Filter exceeds 96%, even for virions smaller than 80 nm [4]. Our own data demonstrate 99.9% and 99.94% retention of T1 phages and T3 phages, respectively [2,3]. The GMF method has also been used as the reference method for comparing the physical and biological sampling efficiency of samplers for virus sampling [4,6]. Please refer to our Application Notes on virus sampling for further information.

Benefits of the Gelatin Filtration Method

- Detect low microbial loads as typically met in cleanrooms by increasing the flow rate within the specific time or extending the sampling period. This allows for large volume sampling and / or long-term sampling and to continuously collect air over 8 hours without adversely affecting microbial recovery.
- Avoid the introduction of nutrient media into an isolator/ filling line.
- No water loss from nutrient media through dehydration.
- Easy microbial recovery from within the pore structure is ensured since the Gelatin Membrane Filters readily dissolve on the agar surface.
- Avoid operator intervention (e.g., frequent change of medium plates) during 8 hours of sampling in a typical working shift. The 2020 revision of the EU GMP Annex 1 specifies that any risk caused by interventions of the environmental monitoring operations be avoided. It also strongly recommends monitoring personnel following interventions and on each exit from the cleanroom.
- Proven retention of viruses facilitates the monitoring of airborne adventitious viruses.
- Use of Gelatin Membrane Filters with rapid detection methods (PCR).

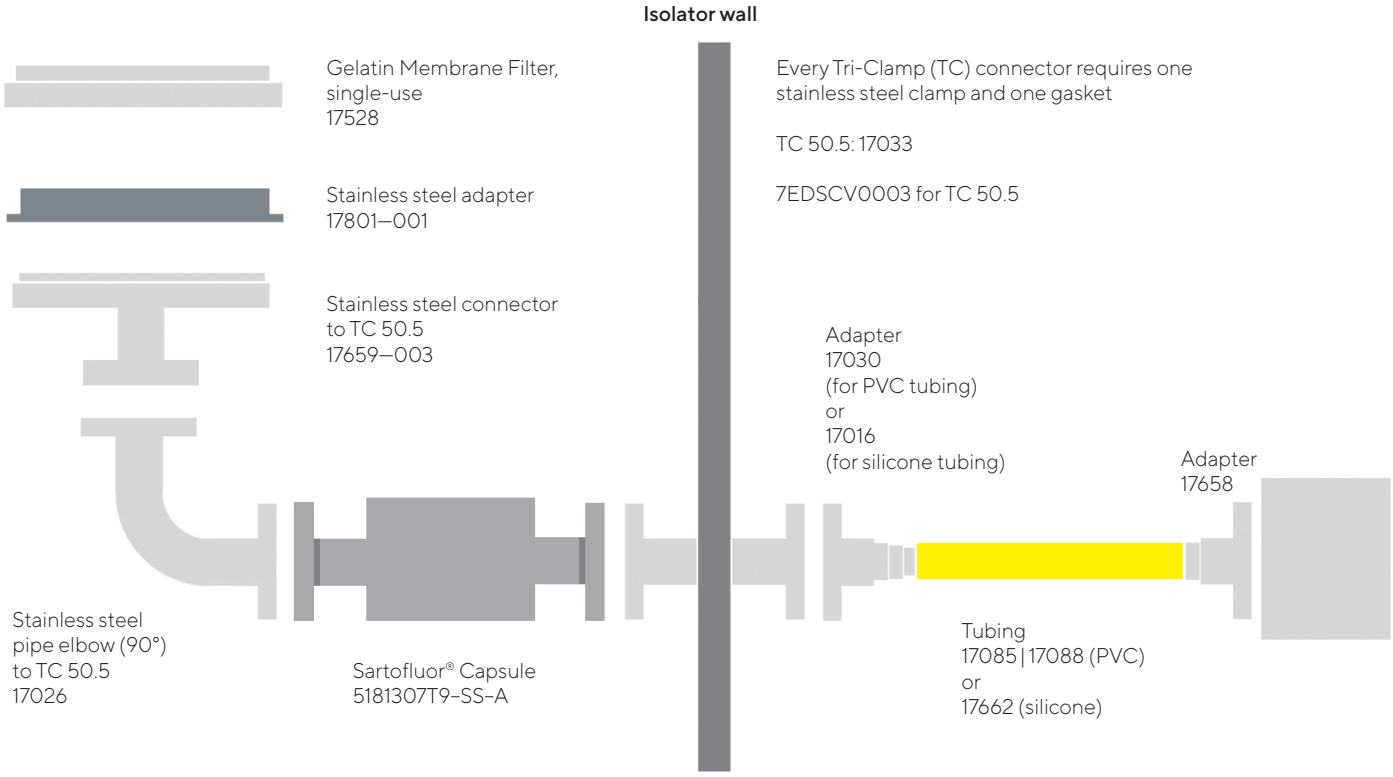
Technical Air Sampler Performance

The MD8 Airscan® is specifically developed to be used in cleanroom environments without disturbing flow patterns. The sampling head can be integrated into an Isolator, Filling Line or any other cleanroom environment at critical control points and enables isokinetic sampling. The sampling head (the entire air flow path) can be sterilized in line with vaporized/ vapor-phase hydrogen peroxide (VHP).

The sampling volume is adjusted by the air flow rate, which is monitored and controlled constantly by an integrated air flowmeter. Multiple configurations of the Gelatin Membrane Filters are available to suit every microbiological testing need. This includes gamma-sterilized single and triple bagged Gelatin Membrane Filters or Gelatin Membrane Filters in Biosafe® bags for aseptic transfer, via a Biosafe® rapid transfer port, into isolators, RABS and cleanrooms. The Biosafe® bags circumvents the need to load an advanced aseptic processing system prior to VHP decontamination.

Figure 6. *Gelatin Filtration method comprising the MD8 Airscan®, a sampling head to be installed in cleanroom environments and pre-sterilzed Gelatin Membrane Filter disposables.*

Figure 7. *Installation scheme of the MD8 Airscan® in an Isolator.*



Conclusion

We have demonstrated that Gelatin Membrane Filters have a retention rate of >97.996% and a physical sampling efficiency >80% compared to a Casella sampler.
In both standards EN 17141 as well as ISO 14698, the membrane filtration method is specified as the reference method for the biological sampling efficiency.
Nevertheless, we have compared the biological efficiency of our GMF method against a Casella slit sampler and demonstrated that there were no differences in the ratio of gram-negative *E. coli* to *B. subtilis* spores compared to a Casella sampler.

The high biological sampling efficiency of the GMF remains unchanged even following an 8 hour sampling period. The mean CFU count on the filters as well as the range of the recovered microbe species are not negatively affected by continuous sampling.

Our results demonstrate that a re/qualification of Sartorius microbial air monitoring instruments and consumables are not required and that the GMF method is compliant with both the EN 17141 and ISO 14698 requirements. This method also caters to the specifications of the 2020 revision of the EU GMP Annex 1, regarding the environmental monitoring of viable particles. View on-demand webinar here.

Click to view on-demand webinar.

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