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Simplifying Progress

Continuous Microbial Air Monitoring

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Agenda

- Introduction – Regulatory landscape
    - Annex 1
- Snapshots
- Gelatin Membrane filters
- Experimental data

Manufacture of Sterile Products

Possible sources of contamination :

- Personnel
- Ventilation (HVAC system)
- Room structure
- Raw materials – including Water
- Machinery, Equipment & tools

EU GMP Annex 1 - Revision

1971

"Manufacturing of sterile medicinal products"

Updates until 2010

2017

- December
- public consultation with over 6000 comments

2020

- 20 February – 3 month phase for commenting
- "Manufacture of Sterile Products"

EU GMP Annex 1 - Revision

Focus:

- Quality Risk Management (QRM)
- Contamination Control Strategy (CCS)
- Barrier technology/ Advanced aseptic processing systems

- Useful aspects for non-sterile manufacturing

- Impacts manufacturing in Europe & products exported to Europe

EU GMP Annex 1 - Revision

Section 4. Cleanroom and clean air equipment qualification

Qualification of clean room:

i. Installed filter leakage and integrity testing.

ii. Airflow measurement - Volume and velocity.

iii. Air pressure difference measurement.

iv. Airflow direction and visualisation.

v. **Microbial airborne and surface contamination**.

vi. Temperature measurement.

vii. Relative humidity measurement.

viii. Recovery testing.

ix. Containment leak testing.

EU GMP Annex 1 - Revision

Table 2: Limits for microbial contamination during qualification

Grade	Air sample cfu/m^3	Settle plates (diameter 90 mm) cfu/4 hours [a]	Contact plates (diameter 55 mm) cfu/plate
A[b]	No growth[b]		
B	10	5	5
C	100	50	25
D	200	100	50

The requalification of cleanrooms and clean air equipment should be carried out periodically:

- For Grade A & B areas, the maximum time interval for requalification is 6 months.
- For Grade C & D areas, the maximum time interval for requalification is 12 months.

EU GMP Annex 1 - Revision

Section 9. Environmental and process monitoring program

- Part of the CCS
- Monitors the controls designed to minimise the risk of particulate and microbial contamination

i. Environmental monitoring – non-viable particles.

ii. **Environmental and personnel monitoring – viable particles.**

iii. **Aseptic process simulation (aseptically manufactured product only).**

EU GMP Annex 1 - Revision

ii. Environmental and personnel monitoring – viable particles:

- Where aseptic operations are performed, microbial monitoring should be frequent using **a combination of methods** such as settle plates, **volumetric air sampling**, glove, gown and surface sampling (e.g. swabs and contact plates).

- Continuous **viable air monitoring in the Grade A zone** (e.g. air sampling or settle plates) should be undertaken for the **full duration of critical processing**, including equipment (aseptic set-up) assembly and filling operations. **A similar approach should be considered for Grade B cleanrooms** based on the risk of impact on the aseptic processing. The monitoring should be performed in such a way that all interventions, transient events and any system deterioration would be captured and **any risk caused by interventions of the monitoring operations is avoided.**

EU GMP Annex 1 - Revision

2020 draft

- 9.29 Sampling methods and equipment used should be fully understood and procedures should be in place for the correct operation and interpretation of results obtained. The recovery efficiency of the sampling methods chosen should be qualified.
- 9.7 Sampling methods should not pose a risk of contamination to the manufacturing operations.

EU GMP Annex 1 - Revision

ii. Environmental and personnel monitoring – viable particles:

- Monitoring should include sampling of personnel at periodic intervals during the process. Sampling of personnel should be performed in such a way that it will not compromise the process. Particular consideration should be given to **monitoring personnel following involvement in critical interventions and on each exit from the Grade B cleanroom**.

- Viable particle monitoring should **also be performed within the cleanrooms when normal manufacturing operations are not occurring** (e.g. post disinfection, prior to start of manufacturing, on completion of the batch and after a shutdown period), and in associated rooms that have not been used, in order to detect potential incidents of contamination which may affect the controls within the cleanrooms. In case of an incident, additional sample locations may be used as a verification of the effectiveness of a corrective action (i.e. cleaning and disinfection).

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iii. Aseptic process simulation (aseptically manufactured product only):

- Process simulation tests should be performed as part of the initial validation, with **at least three** consecutive satisfactory simulation tests that cover all working shifts that the aseptic process may occur in.

- process simulation tests (periodic revalidation) should be repeated **twice a year** (approximately every six months) for each aseptic process, each filling line and each shift.

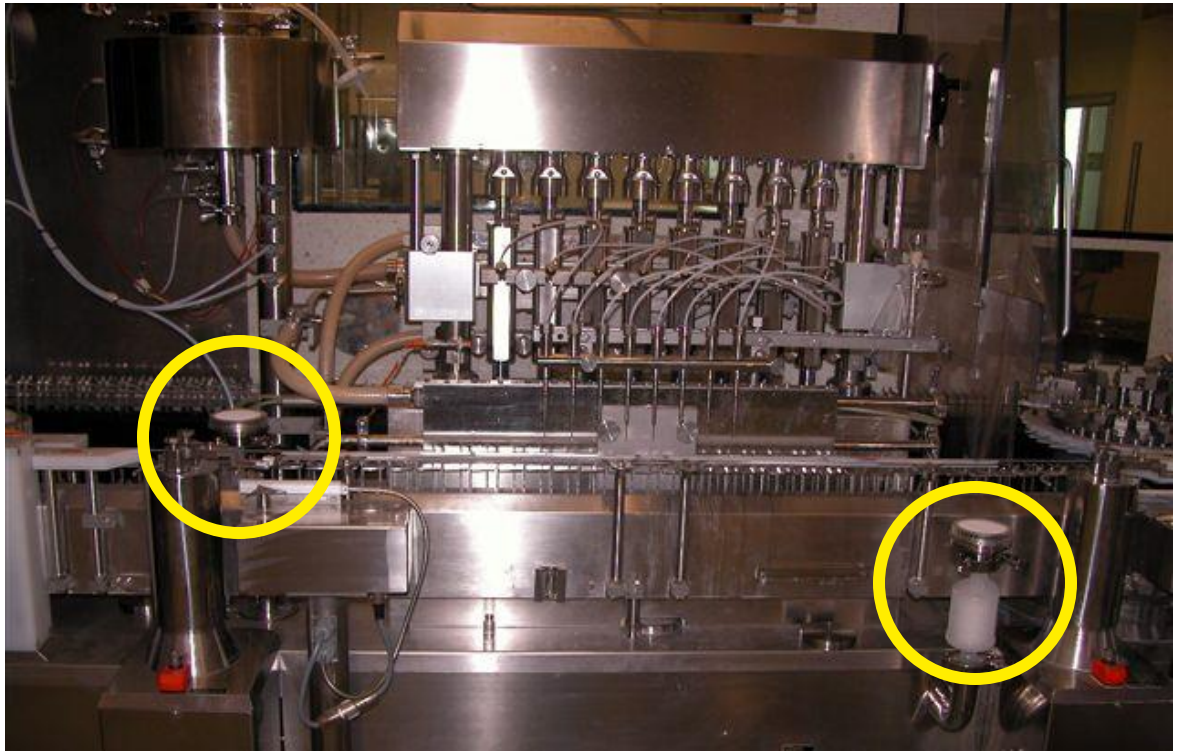
- The process simulation testing should **take into account various aseptic manipulations and interventions** known to occur during normal production

EU GMP Annex 1 - Revision

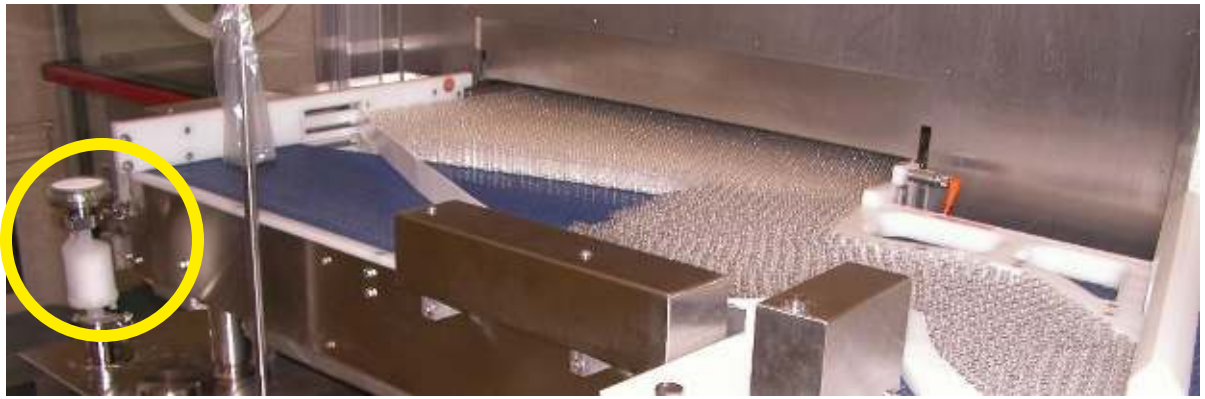
iii. Aseptic process simulation (aseptically manufactured product only):

- The target should be **zero growth**.
- A sufficient number of successful, consecutive repeat media fills (normally a **minimum of 3**) should be conducted in order to demonstrate that the process has been returned to a state of control.
- All products that have been manufactured on a line subsequent to a process simulation failure should be **quarantined** until a successful resolution

Snapshots – Multipoint sampling



Multipoint sampling of a filling line



Depyrogenation of vials

Exit after depyrogenation

*Source

[1]Reference

Snapshots

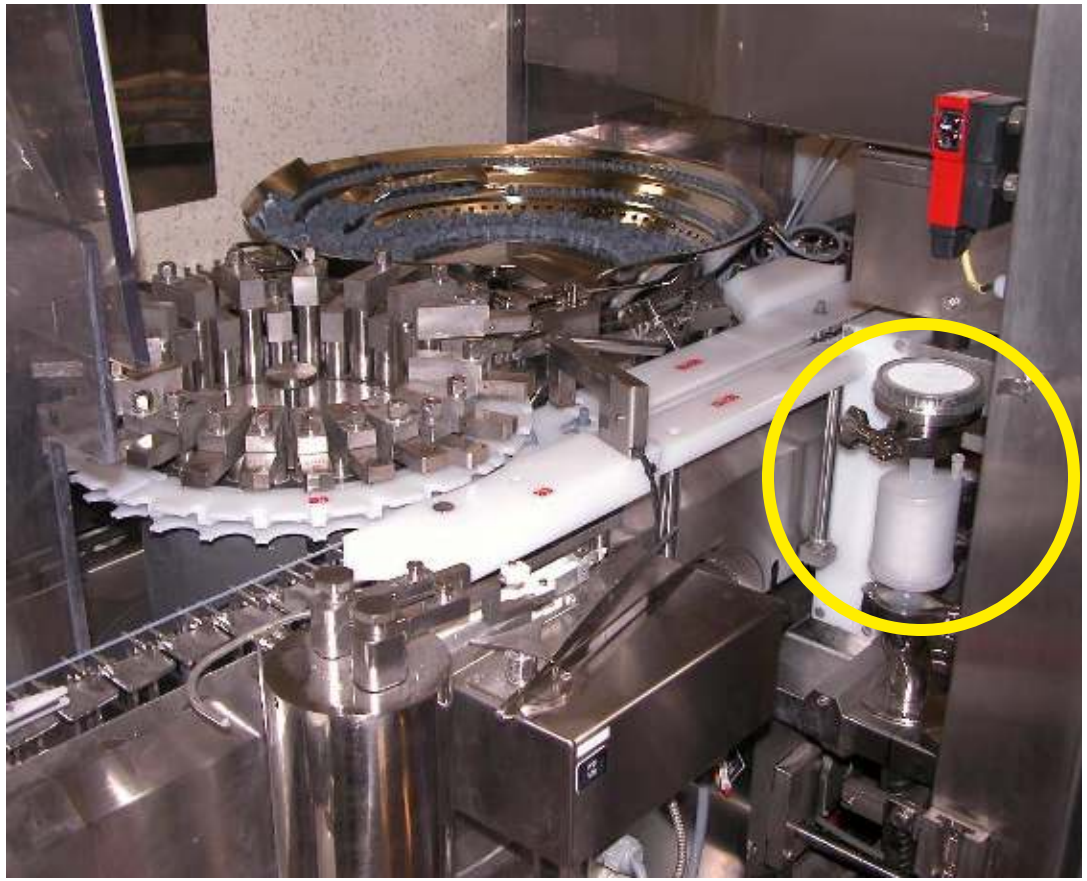
Conveyer

Aseptic filling line

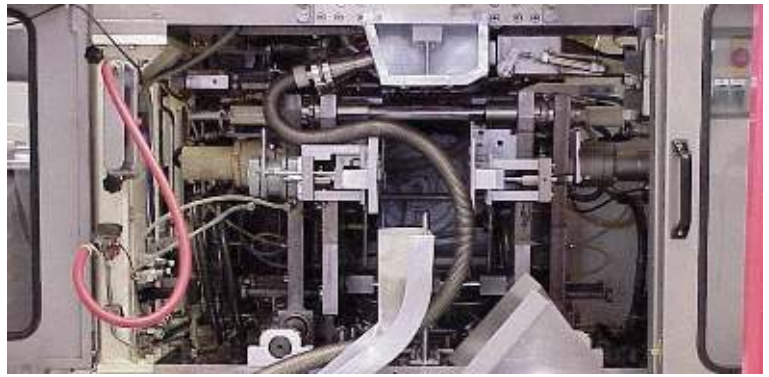
*Source

[1]Reference

Snapshots



Stopper application



Filling zone
Blow-fill seal

*Source

[1]Reference

Microbial Air Monitoring – Continuous or Sequential, Active or Passive?

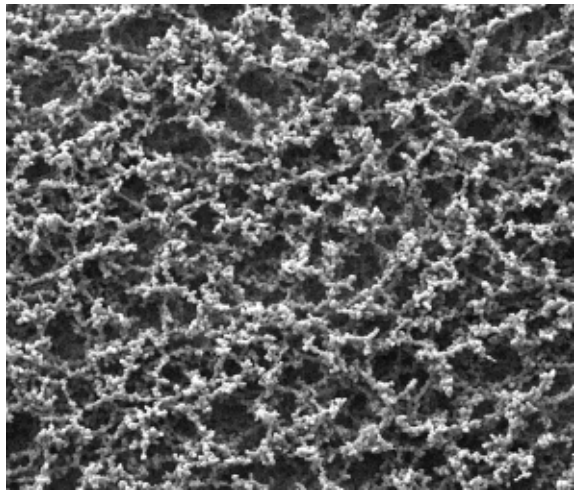
- Agar plates – how much sampling is too much?
  - Change plates (intervention) to allow continuous monitoring
  - Sample periodically (sequentially) on same plate

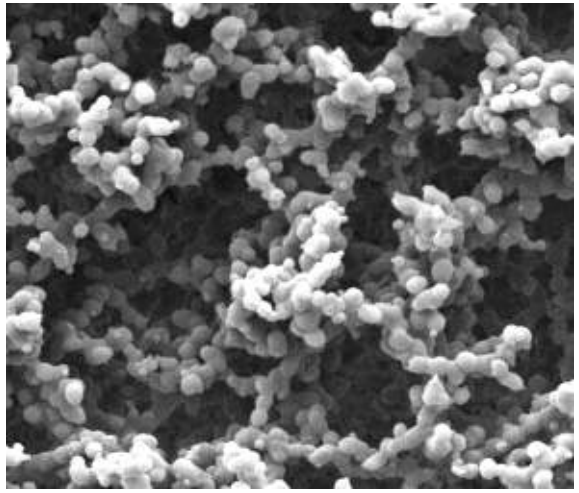
- Alternatives
  - BioAerosol monitoring – fluorescence detection of likely contaminants
  - Filtration techniques to capture and cultivate microbes

Our Solutions

- **Gelatine Filters**
- Gelatine Filters in Biosafe® bags
- MD8 AirScan
- MD8 AirPort

Gelatine Membrane Filters





Pore size 3 µm

Filter diameter 25, 37, 47, 50, **80** mm

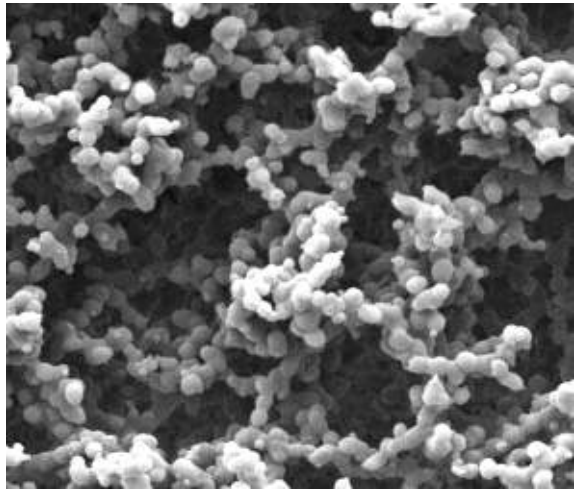
Retentive capacity 99.9995% for *B. subtilis niger*
99.94% for T3 phages

Operating condition Temperature: 30°C | Humidity: 85%

Key Benefits:

- High retention capacity
- Water soluble filter
- Continuous active monitoring for **up to 8 hours**

Gelatine Membrane Filters



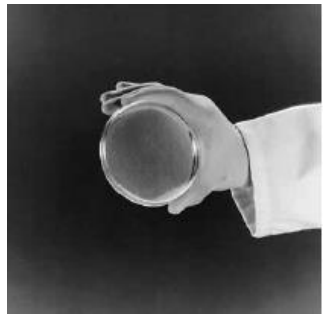
USP <1116> Microbiological Evaluation Of Clean Rooms And Other Controlled Environments

Methodology and instrumentation for Quantitation of Viable Airborne Microorganisms

Gelatine Filter Sampler:
The unit consists of a vacuum pump with an extension hose terminating in a filter holder that can be located remotely in the critical space. The filter consists of random fibres of gelatine capable of retaining airborne microorganisms. After a specified exposure time, the filter is aseptically removed and dissolved in an appropriate diluent and then plated on an appropriate agar medium to estimate its microbial content.

Not the case:




Filtration methods – A common misconception

"Filtration methods are **accurate** and **reliable** and portable filtration samplers designed for the pharmaceutical industry are available. **However**, filtration is less convenient than impaction-based sampling and may cause dehydration stress in the trapped microorganisms."

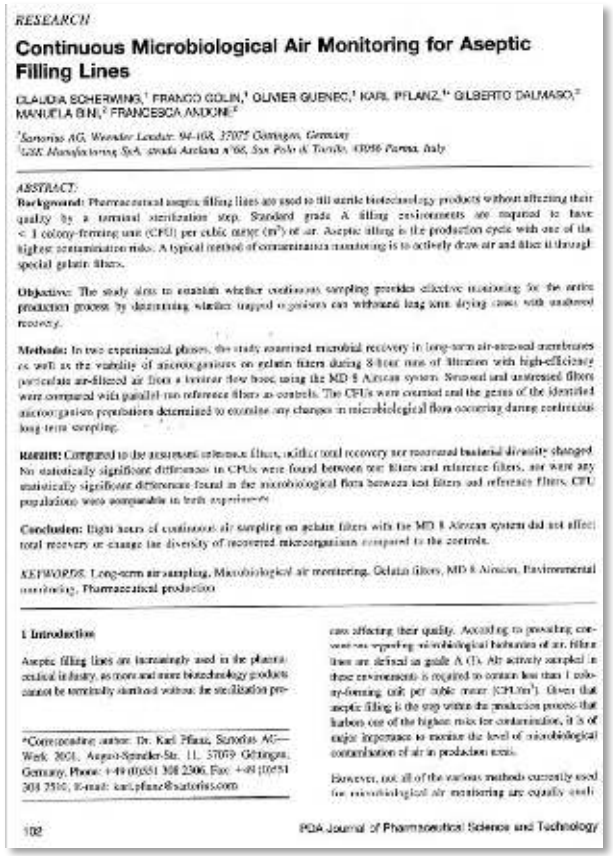
Continuous Microbiological Air Monitoring for Aseptic Filling Lines

- **Trial 1**: Microbial recovery on 'air-stressed' membranes
  - After 8 hours of continuous sampling, can the Gelatine membrane effectively retain microorganisms?
- **Trial 2**: Viability of microorganisms following 8 hours of air filtration
  - After 8 hours of continuous sampling, are microorganisms retained early during sampling still viable?

In collaboration with GlaxoSmithKline

Continuous, Intervention Free Air Monitoring – 8 hours

Trial 1: Recovery on Long-Term Air-Stressed membranes



RESEARCH

Continuous Microbiological Air Monitoring for Aseptic Filling Lines

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ABSTRACT:

Background: Pharmaceutical aseptic filling lines are used to fill sterile biotechnology products without affecting their quality by a terminal sterilization step. Standard grade A filling environments are required to have < 1 colony-forming unit (CFU) per cubic meter (m^3) of air. Aseptic filling is the production cycle with one of the highest contamination risks. A typical method of contamination monitoring is to actively draw air and filter it through special gelatin filters.

Objective: The study aims to establish whether continuous sampling provides effective monitoring for the entire production process by determining whether trapped organisms can withstand long term drying cases with unaltered recovery.

Methods: In two experimental phases, the study examined microbial recovery in long-term air-stressed membranes as well as the viability of microorganisms on gelatin filters during 8-hour runs of filtration with high-efficiency particulate air-filtered air from a laminar flow hood using the MD 8 Airscan system. Stressed and unstressed filters were compared with parallel run reference filters as controls. The CFUs were counted and the genus of the identified microorganism populations determined to examine any changes in microbiological flora occurring during continuous long term sampling.

Results: Compared to the associated reference filters, neither total recovery nor associated bacterial diversity changed. No statistically significant differences in CFUs were found between test filters and reference filters, nor were any statistically significant differences found in the microbiological flora between test filters and reference filters. CFU populations were comparable in both experiments.

Conclusion: Eight hours of continuous air sampling on gelatin filters with the MD 8 Airscan system did not affect total recovery or change the diversity of recovered microorganisms compared to the controls.

KEYWORDS: Long-term air sampling, Microbiological air monitoring, Gelatin filters, MD 8 Airscan, Environmental monitoring, Pharmaceutical production

1 Introduction

Aseptic filling lines are increasingly used in the pharmaceutical industry, as more and more biotechnology products cannot be terminally sterilized without the sterilization process affecting their quality. According to prevailing concepts regarding microbiological behaviors of air, filling lines are defined as grade A (1). Air actively sampled in these environments is required to contain less than 1 colony-forming unit per cubic meter (CFU/m^3). Given that aseptic filling is the step within the production process that harbors one of the highest risks for contamination, it is of major importance to monitor the level of microbiological contamination of air in production areas.

However, not all of the various methods currently used for microbiological air monitoring are equally well

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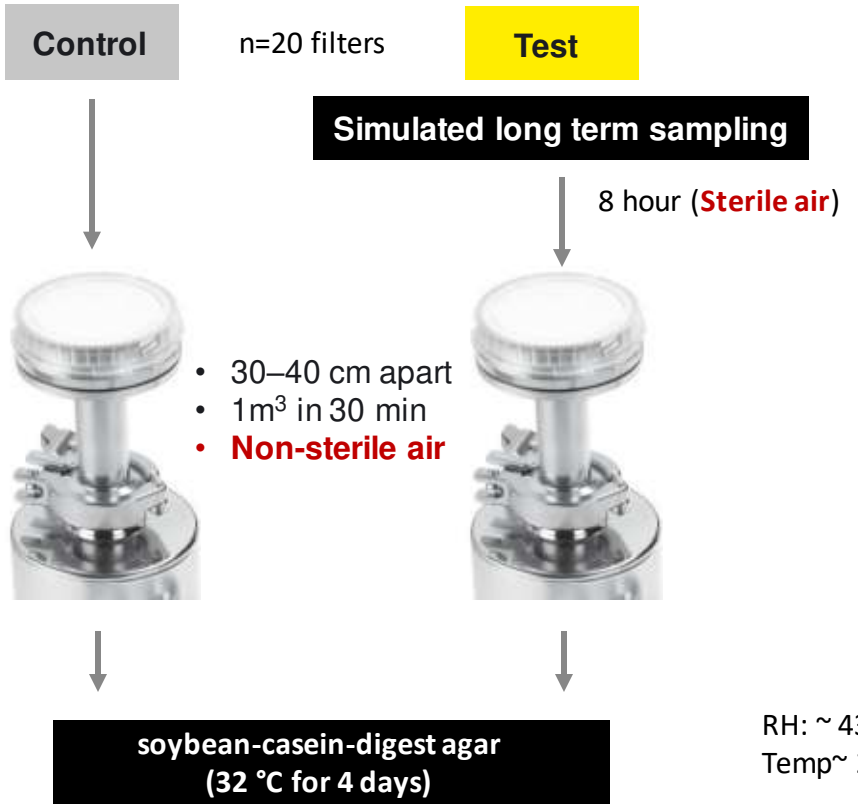
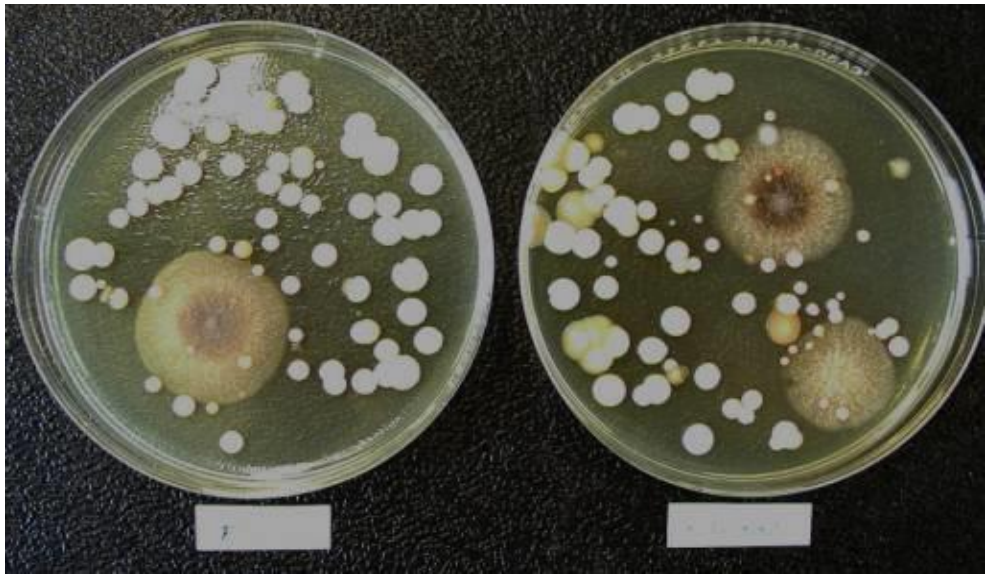


RH: ~ 43 ± 3 %
Temp~ 23 ± 1 °C

Class 2 BSC with a HEPA filter

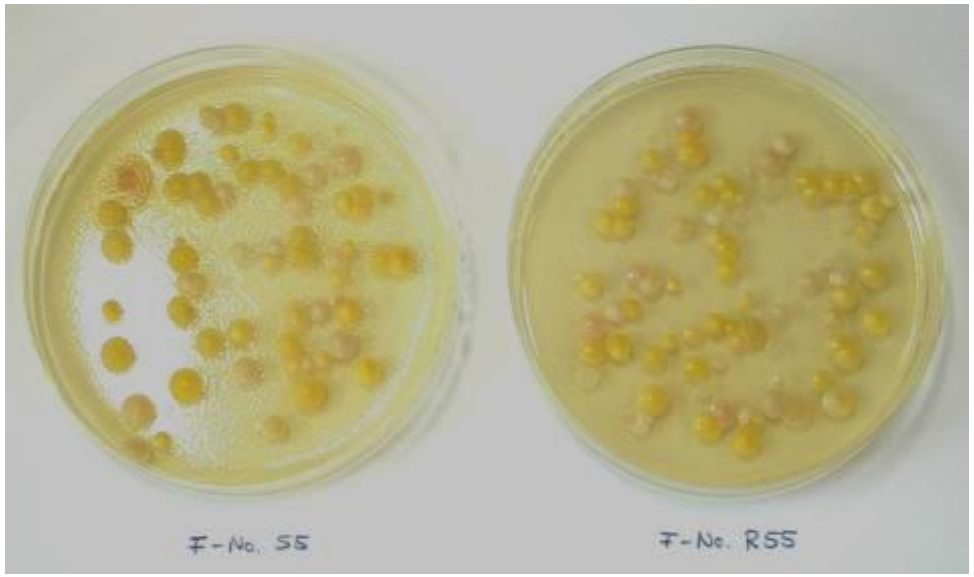
Continuous, Intervention Free Air Monitoring – 8 hours

Trial 1: Recovery on Long-Term Air-Stressed membranes



Test

Control



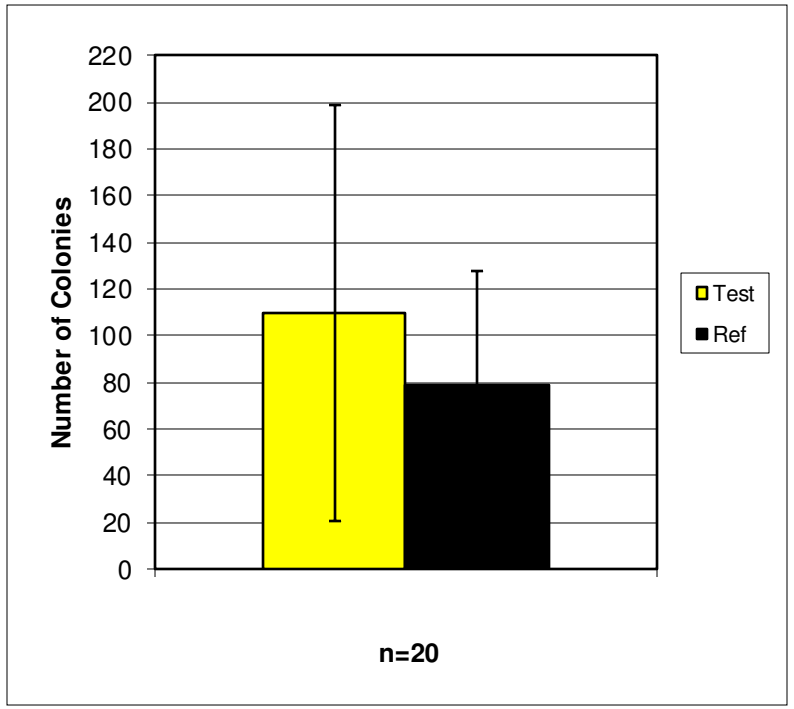
Test

Control

Comparison of the microbiological flora grown on a test filter (left) and its corresponding reference filter (right)

Continuous, Intervention Free Air Monitoring – 8 hours

Trial 1: Recovery on Long-Term Air-Stressed membranes



Comparison of CFU on Test and Reference filters – 20 replicates.

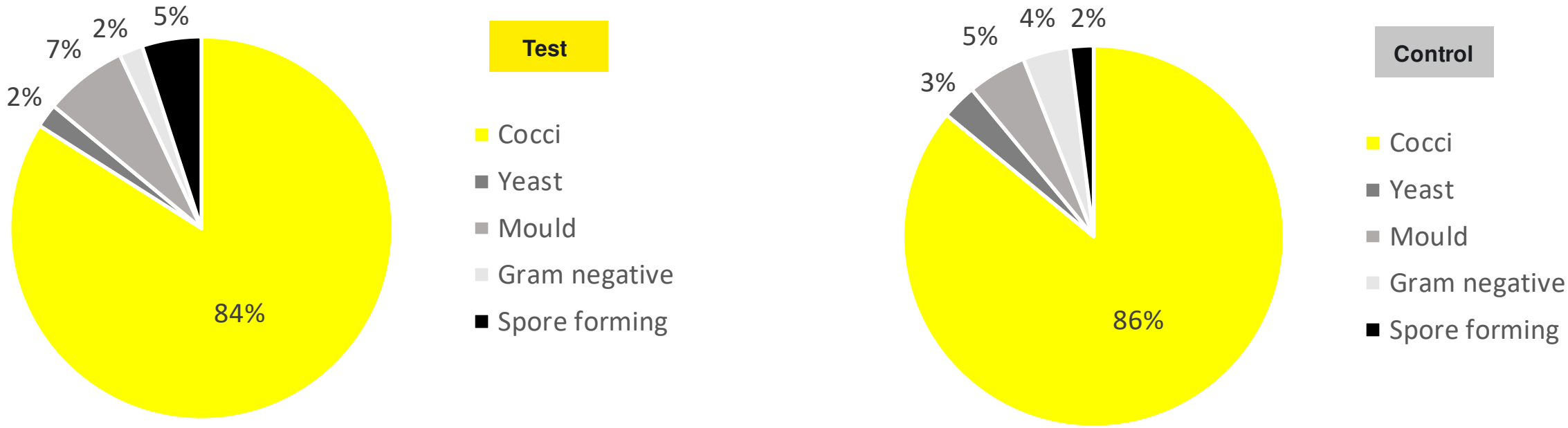
Test	CFU/m3 : 110 colonies
Control	CFU/m3 : 79 colonies

*Source

[1]Reference

Continuous, Intervention Free Air Monitoring – 8 hours

Trial 1: Recovery on Long-Term Air-Stressed membranes



Composition of the microbiological population

*Source

[1]Reference

Continuous, Intervention Free Air Monitoring – 8 hours

Trial 2: Recovery after Long-Term Air-Stressed Microorganisms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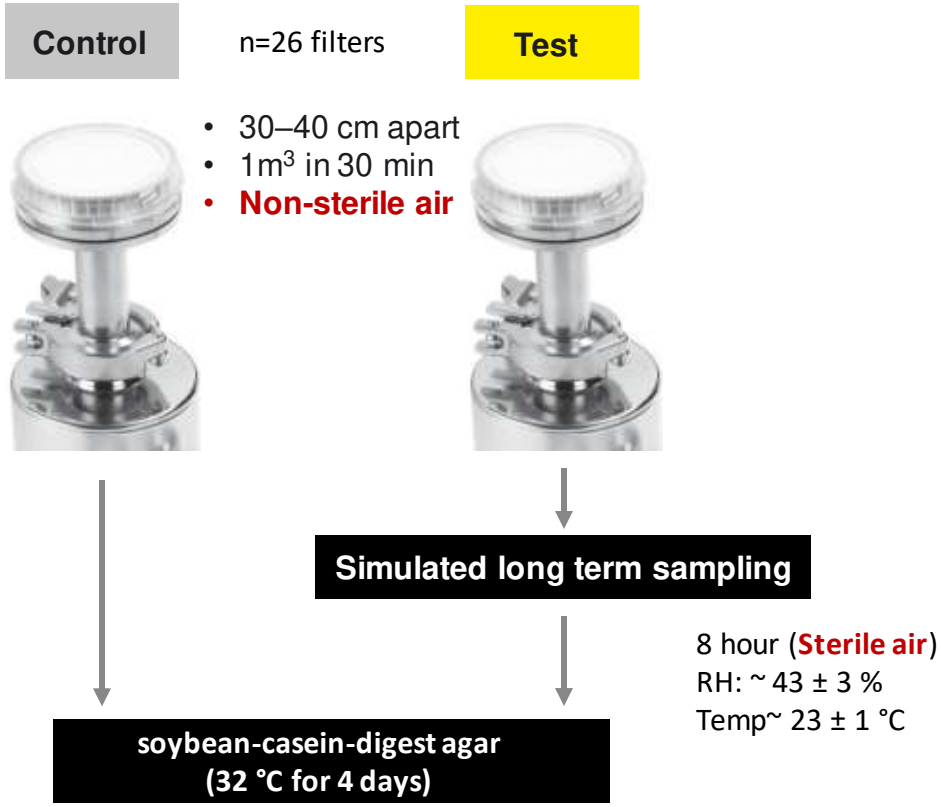
Application Note

February 2019

Keywords:
continuous air monitoring, clean room, gelatin filter, MD8 Airscan®, EU GMP Guideline Annex 1

Continuous Microbial Air Monitoring in Clean Room Environments

Abstract



Class 2 BSC with a HEPA filter

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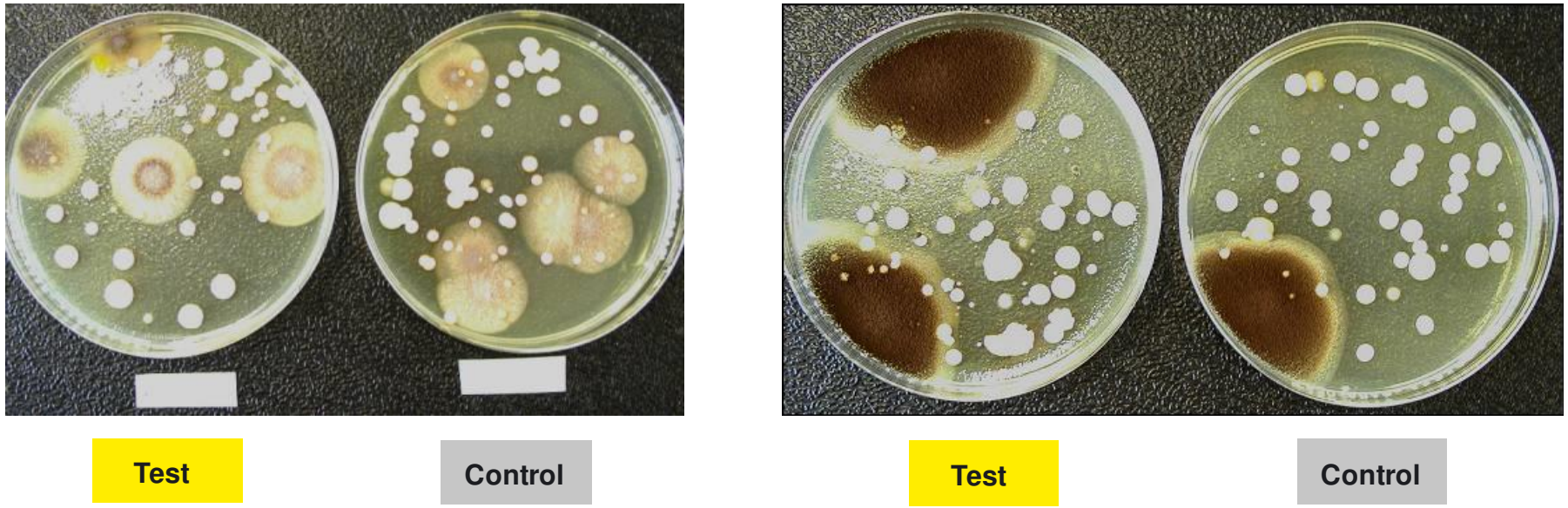
Continuous, Intervention Free Air Monitoring – 8 hours

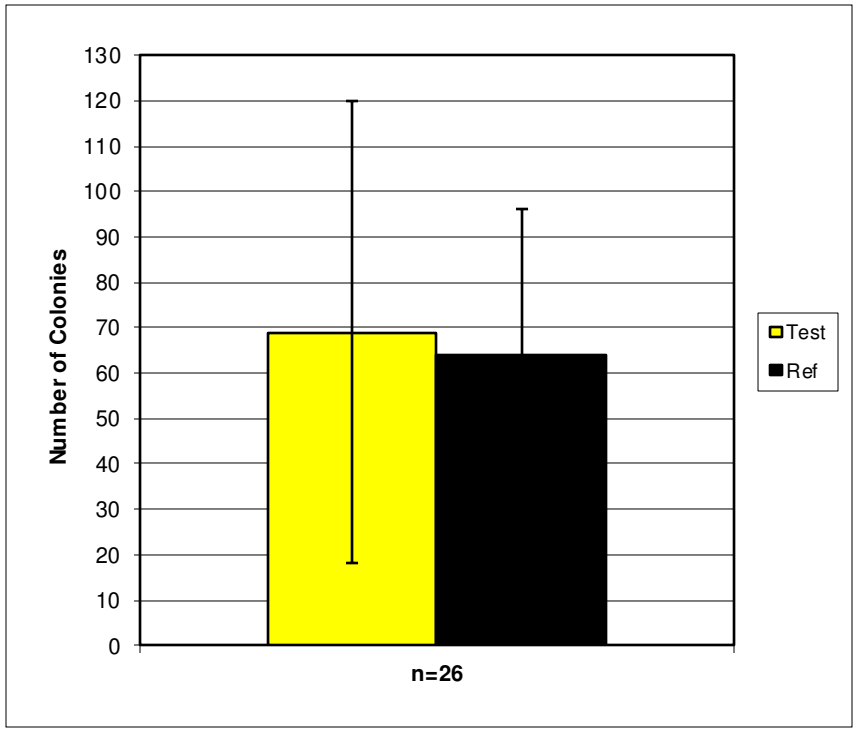
Trial 2: Recovery after Long-Term Air-Stressed Microorganisms



Comparison of the microbiological flora grown on a test filter (left) and its corresponding reference filter (right)

Continuous, Intervention Free Air Monitoring – 8 hours

Trial 2: Recovery after Long-Term Air-Stressed Microorganisms

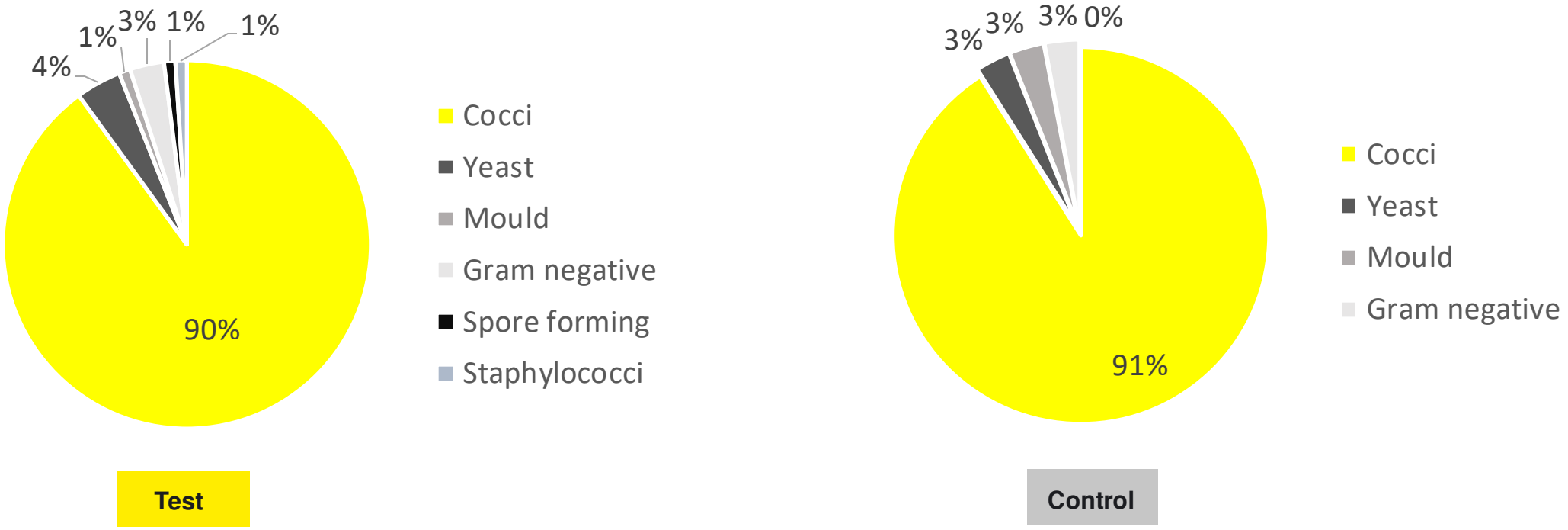


Comparison of CFU on Test and Reference filters collected on different days – 26 replicates.

Test	CFU/m3 : 69 colonies
Control	CFU/m3 : 64 colonies

Continuous, Intervention Free Air Monitoring – 8 hours

Trial 2: Recovery after Long-Term Air-Stressed Microorganisms



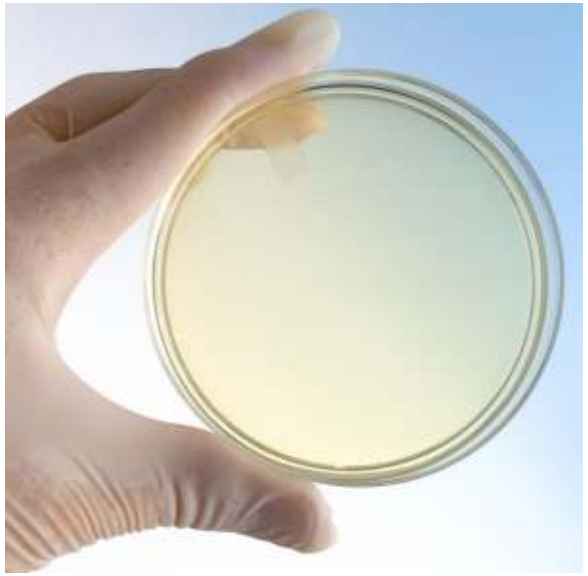
Comparison of the microbiological flora grown on a test filter (left) and its corresponding reference filter (right)

Summary

- Gelatine membrane filters can effectively retain microorganisms following the tested 8 hours of sampling
- No detrimental effect in microbial recovery for the tested period of 8 hours using Gelatine membrane filters
- 0.03% water lost during the sampling of 16 m^3

Advantages:

- No dehydration/desiccation effects - hygroscopic
- No frequent intervention
- Can be paired with traditional & rapid method
- No exposed nutrient source within the cleanroom



MD8 Airscan

- One Command Unit can control up to 5 Sampling Heads
- Fully GMP compliant, calibration data for each sampling head
- Can be integrated into isolators and clean rooms, standard tri-clover connections
- Data saved on board Command Unit, Printer available
- Adjustable flow to achieve iso-kinetic sampling

Summary

Annex 1

- **ANY** risk caused by interventions of the monitoring operation must be avoided
- Sampling methods should not pose a risk of contamination

Gelatine membrane filtration

- Continuous intervention-free monitoring
- No loss of microbial recovery for the tested 8 hours
- Can be paired with traditional and rapid methods
- Compliant with the ISO 14698, EN 17141 and the EU GMP Annex 1

Thank You.

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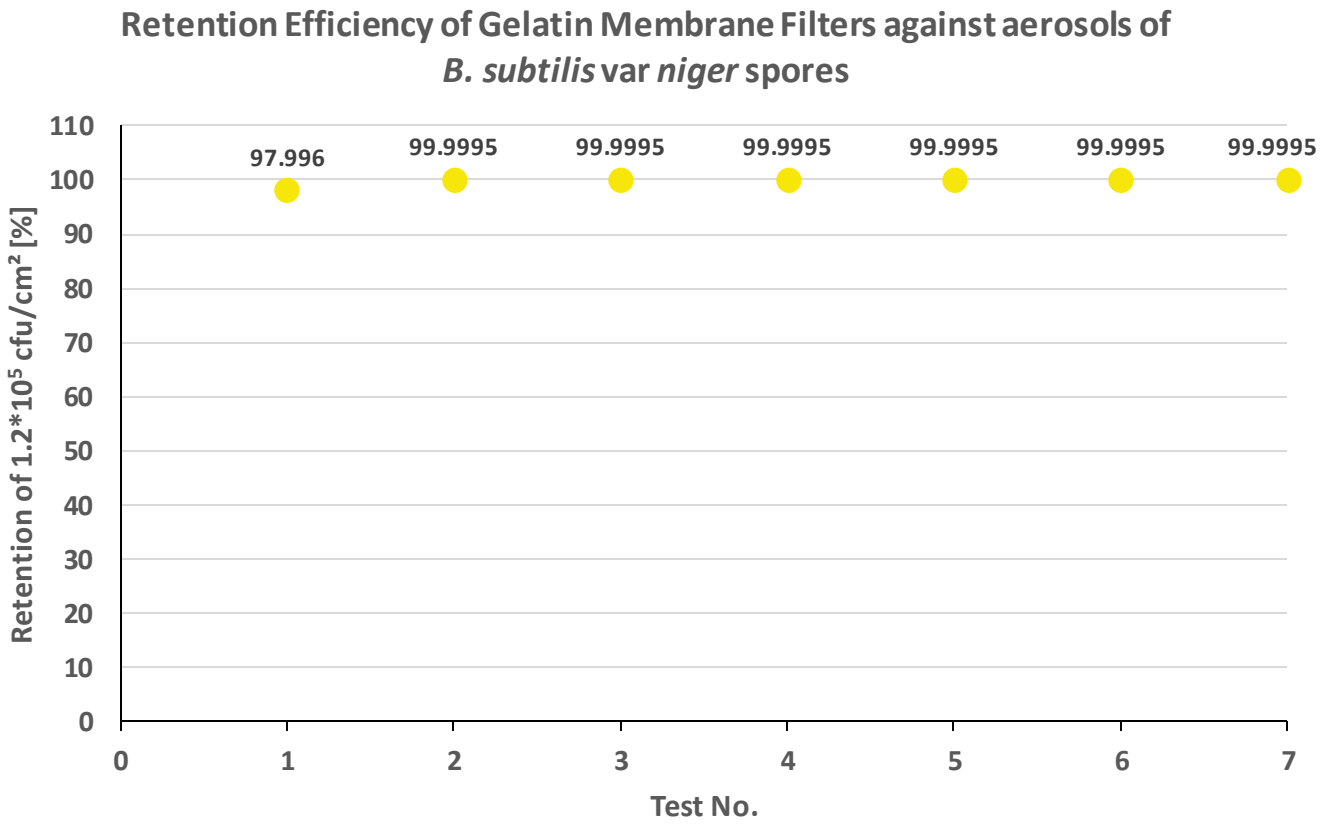
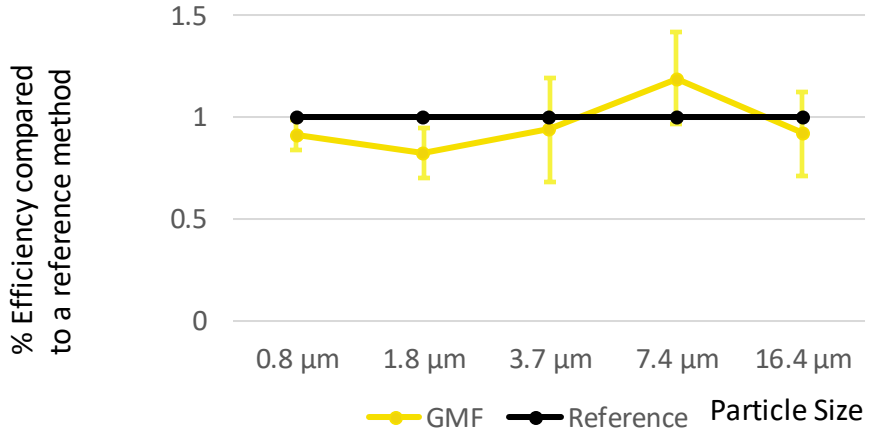
Gelatine Membrane Filters

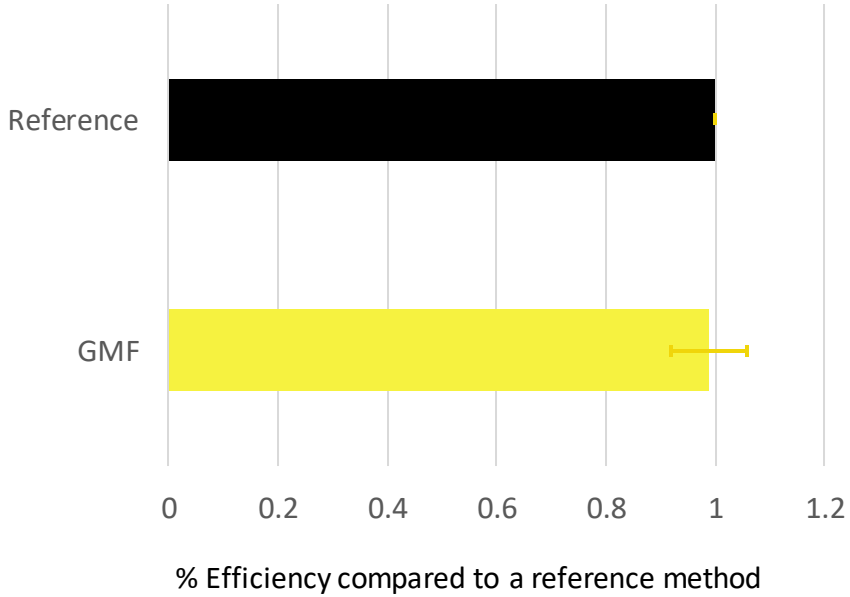
Efficiency of Retention



Gelatine Membrane Filters



Physical Efficiency



Biological Efficiency

Gelatine Filters in Biosafe® Bags

17528--------BFV