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Sartopore Evo®: Sterility Evaluation Following Pre-Use Integrity Testing of Feed-Side Contaminated Filters Using a Bacterial Challenge Test

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Abstract

Final formulation and filling is a critical step in biopharmaceutical manufacturing to ensure drug sterility and patient safety. In this process, sterile filtration of the active pharmaceutical ingredient and preservation of formulation integrity are essential. Sartorius has recently introduced Sartopore Evo®, the latest member of the Sartopore® filter family, specifically developed for final product filtration. Sartopore Evo® combines the excellent throughput and flow rate performance characteristic of Sartopore® filters with exceptionally low adsorption of proteins and excipients such as polysorbate and poloxamer. All materials of construction are PFAS-free, mitigating risks associated with potential future restrictions on PFAS-based materials.[1]

For final filtration, regulatory authorities require pre-use post-sterilization integrity testing (PUPSIT) to confirm filter integrity prior to use. However, there are concerns that pre-use integrity testing may compromise system sterility if bacteria are present on the upstream side. To address this, a bacterial challenge test was performed in which Sartopore Evo® filters were deliberately wetted with bacteria-contaminated water before integrity testing. Results demonstrated the filter's robust bacterial retention capability, confirming reliable performance under worst-case aseptic processing conditions.

For further information, visit
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Introduction

In today's highly regulated pharmaceutical environment, ensuring product sterility throughout manufacturing is not just best practice, it is a regulatory mandate. Among all process steps, final sterile filtration and aseptic filling represent the last and most critical barrier protecting patients from microbial contamination. Any compromise at this stage can have direct consequences for patient safety, product recalls, and regulatory compliance. Therefore, sterile filtration solutions must deliver exceptional reliability, proven microbial retention, and full regulatory alignment.

As the global pharmaceutical industry adapts to evolving expectations ranging from enhanced microbial safety standards to emerging environmental and chemical substance regulations, manufacturers are under growing pressure to adopt forward-compatible technologies. Recognizing these needs, Sartorius introduced Sartopore Evo® (Figure 1), a next-generation, sterile filtration solution manufactured without the intentional use of PFAS, specifically for final filling applications in biopharmaceutical manufacturing.

Sartopore Evo® filters are engineered for outstanding total throughput, low protein and excipient adsorption, and robust microbial retention. Importantly, the materials of construction are PFAS-free, supporting future readiness for environmental and chemical safety regulations such as EU REACH and other global initiatives limiting the use of per- and polyfluoroalkyl substances. Sartopore Evo® provides not only strong technical performance but also strategic regulatory confidence, enabling manufacturers to meet both current and future compliance requirements.

Figure 1: *Sartopore Evo® Filter*

Among the most discussed regulatory expectations in final filtration is the requirement for pre-use post-sterilization integrity testing (PUPSIT). As outlined in EU GMP Annex 1 (2022 update) and reflected in global best practices, PUPSIT is increasingly mandatory to verify filter integrity immediately prior to use. However, its implementation raises concerns: could the application of pressure during pre-use integrity testing compromise filter performance or force microorganisms through the membrane, especially in cases where contamination is inadvertently present on the upstream side?

To investigate this critical concern, we conducted a rigorous bacterial challenge test (BCT) using Sartopore Evo® filters. Under worst-case conditions, filters were intentionally wetted with *Brevundimonas diminuta* contaminated water at a concentration of 10^7 CFU/cm². A non-destructive integrity test was then performed using the Sartocheck® system without disassembling the setup, followed immediately by a BCT in accordance with ASTM F838-15a, the recognized industry standard for evaluating bacterial retention of membrane filters.[2]

This study was designed to evaluate whether pre-use integrity testing poses any risk to filter performance or sterility in practice. The results provide important insights for manufacturers seeking to comply with PUPSIT requirements without compromising aseptic assurance. They further affirm Sartopore Evo®'s suitability for final filtration under the most demanding regulatory and operational conditions.

Materials

In this study, three lots of Sartopore Evo® filters were tested in triplicate. The filters were evaluated in the Size 9 capsule format, which has an effective filtration area (EFA) of 0.26 m². Details of the test filters are provided in Table 1.

Test Organism

Brevundimonas diminuta (ATCC 19146) was selected as the test organism because it is one of the smallest motile bacteria (~0.3 µm), providing a stringent challenge for sterilizing-grade filters.

Regulatory guidelines (USP, Ph. Eur., FDA) and standards like ASTM F838-20 require its use to demonstrate that 0.2 | 0.22 µm rated filters reliably remove microorganisms and ensure sterility.

Table 1: *Details of Three lots of Sartopore Evo® Filters Tested*

Order Code	Lot Number	Membrane Material	Pore Size [µm]	Filtration Area [m²]	Material Treatment
5995307G9G-SS	2447020283	Polyethersulfone (PES)	0.2	0.26	Gamma irradiated
5995307G9G-SS	2447020383	Polyethersulfone (PES)	0.2	0.26	Gamma irradiated
5995307G9G-SS	2447020483	Polyethersulfone (PES)	0.2	0.26	Gamma irradiated

Methods

A bacterial challenge test (BCT) was performed using three different lots of Sartopore® Evo filters to evaluate their bacterial retention performance. Each filter underwent a pre-use integrity test (IT) to ensure membrane integrity prior to testing. The filters were then wetted with contaminated water to simulate worst-case conditions.

Integrity testing was conducted at three critical stages of the study:

- Before the BCT (to verify initial filter integrity),
- After wetting with contaminated water (to assess the impact of potential fouling or pre-conditioning), and
- After completion of the BCT (to confirm continued integrity throughout the challenge).

The bacterial retention efficiency of the filters was assessed based on the Log Reduction Value (LRV), a quantitative measure indicating the logarithmic reduction of the bacterial load. A high LRV reflects effective microbial retention and confirms the filter's performance in maintaining sterility under demanding conditions.

The details of test method are explained in the following flow chart.

Figure 2: *Test Method*

Filter Wetting	▪ Test filters were connected to the test rig ▪ Filter wetting was performed as per a standard wetting procedure
Integrity Test	▪ Bubble point and diffusion were tested using the Sartocheck® system without removing the filters from the test setup
Contaminated Water Wetting	▪ Contaminated water containing 1×10^7 CFU/cm² of *B. diminuta* culture was passed through the filter ▪ A growth test was performed on the challenge solution to confirm the challenge level
Integrity Test	▪ Bubble point and diffusion were tested without removing the filters from the test setup
BCT	▪ A standard BCT was performed by challenging 1×10^7 CFU/cm² ▪ A growth test was performed on the challenge solution to confirm the challenge level
Integrity Test	▪ Bubble point and diffusion were tested using the Sartocheck® system
Incubation	▪ Analytical filters were transferred aseptically to the growth media (tryptic soy agar) ▪ Analytical filters and growth test plates were incubated at 30 °C for 5 days
Result	▪ Integrity test results were compared before and after the test ▪ After the incubation period, analytical filters were checked for, with sterile results indicating a passed test

Note. CFU = colony forming units

Results

Integrity Test

Diffusion and bubble point test results confirmed the structural integrity of the test filters. Integrity test values were measured at three stages before contamination, after contamination, and after the BCT. These values are summarized in Table 2. All integrity test values remained within the acceptable range for each filter type, indicating that the filtersw maintained their integrity even after undergoing two BCT and integrity test cycles.

To ensure the appropriate bacterial load in the challenge solution, a growth promotion test was conducted. The test confirmed that a challenge concentration exceeding 1×10^7 CFU/cm² was achieved during the experiment. Bacterial challenge results are also expressed as the LRV, which quantifies the microbial removal efficiency of a filter. An LRV of 7 indicates that the filter reduces the bacterial load in the feed stream by seven orders of magnitude.

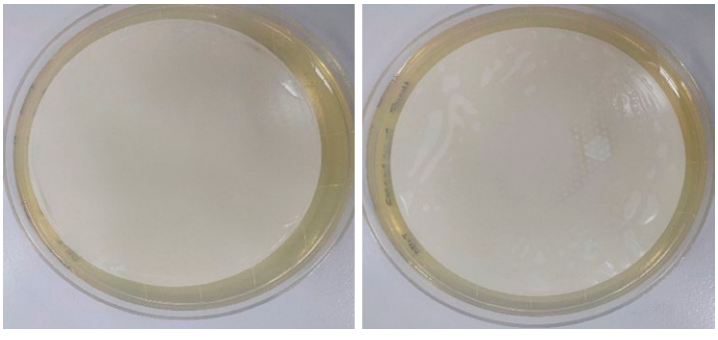
Table 2: *Diffusion and Bubble Point Values of the Test Filters Before the Test, After Contaminated Wetting, and After BCT*

Filter Details		Diffusion [mL/min]			Bubble Point [mbar]		
		Before Test	After Contamination	After BCT	Before Test	After Contamination	After BCT
2447020283	#0006	4.6	5.1	5.0	4,029	4,129	4,179
	#0017	4.2	5.3	4.4	3,999	3,999	4,099
	#0019	4.4	5.3	4.2	4,048	4,098	4,078
2447020483	#0004	4.6	4.2	4.2	3,948	4,049	4,099
	#0016	4.2	5.6	4.0	4,128	4,378	4,228
	#0017	4.3	5.0	4.1	4,148	4,228	4,199
2447020383	#0016	4.6	5.6	4.3	3,878	3,949	4,027
	#0006	4.8	5.9	5.1	3,977	3,999	4,079
	#0007	4.4	5.4	4.7	4,079	4,178	4,128

BCT

The bacterial retention capability of Sartopore Evo® filters was assessed using a BCT. Analytical (retentate) filters were incubated at 30 °C for 5 – 7 days. Post-incubation, no visible colony-forming units (CFUs) were detected on any of the filters, confirming sterility across all test samples. These results demonstrate that Sartopore Evo® filters effectively retained the challenge organism. Representative images showing the absence of CFUs after incubation are provided in Figure 3.

Figure 3: *Sartopore Evo® Filter*



The BCT outcomes — including challenge concentration, colony counts on the analytical filters, and corresponding LRV values — are summarized in Table 3.

Table 3: *BCT Result*

Filter Details		Challenge by Test Solution [$\times 10^7$ CFU/cm²]	Colonies | Analytical Filter	LRV	Test Results
2447020283	#0006	1.0	0	7.0	Pass
	#0017	1.0	0	7.0	Pass
	#0019	1.1	0	7.02	Pass
2447020483	#0004	1.1	0	7.03	Pass
	#0016	1.1	0	7.03	Pass
	#0017	1.1	0	7.02	Pass
2447020383	#0016	1.1	0	7.03	Pass
	#0006	1.0	0	7.0	Pass
	#0007	1.0	0	7.0	Pass

Discussion

The results of this study confirm the robust bacterial retention performance of Sartopore Evo® filters under challenging conditions. Notably, the filters-maintained integrity throughout all critical stages of the process, including PUPSIT, wetting with contaminated water, and post-BCT verification. This demonstrates that the pressure applied during PUPSIT does not compromise the sterility performance of the filters. Even after exposure to high differential pressures and worst-case wetting conditions, the filters retained their full microbial retention capability.

The high LRVs observed across all three lots and replicates further confirm the filters' consistent ability to remove microorganisms and ensure sterility. This consistency across multiple production lots underlines the reproducibility and reliability of the filter manufacturing process.

In addition to their validated bacterial retention performance, Sartopore Evo® filters are manufactured without the intentional use of PFAS, addressing growing regulatory and environmental concerns related to per- and polyfluoroalkyl substances. This feature supports sustainable manufacturing practices while meeting stringent sterility requirements.

Overall, these results support the suitability of Sartopore Evo® filters for final sterile filtration applications in biopharmaceutical manufacturing. Their demonstrated bacterial retention capability, PFAS-free construction, and compliance with relevant regulatory standards (USP, Ph. Eur., FDA, ASTM F838-20) provide confidence in their performance as a critical control point for ensuring product sterility and patient safety.

Conclusion

The data presented demonstrates that the pressure applied during the integrity test, even after wetting the filters with highly contaminated water, does not result in bacterial breakthrough on the filtrate side. With proven high microbial retention capacity, Sartopore Evo® ensures the sterility of the final product, even under the most demanding conditions. Customers can confidently perform PUPSIT or product recovery using compressed air pressures up to the bubble point without impacting product sterility, filter integrity, or overall quality. Sartopore Evo® filters thus offer reliable sterility assurance, supporting risk mitigation during shipping, installation, and operation—without compromising product quality or patient safety.

References

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