BIOTECH

Validation Guide

USTR 2863

Emflon® HTPFR Filter Cartridges

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Contents

1 Overview

This report is designed to assist the filter user in meeting the validation requirements of regulatory authorities within the pharmaceutical industry for the use of Emflon HTPFR sterilizing air/gas/vent filter cartridges. The filter styles that are in scope of this report are 254 mm (10 in.) Emflon HTPFR filter cartridges (part number (p/n) AB1HTPFR7PVH4), each containing 0.84 m^2 (9.04 ft^2) of effective filtration area (EFA), and 127 mm (5 in.) Emflon HTPFR filter cartridges (p/n AB05HTPFR2PVH4), each containing 0.42 m^2 (4.52 ft^2) of effective filtration area. The validation report also covers Emflon HTPFR multi-module assemblies based on the 254 mm (10 in.) modules, i.e. 508 mm (20 in.) and 762 mm (30 in.) filter cartridges (p/n AB2HTPFR7PVH4 and AB3HTPFR7PVH4).

Emflon HTPFR filter cartridges have been specifically designed for the sterilizing filtration of compatible gases in critical high-temperature air or oxidizing gas applications. High-temperature air applications include autoclave venting, fermentation inlet air, and hot water for injection (WFI) tank vents. Oxidizing gas applications include ozonated water tank venting and oxygen-enriched air applications in the biopharmaceutical and biotechnology industry. Oxygen enrichment of air is common in fermenter or bioreactor applications where improved aeration enables higher product yield.

Emflon HTPFR filter cartridges incorporate a proprietary double-layer 0.2 µm sterilizing grade membrane made of inherently hydrophobic and non-oxidizing polytetrafluoroethylene (PTFE), manufactured by Pall. The polypropylene hardware is specially formulated with protective antioxidants, and the filter's support and drainage layers are made of oxidation-resistant polyphenylene sulfide (PPS) polymer to increase the resistance to oxidation and to minimize combustibility in oxygen-enriched service.

Emflon HTPFR filter cartridges are designed, developed, and manufactured in accordance with an ISO 9001 certified quality management system. These filters are manufactured in a controlled environment that meets the air quality standards of an ISO class 8 room with respect to viable and nonviable particulate and positive pressure.

Emflon HTPFR filter cartridges may be used in conformance with current Good Manufacturing Practices (cGMP) in manufacturing, processing, packing or holding of Drugs per Title 21 of the U.S. Code of Federal Regulations (21 CFR Part 210) and cGMP for Finished Pharmaceuticals (21 CFR Part 211). These filters also are made from materials listed for food contact usage per 21 CFR Parts 170-199.

Note:

The units of pressure quoted in this document are "bar" and "pounds force per square inch (psi)."

For conversion to pascal (Pa) use:

- 1 bar = 1 x 10^5 Pa
- 1 psi = 6.89476 x 10^3 Pa

All air flow measurements were corrected to standard conditions for gases:

- 1013.25 mbara (14.7 psia)
- 20 °C (68 °F)

The units of airflow rates quoted in this document are standard liters per minute (SLPM), normal cubic meters per hour (Nm^3/h), and standard cubic feet per minute (SCFM). For conversion use:

- Standard cubic feet per minute (SCFM) to normal cubic meters per hour (Nm3/h) and vice versa:
  1 SCFM = 1.69901 Nm3/h
  1 Nm3/h = 0.59 SCFM
- Standard cubic feet per minute (SCFM) to standard liters per minute (sL/min) and vice versa:
  SCFM = 28.3168 sL/min
  1 sL/min = 0.0353 SCFM

2 Summary of Conclusions

2.1 Microbial Removal Efficiency Validation Tests

Emflon HTPFR filter cartridges were tested for bacterial retention using *Brevundimonas diminuta* (*B. diminuta*) (American Type Culture Collection (ATCC◆) 19146), in accordance with the American Society for Testing and Materials (ASTM) Standard Test Method F838-05, the U.S. Federal Drug Administration (FDA) Guidelines on Sterile Products Produced by Aseptic Processing (1987) and FDA Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice (2004). These tests demonstrated that Emflon HTPFR filter cartridges provide a sterile effluent when challenged with > 10^7 colony forming units (CFU) of *B. diminuta* per cm^2 of effective filtration area (CFU/cm^2) suspended in liquid (water).

Forward flow and water intrusion integrity tests were validated as non-destructive integrity tests for Emflon HTPFR filter cartridges. Test parameters correlated to 100% liquid retention of *B. diminuta* were set.

Aerosol microbial challenge tests were also performed on filter cartridges produced under standard manufacturing conditions, using spores of *Bacillus subtilis (B. subtilis)* (National Collection of Type Cultures (NCTC) 10073) and MS2 bacteriophage (National Collection of Industrial, Food and Marine (NCIMB) 0108). These tests demonstrated that integral Emflon HTPFR filter cartridges also retain 100% of aerosolized bacteriophage and bacterial spores.

Table 1 summarizes the demonstrated microbial removal efficiency in liquid and aerosol challenge testing.

Table 1

Summary of the microbial removal efficiency data for Emflon HTPFR filter cartridges

Filter Part Number	Challenge Organism	Challenge Description	Challenge Level	Microbial Recovery	Titer Reduction
AB1HTPFR7PVH4	*B. diminuta* (ATCC 19146)	Liquid challenge	1.6×10^{11}	No	$> 1.6 \times 10^{11}$
AB1HTPFR7PVH4	*B. subtilis* spores (NCTC 10073)	Aerosol challenge	1.0×10^{10}	No	$> 1.0 \times 10^{10}$
AB1HTPFR7PVH4	MS2 bacteriophage (NCIMB 10108)	Aerosol challenge	1.4×10^{11}	No	$> 1.4 \times 10^{11}$
AB05HTPFR2PVH4	*B. diminuta* (ATCC 19146)	Liquid challenge	8.4×10^{10}	No	$> 8.4 \times 10^{10}$

2.2 Sodium Chloride Aerosol

This study aimed to perform aerosol challenge tests with sodium chloride to define the particulate removal efficiency for Emflon HTPFR filter cartridges in gases. The results show that Emflon HTPFR filter cartridges retain particles down to a size of 0.003 µm with high efficiency (> 99.999999%) from a gas stream when challenged with a particle load of $>10^9$ particles per ft^3.

2.3 Resistance to Steam Exposure

Emflon HTPFR filter cartridges have been demonstrated to be capable of withstanding multiple steam-in-place sterilization cycles in the forward ('out to in') direction. Exposure to steam-in-place cycles can be considered equally or more challenging regarding exposure to saturated steam and differential pressure than autoclaving, thus also providing the data basis for a respective autoclave exposure claim.

- The filter cartridges have been shown to maintain integrity as demonstrated by passing forward flow and water intrusion testing after steam exposure, maintaining the retention performance of a sterilizing filter as demonstrated by providing sterile effluent in liquid challenge testing with *B. diminuta* and maintaining the ability to pass applicable effluent quality testing.
- The data presented in this section support the following product claim for steam in place in the forward direction or autoclaving of Emflon HTPFR filter cartridges with a 100% safety margin.

Table 2

Claim for maximum steam exposure by steam-in-place cycles in the forward ('out to in') direction or autoclaving

Filter Part Number	Steaming Temperature	Cycle Duration	Number of Cycles
AB1HTPFR7PVH4, AB05HTPFR2PVH4	142 °C (288 °F)	One hour	100

2.4 Resistance to Hot Air

Based on accelerated aging tests, where Emflon HTPFR filter cartridges were exposed to hot air (140 ºC, 284 °F), the filters can be used in hot-air applications up to 100 ºC (212 °F) for up to one (1) year while maintaining integrity.

2.5 Airflow / Differential Pressure Characteristic

Emflon HTPFR filter cartridges were evaluated for airflow versus differential pressure under four (4) different conditions: venting applications (at atmospheric pressure) and in compressed air systems at 1.0 bar, 2.0 bar, and 4.0 bar (14.5 psi, 29.0 psi, 58.0 psi) system pressure. This data can be used for system sizing calculations.

2.6 Extractables Testing

The non-volatile gravimetric residue (NVR), when extracting autoclaved filters in different extracting fluids, was determined using typical Emflon HTPFR production filter cartridges (p/n AB1HTPFR7PVH4) that contain 0.84 m^2 (9.04 ft^2) of effective filtration area (EFA). Total non-volatile extractables in water were less than 9 mg. Total non-volatile extractables in 50:50 (v/v) ethanol/water were less than 27 mg.

2.7 Biological Safety Tests

The materials of construction of Emflon HTPFR filter cartridges were tested and found to meet the requirements for biological reactivity, *in vivo*, under United States Pharmacopeia (USP) <88> (for Class VI–121 °C plastics) and *in vitro*, under USP <87>. *In vivo* tests included the Systemic Toxicity Test, the Intracutaneous Test, and the Implantation Test. *In vitro* testing was carried out as Minimum Essential Medium (MEM) Elution Cytotoxicity Test.

2.8 Shelf-Life Studies

Shelf-life studies post five (5) years of real-time storage were performed to support Pall's recommended storage period for polymeric filters. Emflon HTPFR filter cartridges were stored for five (5) years under recommended storage conditions. After the storage period, filter samples were subjected to all quality control testing in accordance with the applicable Pall "Certificate of Test for Pharmaceutical Grade Filters," to resistance to steam exposure testing, and to airflow/differential pressure measurements and confirmed that relevant product claims continued to be met after the applied storage period.

3 Bacterial Retention Validation Using *B. diminuta* Liquid Challenge Tests

3.1 Overview

FDA's Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice (2004) states: 'A sterilizing filter should be validated to reproducibly remove viable microorganisms from the process stream, producing a sterile effluent." The guideline also states, 'the microorganism *B. diminuta* (ATCC 19146), when properly grown, harvested and used, is a common challenge organism for 0.2 µm rated filters because of its small size (0.3 µm mean diameter).' These statements apply to sterilizing filters intended for aseptic processing of liquids.

For a filter to be defined as a sterilizing grade liquid filter, it must meet the industry requirements of removing *B. diminuta* (ATCC 19146) at a minimum level of ≥ 1.0 x 10^7 colony forming units (CFU) per cm^2 of effective filtration area (EFA). Although FDA's 'Guidance for Industry' was updated in 2004, the minimum bacterial challenge level for a sterilizing grade filter, as defined in the previous version (1987), remains the industry standard for performance characterization of sterilizing grade filters for liquids.

While Emflon HTPFR filter cartridges were developed for microbial retention from gases, Pall applied for its bacterial retention validation the stringent requirements for a sterilizing filter for the microbial retention from liquids.

Thus, the filters were tested for retention of *B. diminuta* (ATCC 19146) using bacterial challenge tests in accordance with ASTM Standard Test Method F838-05 and the FDA's Guidance for Industry - Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice (September 2004) using a minimum of 1 x 10^7 CFU/cm^2 of effective filtration area.

The FDA guideline further states, 'After a filtration process is properly validated for a given product, process and filter, it is important to ensure that identical filters (e.g., of identical polymer construction and pore size rating) are used in production runs integrity testing of the filter(s) can be performed prior to processing, and should be routinely performed post-use. Forward Flow and Bubble Point tests, when appropriately employed, are two integrity tests that can be used. A production filters' integrity test specification should be consistent with the data generated during bacterial retention validation studies.'

The correlation between *B. diminuta* bacterial retention and a non-destructive integrity test is an essential aspect of the validation of sterilizing grade filters. The integrity tests employed in this study were the forward flow and water intrusion tests.

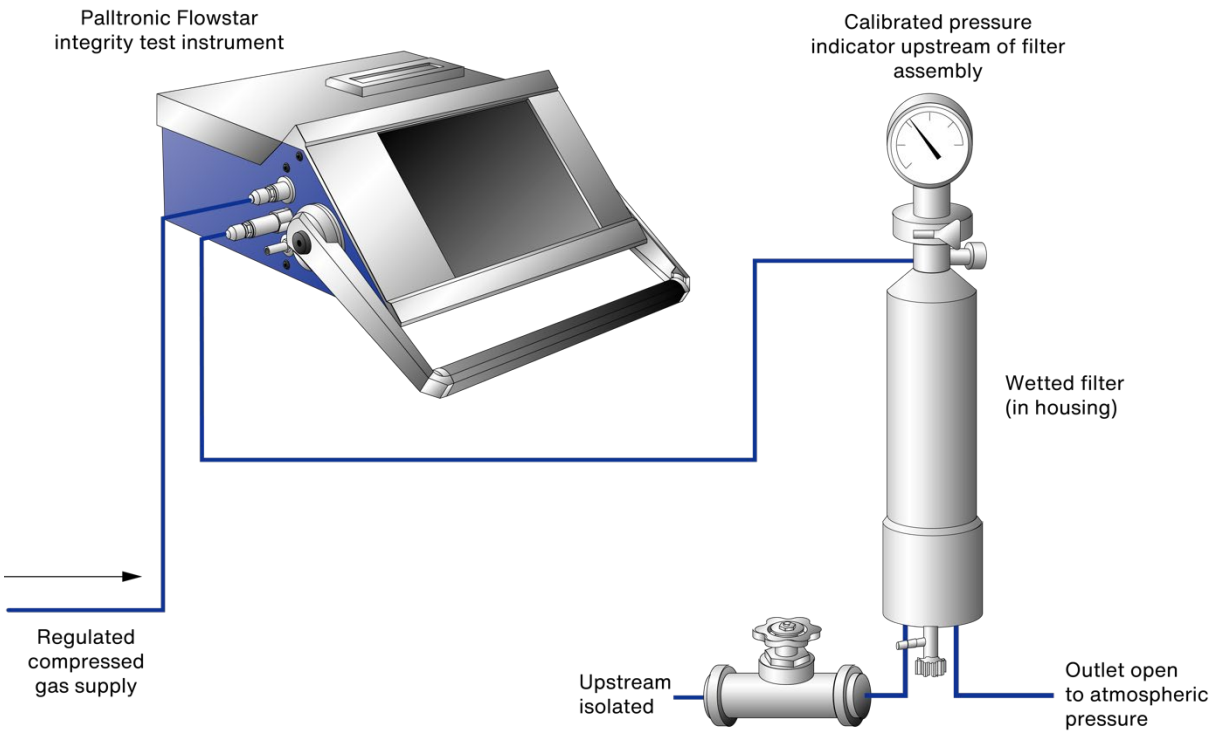
The studies in this chapter aimed to evaluate the microbial removal efficiency of typical Emflon HTPFR filter cartridges in liquid challenge tests using *B. diminuta* (ATCC 19146) and to demonstrate a direct correlation of the measured forward flow and water intrusion integrity test values to the microbial removal efficiency.

3.1.1 The Forward Flow Test

In the forward flow test, the pores of a filter are fully wetted with an appropriate test liquid, and pre-determined gas pressure is applied to the upstream side of the filter assembly. After a stabilization period, the gas flow through the wetted membrane can be measured on the upstream side, using sensitive flow measurement equipment such as the Palltronic® Flowstar filter integrity test instrument (see Figure 1). This gas flow comprises diffusion across the wetted membrane, plus bulk gas flow through any non-wetted pores or defects.

Figure 1

The automated integrity test



3.1.2 The Water Intrusion Integrity Test

The water intrusion test is performed on a non-wetted (dry), preferably clean, hydrophobic membrane filter. The upstream side of the filter assembly is filled with water until all air is removed and pre-determined gas pressure is applied to the water through the top vent on the housing. The water flow through the membrane can be measured directly as gas flow on the upstream side using sensitive direct flow measurement equipment, such as the Palltronic AquaWIT filter integrity test system, which incorporates the Palltronic Flowstar filter integrity test instrument (see Figure 1).

The aim of these bacterial retention studies was to evaluate the microbial removal efficiency of typical Emflon HTPFR filter cartridges in liquid challenge tests using *B. diminuta* (ATCC 19146) and to demonstrate a direct correlation of the measured forward flow and water intrusion integrity test values to the microbial removal efficiency.

3.2 Methods

Sixty-seven (67) typical production 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) were used for the bacteria retention studies using an aqueous suspension of *B. diminuta* (ATCC 19146). All filters underwent forward flow testing prior to and after the bacteria challenge test. In addition, thirty-two (32) of these filter cartridges underwent water intrusion testing prior to and after bacteria challenge testing.

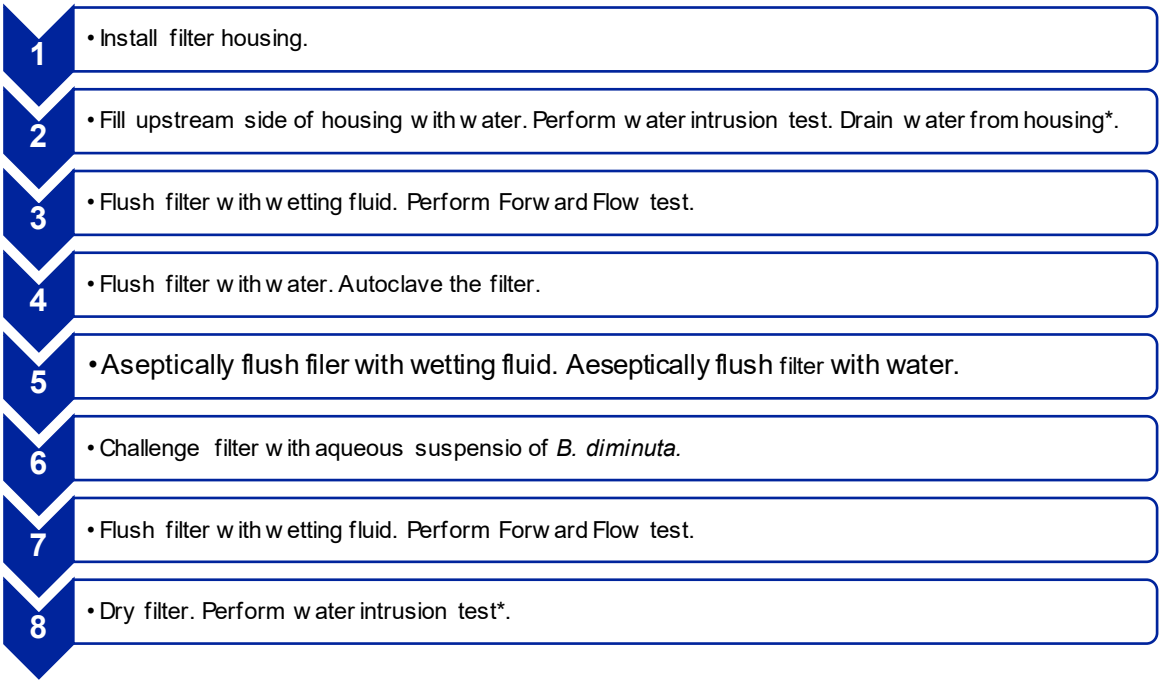
Twenty-four (24) typical production 127 mm (5 in.) Emflon HTPFR filter cartridges (p/n AB05HTPFR2PVH4) were used for the bacteria retention studies using an aqueous suspension of *B. diminuta* (ATCC 19146). All twenty-four (24) filters underwent forward flow and water intrusion testing prior to and after the bacteria challenge test.

The filter cartridges tested from each part number represented three (3) different sub-batches (designated 1, 2, and 3). Three (3) different membrane roll pairs were used to manufacture three (3) different sub-batches of p/n AB1HTPFR7PVH4, and two (2) different membrane roll pairs were used to manufacture the three (3) different sub-batches of p/n AB05HTPFR7PVH4, i.e., a total of five (5) rolls pairs were used for filter built.

Figure 2 shows the general workflow for integrity testing and bacteria challenge testing. An asterisk indicates process steps associated with the water intrusion test omitted for a sub-population of p/n AB1HTPFR7PVH4.

Figure 2

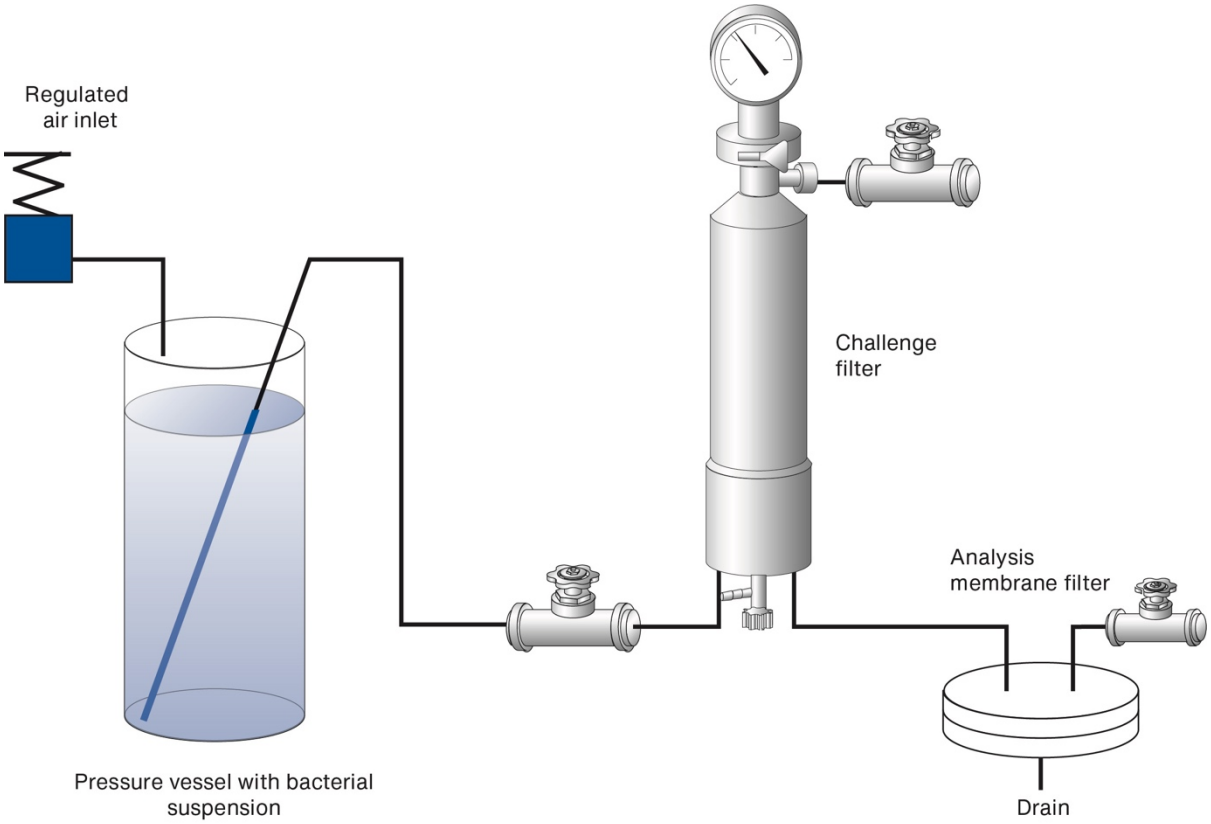
Test sequence of forward flow, water intrusion, and liquid microbial (*B. diminuta*) challenge testing of typical Emflon HTPFR filter cartridges



After the pre-challenge integrity testing, the filter cartridges were flushed with water and autoclaved at 121 ºC (250 ºF) for 60 minutes. After autoclaving, the filters were flushed with alcohol, followed by water, then aseptically connected to a downstream membrane housing containing a pre-sterilized recovery membrane disc and then aseptically connected to the challenge reservoir, as shown in Figure 3.

Figure 3

Microbial challenge apparatus



A total volume of ten (10) liters containing > 1 x 10^7 CFU/mL of *B. diminuta* was passed through each test filter cartridge to achieve a challenge level of > 1.0 x 10^7 CFU/cm^2. A total challenge level of > 1.0 x 10^{11} CFU per filter cartridge was achieved for all tests performed. During the bacterial challenge test, the entire filter effluent was passed through a 0.2 µm rated recovery membrane located on the downstream side of the test filter assembly. Following the challenge test, the recovery membrane was aseptically removed from the filter housing in a laminar flow cabinet and placed onto tryptic soy agar (TSA) plates. All agar plates were incubated at 30 ± 2 ºC (86 ± 3.6 ºF) for a minimum of two (2) days.

After incubation, the recovery membranes were examined for growth to determine whether bacteria had passed through the test filter during the challenge. The titer reduction (T_R) ratio for each filter was determined as follows:

$$T_r = \frac{Total\ number\ of\ bacteria\ influent\ to\ the\ filter}{Number\ of\ colonies\ recorded\ on\ the\ downstream\ recovery\ disc}$$

When no colonies were detected downstream, the titer reduction was expressed as greater than the total number of bacteria influent to the filter (e.g., > 1 x 10^{11}).

3.3 Results

3.3.1 Forward Flow Correlation for Part Number AB1HTPFR7PVH4

Forward flow testing of 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) was performed before and after the microbial challenge. The respectively higher of the two (2) forward flow results was selected and is shown in Table 3 together with the *B. diminuta* retention test results. The results are presented in order of increasing forward flow values. It was found that all filter cartridges tested gave sterile effluent when challenged with > 1 x 10^{11} CFU of *B. diminuta* per filter cartridge (> 1.0 x 10^7 CFU/cm^2).

Table 3

Results of forward flow (in increasing order) and *B. diminuta* retention tests (liquid challenge) for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4)

Filter Serial Number	Sub-Batch (# 1, 2, 3)	Forward Flow (mL/min) *	Challenge Level per Filter (CFU)	Sterile Effluent	Titer Reduction
PB2426/040	2	7.33	2.0×10^{11}	Yes	$> 2.0 \times 10^{11}$
PB2426/054	3	8.37	2.5×10^{11}	Yes	$> 2.5 \times 10^{11}$
PB2426/066	3	8.43	3.0×10^{11}	Yes	$> 3.0 \times 10^{11}$
PB2426/033	3	8.55	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2426/038	2	8.67	1.8×10^{11}	Yes	$> 1.8 \times 10^{11}$
PB2426/050	2	8.72	1.9×10^{11}	Yes	$> 1.9 \times 10^{11}$
PB2426/025	1	8.73	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/032	2	9.04	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2426/093	3	9.43	3.2×10^{11}	Yes	$> 3.2 \times 10^{11}$
PB2426/014	2	9.49	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/036	3	9.56	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/095	2	9.78	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/103	1	9.84	1.9×10^{11}	Yes	$> 1.9 \times 10^{11}$
PB2426/079	1	9.95	2.4×10^{11}	Yes	$> 2.4 \times 10^{11}$
PB2426/102	3	10.0	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2426/111	3	10.2	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/060	3	10.2	2.1×10^{11}	Yes	$> 2.1 \times 10^{11}$
PB2426/007	1	10.3	3.0×10^{11}	Yes	$> 3.0 \times 10^{11}$
PB2426/078	3	10.4	2.9×10^{11}	Yes	$> 2.9 \times 10^{11}$
PB2426/100	1	10.4	2.6×10^{11}	Yes	$> 2.6 \times 10^{11}$
PB2426/089	2	10.4	2.5×10^{11}	Yes	$> 2.5 \times 10^{11}$
PB2426/105	3	10.5	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/107	2	10.6	1.6×10^{11}	Yes	$> 1.6 \times 10^{11}$
PB2426/101	2	10.7	2.6×10^{11}	Yes	$> 2.6 \times 10^{11}$
PB2426/053	2	10.7	2.1×10^{11}	Yes	$> 2.1 \times 10^{11}$
PB2426/055	1	10.7	3.1×10^{11}	Yes	$> 3.1 \times 10^{11}$
PB2426/005	2	10.7	1.7×10^{11}	Yes	$> 1.7 \times 10^{11}$
PB2426/090	3	10.8	1.9×10^{11}	Yes	$> 1.9 \times 10^{11}$
PB2426/034	1	10.8	2.1×10^{11}	Yes	$> 2.1 \times 10^{11}$
PB2426/076	1	11.0	2.4×10^{11}	Yes	$> 2.4 \times 10^{11}$
PB2426/108	3	11.0	2.6×10^{11}	Yes	$> 2.6 \times 10^{11}$
PB2426/008	2	11.0	2.7×10^{11}	Yes	$> 2.7 \times 10^{11}$

PB2426/021	3	11.1	1.7×10^{11}	Yes	$> 1.7 \times 10^{11}$
PB2426/065	2	11.1	2.1×10^{11}	Yes	$> 2.1 \times 10^{11}$
PB2426/088	1	11.2	2.4×10^{11}	Yes	$> 2.4 \times 10^{11}$
PB2426/006	3	11.2	2.6×10^{11}	Yes	$> 2.6 \times 10^{11}$
PB2426/027	3	11.2	2.0×10^{11}	Yes	$> 2.0 \times 10^{11}$
PB2426/058	1	11.3	2.7×10^{11}	Yes	$> 2.7 \times 10^{11}$
PB2426/028	1	11.3	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/113	2	11.6	1.9×10^{11}	Yes	$> 1.9 \times 10^{11}$
PB2426/074	2	11.6	2.1×10^{11}	Yes	$> 2.1 \times 10^{11}$
PB2426/086	2	11.6	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/073	1	11.6	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2426/017	2	11.7	3.1×10^{11}	Yes	$> 3.1 \times 10^{11}$
PB2426/023	2	11.7	2.1×10^{11}	Yes	$> 2.1 \times 10^{11}$
PB2426/110	2	11.7	2.6×10^{11}	Yes	$> 2.6 \times 10^{11}$
PB2426/047	2	12.0	3.4×10^{11}	Yes	$> 3.4 \times 10^{11}$
PB2426/057	3	12.1	3.0×10^{11}	Yes	$> 3.0 \times 10^{11}$
PB2426/071	2	12.1	2.4×10^{11}	Yes	$> 2.4 \times 10^{11}$
PB2426/069	3	12.1	2.0×10^{11}	Yes	$> 2.0 \times 10^{11}$
PB2426/094	1	12.3	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/051	3	12.4	1.7×10^{11}	Yes	$> 1.7 \times 10^{11}$
PB2426/037	1	12.5	2.5×10^{11}	Yes	$> 2.5 \times 10^{11}$
PB2426/039	3	12.6	2.4×10^{11}	Yes	$> 2.4 \times 10^{11}$
PB2426/063	3	12.6	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2426/061	1	12.7	3.2×10^{11}	Yes	$> 3.2 \times 10^{11}$
PB2426/070	1	12.7	1.8×10^{11}	Yes	$> 1.8 \times 10^{11}$
PB2426/064	1	12.8	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/056	2	12.8	1.8×10^{11}	Yes	$> 1.8 \times 10^{11}$
PB2426/030	3	12.9	1.8×10^{11}	Yes	$> 1.8 \times 10^{11}$
PB2426/092	2	12.9	2.6×10^{11}	Yes	$> 2.6 \times 10^{11}$
PB2426/075	3	13.4	1.8×10^{11}	Yes	$> 1.8 \times 10^{11}$
PB2426/018	3	13.5	1.6×10^{11}	Yes	$> 1.6 \times 10^{11}$
PB2426/114	3	14.2	2.4×10^{11}	Yes	$> 2.4 \times 10^{11}$
PB2426/091	1	14.5	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2426/016	1	15.0	3.3×10^{11}	Yes	$> 3.3 \times 10^{11}$
PB2426/020	2	16.4	2.9×10^{11}	Yes	$> 2.9 \times 10^{11}$

** Forward flow values measured at 15.0 psi air test pressure, wetted with 60:40 (v/v) isopropyl alcohol/water at ambient temperature.*

3.3.2 Water Intrusion Correlation for Part Number AB1HTPFR7PVH4

Water intrusion testing of 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) was performed before and after microbial challenge. The respectively higher of the two water intrusion results was selected and is shown in Table 4 together with the *B. diminuta* retention results. The results are presented in order of increasing water intrusion values. All filters tested gave sterile effluent when challenged with > 1 x 10^{11} CFU of *B. diminuta* per filter (> 1 x 10^{7} CFU/cm^2).

Table 4

Results of water intrusion (in increasing order) and *B. diminuta* retention testing (liquid challenge) for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4)

Filter Serial Number	Sub-Batch (# 1, 2, 3)	Water Intrusion (mL/min) *	Challenge Level per Filter (CFU)	Sterile Effluent	Titer Reduction
PB2426/014	2	0.19	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/017	2	0.20	3.1×10^{11}	Yes	$> 3.1 \times 10^{11}$
PB2426/110	2	0.21	2.6×10^{11}	Yes	$> 2.6 \times 10^{11}$
PB2426/023	2	0.21	2.1×10^{11}	Yes	$> 2.1 \times 10^{11}$
PB2426/060	3	0.23	2.1×10^{11}	Yes	$> 2.1 \times 10^{11}$
PB2426/040	1	0.23	2.0×10^{11}	Yes	$> 2.0 \times 10^{11}$
PB2426/076	1	0.23	2.4×10^{11}	Yes	$> 2.4 \times 10^{11}$
PB2426/058	1	0.23	2.7×10^{11}	Yes	$> 2.7 \times 10^{11}$
PB2426/025	1	0.25	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/056	2	0.25	1.8×10^{11}	Yes	$> 1.8 \times 10^{11}$
PB2426/032	2	0.26	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2426/036	3	0.26	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/050	2	0.26	1.9×10^{11}	Yes	$> 1.9 \times 10^{11}$
PB2426/074	2	0.26	2.1×10^{11}	Yes	$> 2.1 \times 10^{11}$
PB2426/102	3	0.27	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2426/090	3	0.27	1.9×10^{11}	Yes	$> 1.9 \times 10^{11}$
PB2426/089	2	0.27	2.5×10^{11}	Yes	$> 2.5 \times 10^{11}$
PB2426/088	1	0.28	2.4×10^{11}	Yes	$> 2.4 \times 10^{11}$
PB2426/020	2	0.28	2.9×10^{11}	Yes	$> 2.9 \times 10^{11}$
PB2426/038	2	0.28	1.8×10^{11}	Yes	$> 1.8 \times 10^{11}$
PB2426/033	3	0.28	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2426/054	3	0.28	2.5×10^{11}	Yes	$> 2.5 \times 10^{11}$
PB2426/057	3	0.28	3.0×10^{11}	Yes	$> 3.0 \times 10^{11}$
PB2426/091	1	0.29	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2426/100	1	0.29	2.6×10^{11}	Yes	$> 2.6 \times 10^{11}$
PB2426/007	1	0.29	3.0×10^{11}	Yes	$> 3.0 \times 10^{11}$
PB2426/064	1	0.29	2.3×10^{11}	Yes	$> 2.3 \times 10^{11}$
PB2426/101	2	0.30	2.6×10^{11}	Yes	$> 2.6 \times 10^{11}$

PB2426/019	1	0.30	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2426/047	2	0.31	3.4×10^{11}	Yes	$> 3.4 \times 10^{11}$
PB2426/061	1	0.33	3.2×10^{11}	Yes	$> 3.2 \times 10^{11}$
PB2426/066	3	0.34	3.0×10^{11}	Yes	$> 3.0 \times 10^{11}$

** Water intrusion values measured at 2500 mbar test pressure, using deionized water at ambient temperature.*

3.3.3 Forward Flow Correlation for Part Number AB05HTPFR2PVH4

Forward flow testing of 127 mm (5 in.) Emflon HTPFR filters (p/n AB05HTPFR7PVH4) was performed before and after microbial challenge. The respectively higher of the two forward flow results was selected and is shown in Table 5 together with the *B. diminuta* retention results. The results are presented in order of increasing forward flow values. All filters tested gave sterile effluent when challenged with $> 5 \times 10^{10}$ CFU of *B. diminuta* per filter ($> 1.0 \times 10^{7}$ CFU/cm^2).

Table 5

Results of forward flow (in increasing order) and *B. diminuta* retention testing (liquid challenge) for 127 mm (5 in.) Emflon HTPFR filter cartridges (p/n AB05HTPFR2PVH4)

Filter Serial Number	Sub-Batch (# 1, 2, 3)	Forward Flow (mL/min) *	Challenge Level per Filter (CFU)	Sterile Effluent	Titer Reduction
IT4653/009	3	4.85	4.4×10^{11}	Yes	$> 4.4 \times 10^{11}$
IT4653/045	3	4.89	3.9×10^{11}	Yes	$> 3.9 \times 10^{11}$
IT4652/005	2	5.29	8.4×10^{10}	Yes	$> 8.4 \times 10^{10}$
IT4652/055	2	5.48	3.7×10^{11}	Yes	$> 3.7 \times 10^{11}$
IT4653/006	3	5.78	3.9×10^{11}	Yes	$> 3.9 \times 10^{11}$
IT4653/017	3	5.89	3.7×10^{11}	Yes	$> 3.7 \times 10^{11}$
IT4652/004	2	6.07	3.5×10^{11}	Yes	$> 3.5 \times 10^{11}$
IT4653/060	3	6.12	3.8×10^{11}	Yes	$> 3.8 \times 10^{11}$
IT4652/009	2	6.31	3.2×10^{11}	Yes	$> 3.2 \times 10^{11}$
IT4652/008	2	6.43	3.4×10^{11}	Yes	$> 3.4 \times 10^{11}$
IT4652/010	2	6.43	9.9×10^{10}	Yes	$> 9.9 \times 10^{10}$
IT4651/024	1	5.61	3.3×10^{11}	Yes	$> 3.3 \times 10^{11}$
IT4653/036	3	6.69	3.6×10^{11}	Yes	$> 3.6 \times 10^{11}$
IT4652/015	1	6.71	9.7×10^{10}	Yes	$> 9.7 \times 10^{10}$
IT4651/001	1	6.77	1.2×10^{11}	Yes	$> 1.2 \times 10^{11}$
IT4651/038	1	7.14	1.6×10^{11}	Yes	$> 1.6 \times 10^{11}$
IT4652/022	2	7.28	1.2×10^{11}	Yes	$> 1.2 \times 10^{11}$
IT4651/032	1	7.33	1.2×10^{11}	Yes	$> 1.2 \times 10^{11}$
IT4651/049	1	7.42	8.8×10^{10}	Yes	$> 8.8 \times 10^{10}$
IT4651/004	1	7.48	1.1×10^{11}	Yes	$> 1.1 \times 10^{11}$
IT4651/012	1	7.48	9.4×10^{10}	Yes	$> 9.4 \times 10^{10}$
IT4653/063	3	7.58	3.7×10^{11}	Yes	$> 3.7 \times 10^{11}$
IT4651/021	1	7.70	1.1×10^{11}	Yes	$> 1.1 \times 10^{11}$
IT4653/053	3	7.92	3.0×10^{11}	Yes	$> 3.0 \times 10^{11}$

** Forward flow values measured at 15.0 psi air test pressure, wetted with 60:40 (v/v) isopropyl alcohol/water at ambient temperature.*

3.3.4 Water Intrusion Correlation for Part Number AB05HTPFR2PVH4

Water intrusion testing of 127 mm (5 in.) Emflon HTPFR filters (p/n AB05HTPFR7PVH4) was performed before and after microbial challenge. The respectively higher of the two (2) water intrusion results was selected and is shown in Table 6 together with the *B. diminuta* retention results. The results are presented in order of increasing water intrusion values. All filters tested gave sterile effluent when challenged with > 5 x 10^{10} CFU of *B. diminuta* per filter (> 1.0 x 10^{7} CFU/cm^{2}).

Table 6

Results of water intrusion (in increasing order) and *B. diminuta* retention testing (liquid challenge) for 127 mm (5 in.) Emflon HTPFR filter cartridges (p/n AB05HTPFR2PVH4)

Filter Serial Number	Sub-Batch (# 1, 2, 3)	Water Intrusion (mL/min) *	Challenge Level per Filter (CFU)	Sterile Effluent	Titer Reduction
IT4653/060	3	0.083	3.8 x 10^{11}	Yes	> 3.8 x 10^{11}
IT4653/063	3	0.085	3.7 x 10^{11}	Yes	> 3.7 x 10^{11}
IT4652/055	2	0.094	3.7 x 10^{11}	Yes	> 3.7 x 10^{11}
IT4651/024	1	0.094	3.3 x 10^{11}	Yes	> 3.3 x 10^{11}
IT4651/038	1	0.10	1.6 x 10^{11}	Yes	> 1.6 x 10^{11}
IT4651/012	1	0.10	9.4 x 10^{10}	Yes	> 9.4 x 1010
IT4653/009	3	0.10	4.4 x 10^{11}	Yes	> 4.4 x 10^{11}
IT4652/009	2	0.10	3.2 x 10^{11}	Yes	> 3.2 x 10^{11}
IT4651/032	1	0.11	1.2 x 10^{11}	Yes	> 1.2 x 10^{11}
IT4651/004	1	0.11	1.1 x 10^{11}	Yes	> 1.1 x 10^{11}
IT4651/021	1	0.11	1.1 x 10^{11}	Yes	> 1.1 x 10^{11}
IT4653/045	3	0.11	3.9 x 10^{11}	Yes	> 3.9 x 10^{11}
IT4651/049	1	0.12	8.8 x 10^{10}	Yes	> 8.8 x 10^{10}
IT4652/022	2	0.12	1.2 x 10^{11}	Yes	> 1.2 x 10^{11}
IT4652/010	2	0.12	9.9 x 10^{10}	Yes	> 9.9 x 10^{10}
IT4652/004	2	0.12	3.5 x 10^{11}	Yes	> 3.5 x 10^{11}
IT4652/008	2	0.12	3.4 x 10^{11}	Yes	> 3.4 x 10^{11}
IT4652/005	2	0.12	8.4 x 10^{10}	Yes	> 8.4 x 10^{10}
IT4653/053	3	0.12	1.2 x 10^{11}	Yes	> 1.2 x 10^{11}
IT4653/006	3	0.12	3.0 x 10^{11}	Yes	> 3.0 x 10^{11}
IT4651/001	1	0.13	1.2 x 10^{11}	Yes	> 1.2 x 10^{11}
IT4652/015	2	0.13	9.7 x 10^{10}	Yes	> 9.7 x 10^{10}
IT4653/017	3	0.13	3.7 x 10^{11}	Yes	> 3.7 x 10^{11}
IT4653/036	3	0.17	3.6 x 10^{11}	Yes	> 3.6 x 10^{11}

** Water intrusion values at 2500 mbar air test pressure, using deionized water at ambient temperature.*

3.4 Conclusions

Based on the results of the microbial retention studies, the forward flow and water intrusion test methods were validated as non-destructive integrity tests for Emflon HTPFR filter cartridges.

Integrity test parameters for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) were set as follows, based on the test data generated.

Table 7

Forward flow integrity test parameters for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4)

Wetting Liquid	**60:40 (v/v) Isopropyl Alcohol/Water**
Temperature	20 ± 5 °C (68 ± 9 °F)
Test pressure	1040 mbar (15.0 psi)
Test gas	Air
Maximum allowable forward flow limit	16.0 mL/min

Table 8

Water intrusion integrity test parameters for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4)

Test Liquid	**Deionized Water**
Temperature	20 ± 2 °C (68 ± 4 °F)
Test pressure	2500 mbar (36.0 psi)
Maximum allowable water intrusion limit	0.33 mL/min

Integrity test parameters for 127 mm (5 in.) Emflon HTPFR filter cartridges (p/n AB05HTPFR2PVH4) were set as follows, based on the test data generated.

Table 9

Forward flow integrity test parameters for 127 mm (5 in.) Emflon HTPFR filter cartridges (p/n AB05HTPFR2PVH4)

Wetting Liquid	**60:40 (v/v) Isopropyl Aalcohol / Water**
Temperature	20 ± 5 °C (68 ± 9 °F)
Test pressure	1040 mbar (15.0 psi)
Test gas	Air
Maximum allowable forward flow limit	8.00 mL/min

Table 10

Water intrusion integrity test parameters for 127 mm (5 in.) Emflon HTPFR filter cartridges (p/n AB05HTPFR2PVH4)

Test Liquid	**Deionized Water**
Temperature	20 ± 2 °C (68 ± 4 °F)
Test pressure	2500 mbar (36.0 psi)
Maximum allowable water intrusion limit	0.16 mL/min

These forward flow and water intrusion integrity test parameters incorporate a safety margin, provide a high level of assurance that Emflon HTPFR filters perform to their retention claim, i.e., complete retention of *B. diminuta* when challenged with ≥ 1.0×10^7 CFU/cm^2 of effective filtration area, and confirm that Emflon HTPFR filters satisfy the requirements for sterilizing grade liquid filters.

4 Microbial Aerosol Retention Tests Using *B. subtilis* Spores, MS2 Bacteriophage, and *B. diminuta*

The tests aimed to demonstrate the microbial aerosol retention capability of Emflon HTPFR filters using the following challenge organisms:

- *B. subtilis* spores (NCTC 10073)
- MS2 bacteriophage (NCIMB 10108)
- B. diminuta ATCC 19146

4.1 Summary of Methods for Aerosol Challenge

In the microbial aerosol challenge method, the suspension of microorganisms is nebulized by a Collison spray to form a fine aerosol containing the challenge microorganisms. The generated aerosols are injected into a dry air stream flowing into a long stainless-steel tube. This causes liquid droplets surrounding the microorganisms to evaporate, leaving the challenge organisms free in the air stream at their minimum aerodynamic size. The efficiencies of the filters are calculated by determining the airborne microbial concentration upstream and downstream of the test filter.

254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) from standard manufacturing production lots were used for the tests. Before and after the challenge tests, filter integrity was confirmed using the water intrusion and forward flow test methods. Prior to the challenge test, the filter cartridges were dried by oven drying at a suitable temperature (50 °C (122 °F) up to 65 °C (149 °F) for sixteen (16) hours ("overnight")). After completing the microbial challenges, the test filter cartridges were sanitized, flushed with water, and then dried as above prior to performing the final set of water intrusion and forward flow integrity tests.

The titer reduction (TR) for each filter was determined as follows:

$$T_R = \frac{Number\ of\ bacteria\ or\ coliphage\ in\ the\ challenge}{Number\ of\ bacteria\ or\ coliphage\ assayed\ in\ the\ recovery\ buffer}$$

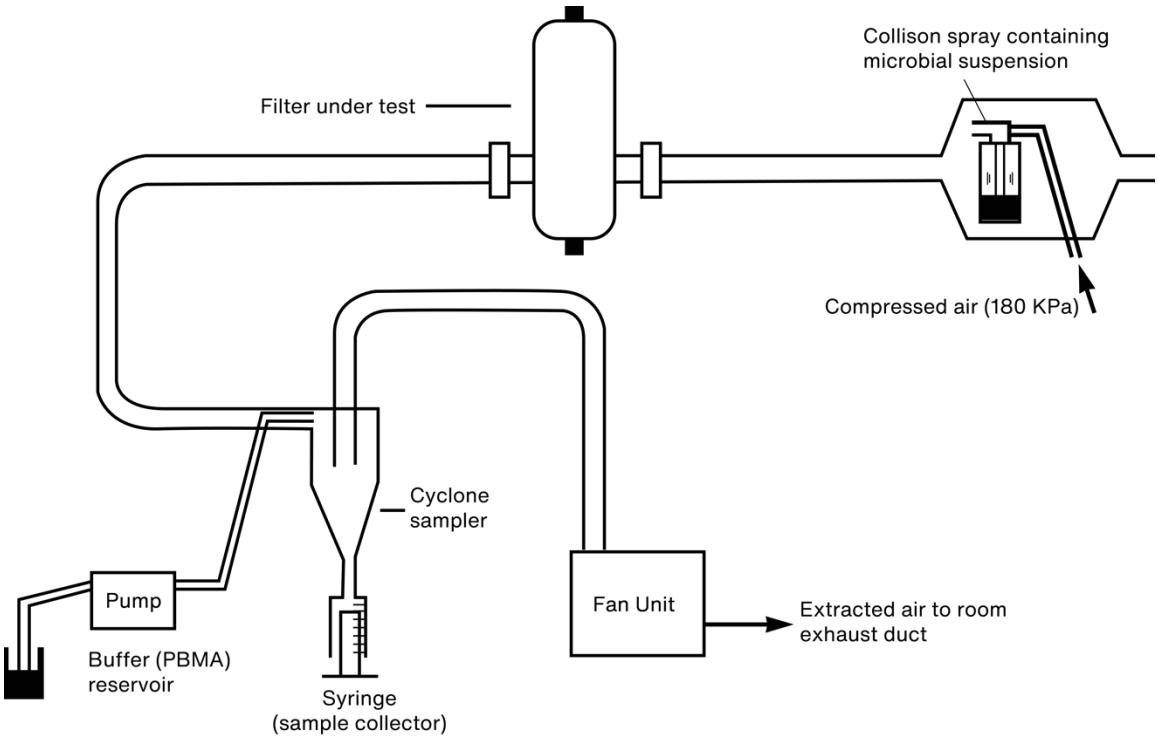
When no colonies were detected downstream, the titer reduction was expressed as
> Total number of microorganism influent to the filter (e.g., > 1 x 10^{10}).

4.2 *B. Subtilis* Spores and MS2 Bacteriophage Aerosol Challenge

Six (6) 254 mm (10 in.) Emflon HTPFR cartridges (p/n AB1HTPFR7PVH4) were used for challenge tests using *B. subtilis* spores and further six (6) 254 mm (10 in.) Emflon HTPFR cartridges (p/n AB1HTPFR7PVH4) were used for MS2 bacteriophage challenge tests. The testing assembly was sterilized by autoclaving and aseptically connected to the sterile challenge apparatus, as shown schematically in Figure 4. The assembly consisted of a Collison nebulizer used to generate the microbial aerosols. This was housed in a chamber with a compressed air supply. The aerosol was generated in the chamber and introduced to the filter housing via stainless steel piping. Aerosol sampling devices and a vacuum pump were used to draw the air containing the microbial aerosols through the system at the required flow rate.

Figure 4

Microbial aerosol challenge apparatus used for *B. subtilis* spores and MS2 bacteriophage



Air containing an aerosol suspension of *B. subtilis* spores or MS2 bacteriophage was passed through each test filter at a flow rate of 700 L/min (25 SCFM) for 15 minutes or 700 L/min (25 SCFM) for five (5) minutes for the *B. subtilis* spores and MS2 bacteriophage, respectively.

Sterile phosphate buffer containing Manucol ♦ and antifoam (PBMA) was used as the collection fluid and was fed into the cyclone inlet at a rate of 1 mL/min using a peristaltic pump. Particles in the airstream were deposited by centrifugal force on the cyclone wall and were collected by the swirling liquid removed by a syringe at the end of the challenge period.

A cyclone sampler was used to collect the organisms generated in the system. Sterile collecting fluid was fed into the cyclone sampler and particles in the air stream were deposited by centrifugal force into the swirling liquid on the wall of the device. On completion of the challenge, the volume of collection fluid was measured and then assayed for the challenge organism using an appropriate technique.

Background levels were measured by operating the system with a filter in place and with the Collison nebulizer sprays switched 'off', and challenge levels were determined by operating the system with the filters removed and the Collison nebulizer sprays switched 'on'.

4.3 *B. diminuta* Challenge

Twelve (12) 254 mm (10 in.) Emflon HTPFR cartridges (p/n AB1HTPFR7PVH4) were used for the challenge tests using *B. diminuta* cells. For this type of aerosol challenge, a concentrated suspension of bacteria was aerosolized using a nebulizer and passed through the test filter. Impingers were used to capture any bacteria that may appear downstream of the test filter. In a parallel stream, the aerosol was also passed directly through the test system without a test filter in line, and the bacterial concentration was established in the downstream flow. This was compared to the bacterial concentration recovered downstream of the test filters to establish a titer reduction.

Each test filter cartridges were subjected to an aerosol challenge using *B. diminuta* (ATCC 19146) at a total challenge level of > 1.0×10^8 CFU. The bacterial challenges were performed at a flow rate of ten (10) standard cubic feet per minute (SCFM) (0.535 sL/min).

The input challenge levels of *B. diminuta* were determined pre- and post-challenge from the nebulizer samples by performing serial dilutions in phosphate-buffered saline (PBS), then passing appropriate dilutions through sterile 0.2 µm rated recovery membranes (p/n NR047100). The recovery membranes were then aseptically transferred onto TSA plates. The impingers were sampled, and the full volume recovered was also passed through a recovery membrane to allow any recoveries of *B. diminuta* to be analyzed. All agar plates were incubated at 30 ± 2 ºC (86 ± 3.6 ºF) for a minimum of two (2) days.

4.4 Results

Forward flow and water intrusion testing of 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) had been performed before and after microbial challenge. The respectively higher of the two (2) forward flow and water intrusion results was selected and is shown in the tables below together with the results of the microbial challenge tests.

Results of the aerosol challenges using *B. subtilis* and MS2 bacteriophage are shown in Table 11 and Table 12, respectively. The challenge levels targeted and achieved for the MS2 challenges were > 10^7 plaque forming units (PFU) per cm^2 of effective filter area (PFU/cm^2). Due to the larger size of the *B. subtilis* spores, the maximum challenge level that could be achieved in these tests was 10^6 CFU/cm^2.

The results show that the filter cartridges retained 100% of the two (2) challenge microorganisms, with minimum titer reductions of *B. subtilis* spores and MS2 bacteriophage being > 1.0 x 10^{10} and > 1.4 x 10^{11}, respectively, reflecting the respective challenge level per filter cartridge.

Table 11

Aerosol (*B. subtilis* spores) challenge results for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4)

Filter Serial Number	Forward Flow* (mL/min)	Water Intrusion ** (mL/min)	Challenge Level per Filter (CFU)	Effluent Recovery	Titer Reduction
BP2426098	12.6	0.17	3.2 x 10^{10}	No	> 3.2 x 10^{10}
BP2426031	13.2	0.18	1.4 x 10^{10}	No	> 1.4 x 10^{10}
BP2426115	13.6	0.18	3.5 x 10^{10}	No	> 3.5 x 10^{10}
BP2426024	13.9	0.20	1.0 x 10^{10}	No	> 1.0 x 10^{10}
BP2426104	14.0	0.19	3.1 x 10^{10}	No	> 3.1 x 10^{10}
BP2426035	15.0	0.17	1.3 x 10^{10}	No	> 1.3 x 10^{10}

** Forward flow values measured at 15.0 psi air test pressure, wetted with 60:40 (v/v) isopropyl alcohol/water at ambient temperature. Maximum allowable forward flow value = 16.0 mL/min.*

*** Water intrusion values at 2500 mbar (36.0 psi), air test pressure, using deionized water at ambient temperature. Maximum allowable flow = 0.33 mL/min.*

Table 12

Aerosol (MS2 bacteriophage) challenge results for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4)

Filter Serial Number	Forward Flow * (mL/min)	Water Intrusion ** (mL/min)	Challenge Level per Filter (CFU)	Effluent Recovery	Titer Reduction
BP2426015	12.4	0.28	1.7 x 10^{11}	No	> 1.7 x 10^{11}
BP2426106	12.8	0.20	1.6 x 10^{11}	No	> 1.6 x 10^{11}
BP2426097	13.1	0.24	1.4 x 10^{11}	No	> 1.4 x 10^{11}
BP2426012	13.5	0.26	1.4 x 10^{11}	No	> 1.4 x 10^{11}
BP2426109	13.9	0.18	1.9 x 10^{11}	No	> 1.9 x 10^{11}
BP2426026	14.1	0.26	1.5 x 10^{11}	No	> 1.5 x 10^{11}

** Forward flow values measured at 15.0 psi air test pressure, wetted with 60:40 (v/v) isopropyl alcohol/water at ambient temperature. Maximum allowable forward flow = 16.0 mL/min.*

*** Water intrusion values at 2500 mbar air test pressure, using deionized water at ambient temperature. Maximum allowable flow = 0.33 mL/min.*

For the *B. diminuta* aerosol challenges, twelve (12) 254 mm (10 in.) Pall Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) were challenged with a total challenge level > 1.0 x 10^8 CFU per filter cartridge. Under the test conditions employed, all the filters yielded a sterile effluent. The results are shown in Table 13.

Table 13

Aerosol (*B. diminuta*) challenge results for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4)

Filter Serial Number	Forward Flow (mL/min) *	Water Intrusion (mL/min) **	Challenge Level per Filter (CFU)	Effluent Recovery	Titer Reduction
IS4936096	10.5	0.20	3.0×10^8	No	$> 3.0 \times 10^8$
IS4935012	11.2	0.17	1.8×10^8	No	$> 1.8 \times 10^8$
IS4936095	11.3	0.19	2.9×10^8	No	$> 2.9 \times 10^8$
IS4936020	11.5	0.18	2.4×10^8	No	$> 2.4 \times 10^8$
S4935079	12.0	0.21	3.4×10^8	No	$> 3.4 \times 10^8$
IS4934103	12.0	0.17	3.1×10^8	No	$> 3.1 \times 10^8$
IS4934106	12.1	0.19	6.0×10^8	No	$> 6.0 \times 10^8$
IS4936032	12.7	0.18	2.1×10^8	No	$> 2.1 \times 10^8$
IS4935010	12.9	0.17	5.0×10^8	No	$> 5.0 \times 10^8$
IS4934108	13.1	0.18	2.1×10^8	No	$> 2.1 \times 10^8$
IS4935019	14.2	0.17	5.6×10^8	No	$> 5.6 \times 10^8$
IS4934115	15.6	0.19	3.5×10^8	No	$> 3.5 \times 10^8$

** Forward flow values measured at 15.0 psi air test pressure, wetted with 60:40 (v/v) isopropyl alcohol/water at ambient temperature. Maximum allowable forward flow = 16.0 mL/min.*

*** Water intrusion values at 2500 mbar air test pressure, using deionized water at ambient temperature. Maximum allowable flow = 0.33 mL/min.*

4.5 Conclusions

The results of the microbial aerosol tests demonstrate that standard production integral Emflon HTPFR filter cartridges will completely retain a very high level of aerosolized small viruses, bacteria and bacterial spores, as demonstrated by the titer reductions of MS2 bacteriophage, *B. diminuta,* and *B. subtilis* spores, respectively.

5 Sodium Chloride Aerosol Challenge Testing

The aim of this study was to perform aerosol challenge tests with sodium chloride to define the particulate removal efficiency for Emflon HTPFR filter cartridges in gases.

5.1 Summary of Methods

Typical production 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) were used in these tests.

Prior to the challenge test, the background of the test rig was established to ensure it did not exceed the maximum acceptable level of 20 counts/ft³. This was performed for three (3) cycles of five (5) minutes steady and ten (10) minutes pulsed flow using a background filter (Pall Ultramet-L®, p/n GLFF4000VM4, s/n 4HS-A976B-12).

Particle data was recorded using a TSI 3025 Condensation Nucleus Counter (CNC) capable of counting particles down to a size of 0.003 µm with one-minute sample intervals. The upstream challenge level was then generated using a Six Jet Atomizer with a 0.04% w/v NaCl solution as described in the Pall publication STR 1432[(1)] "Testing of all-metal filters for high purity semiconductor process gases" by B. Gotlinsky, P. Connor, D. Capitano, L. Johnson, S. Tousi; Pall Corporation 1994.

Afterwards, a background check was performed for fifteen (15) minutes to re-establish background levels in the test stand.

A test filter cartridge was then installed onto the test stand. Airflow was restarted, the test filter was flushed until particle counts downstream of the filter had reached background levels (< 20 counts/ft³) for forty-five (45) minutes with three (3) cycles of five (5) minutes steady and ten (10) minutes pulsed flow. Once the low background levels were achieved the filter cartridge was challenge tested for ninety (90) minutes of steady flow at the challenge level of 9.99 x 10^4 counts/cm³ (or 2.83 x 10^9 counts/ft^3). Testing was performed at a test flow rate of 1.5 standard liter per minute (SLPM).

Particle reduction was calculated as follows:

$$Particle\ reduction = \frac{Upstream\ counts\ per\ unit\ volume}{Downstream\ counts\ per\ unit\ volume}$$

5.2 Results

The sodium chloride aerosol challenge results are shown in Table 14. All Emflon HTPFR filters tested demonstrated high removal efficiencies for sodium chloride particles (> 99.999999%).

Table 14

Sodium chloride aerosol challenge results for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4)

Filter Serial Number	Upstream Counts (counts/ft^3)	Downstream Counts (counts/ft^3)	Particle Reduction	Removal Efficiency (%)
PB2378073	2.83 x 10^9	7.3	3.88 x 10^8	> 99.999999
PB2378186	2.83 x 10^9	18.9	1.50 x 10^8	> 99.999999
PB2378263	2.83 x 10^9	13.6	2.08 x 10^8	> 99.999999
IS6812076	2.83 x 10^9	9.4	3.01 x 10^8	> 99.999999
IS6812047	2.83 x 10^9	9.4	3.01 x 10^8	> 99.999999
IS6812082	2.83 x 10^9	13.6	2.08 x 10^8	> 99.999999

5.3 Conclusions

The results show that Emflon HTPFR filter cartridges retain particles down to a size of 0.003 µm with high efficiency (> 99.999999%) from a gas stream when challenged with a particle load of > 10^9 particles per ft^3.

6 Resistance to Steam Exposure

Steam exposure of filters in a sterilization process creates substantial thermal and mechanical stress and can cause them to lose integrity. These tests were performed to evaluate the ability of Emflon HTPFR filter cartridges to withstand multiple steam sterilization cycles. The tests were run to represent extreme conditions of use and at the upper limit of the temperature range of these filters. A 100% safety factor for the number of cycles was established by exposing the filter cartridges to twice the number of cycles (i.e., 200 cycles) than the target claim (100 cycles).

6.1 Summary of Methods

The procedure for these tests was based on the recommended instructions for steam sterilization described in Pall publication USTR 805 'Steam Sterilization of Pall Filter Assemblies Utilizing Replaceable Filter Cartridges.'

Typical production 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) and 127 mm (5 in.) Emflon HTPFR filter cartridges (p/n AB05HTPFR2PVH4) were used for these tests, three sub-batches of each p/n. The filter cartridges were installed in stainless steel housings and steamed in place in the forward ('out to in') direction using saturated steam at constant pressure and flow while ensuring effective condensate drainage. After each steam-in-place cycle the filters were cooled by passing dry compressed air through them.

Table 15 shows the applied steaming and cooling conditions.

Table 15

Steam exposure test conditions in the forward ('out to in') direction for Emflon HTPFR filters

Condition	Test Parameter
Steam temperature	142 ºC (288 ºF)
Duration of steam-in-place cycle	60 minutes
Air cooling time	30 minutes
Maximum differential pressure during steaming and cooling cycles	300 mbard (4.35 psid)

A total number of twenty-four (24) 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) were used for the steaming tests. Forward flow and water intrusion testing were performed after twenty-five (25) steam cycles, respectively. After having being exposed to fifty (50), hundred (100), or two-hundred (200) cumulative steaming cycles, respectively, six (6) test filter cartridges were flushed with alcohol followed by water and challenged with *B. diminuta* (ATCC 19146) at a minimum challenge level of 1.0×10^7 CFU/cm^2, as described in Section 3.2.

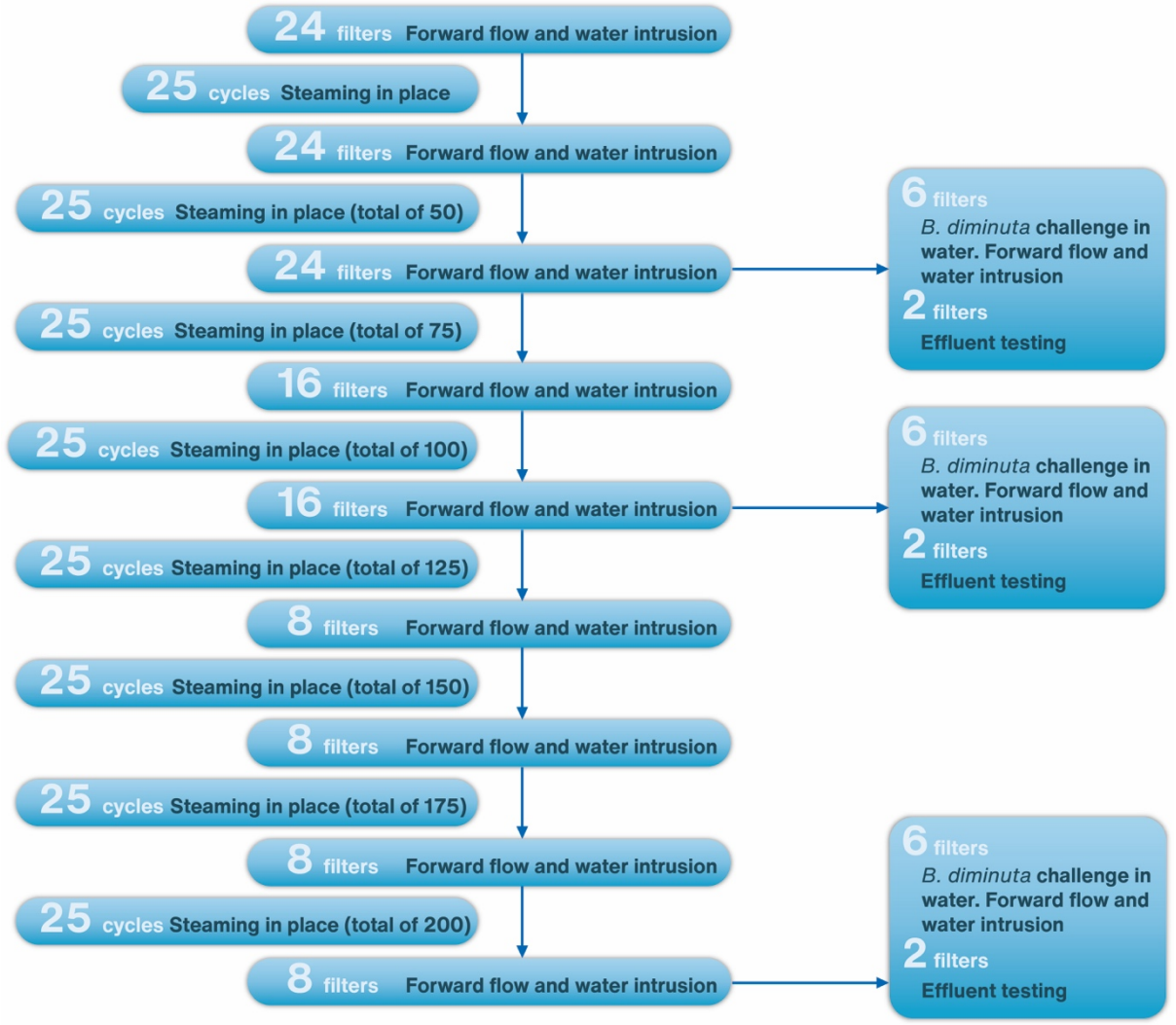
Two (2) test filter cartridges were tested for effluent quality (particulates, pH, oxidizable substances, and conductivity).

- Effluent quality testing was performed to confirm that Emflon HTPFR filter cartridges continue to meet the criteria that are certified in Pall's Certificate of Test (CoT) under Effluent Quality and in addition conductivity testing after exposure to multiple steam cycles:
- Filters meets with adequate safety margin after flushing current limits under USP <788> Particulate Matter in Injections with effluent counts determined microscopically. Counts serve to document conformance with the requirements for a non-fiber-releasing filter per Title 21 of the U.S. **Code of Federal Regulations** (CFR) parts 211.72 and 210.3(b)(6).
- Filters meet the current USP requirement, after flushing, under Sterile Purified Water, as determined by a potassium permanganate test.
- Filters meet internal specification for pH after flushing when tested in accordance with USP <791>.
- Filters meet the current USP <645> requirements under Purified Water after flushing.

The workflow sequence for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) is shown in Figure 5.

Figure 5

The evaluation of resistance to steam exposure in the forward ('out to in') direction of 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PH4)



- A total number of twelve (12) 127 mm (5 in.) Emflon HTPFR filter cartridges (p/n AB05HTPFR2PVH4) from three (3) different sub-batches were exposed to two hundred (200) cumulative steam cycles in the forward ('out to in') direction. Forward flow and water intrusion testing were performed prior to and after the steam exposure.

6.2 Results

6.2.1 Filter Part Number AB1HTPFR7PVH4

After up to two hundred (200) one-hour steam-in-place cycles in the forward ('out to in') direction all test filter cartridges passed the forward flow and water intrusion test. The forward flow and water intrusion test results are shown in Table 16 and Table 17. Fields colored gray indicate that filters were used for bacterial retention or quality effluent testing after exposure to stated number of steam-in-place cycles at 142 °C (288 °F).

Table 16

Forward flow* test results (mL/min) after steam-in-place cycles in the forward ('out to in') direction (one-hour cycles at 142 °C (288 °F)) of 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFRPVH4)

Filter Serial Number	Sub-Batch (# 1, 2 or 3)	Number of One-Hour Cycles							
		25	50	75	100	125	150	175	200
BP2378016	1	6.68	6.06	N/A	N/A	N/A	N/A	N/A	N/A
BP2378017	2	5.73	4.40	N/A	N/A	N/A	N/A	N/A	N/A
BP2378018	3	4.45	4.17	N/A	N/A	N/A	N/A	N/A	N/A
BP2378091	1	6.22	5.12	N/A	N/A	N/A	N/A	N/A	N/A
BP2378279	3	5.51	7.10	N/A	N/A	N/A	N/A	N/A	N/A
BP2378280	1	7.07	7.55	N/A	N/A	N/A	N/A	N/A	N/A
BP2378281	2	7.45	6.50	N/A	N/A	N/A	N/A	N/A	N/A
BP2378173	2	5.28	3.85	N/A	N/A	N/A	N/A	N/A	N/A
BP2378054	3	4.82	9.30	5.75	4.43	N/A	N/A	N/A	N/A
BP2378055	1	6.35	6.98	4.50	1.62	N/A	N/A	N/A	N/A
BP2378056	2	7.69	7.74	4.61	3.10	N/A	N/A	N/A	N/A
BP2378146	2	11.5	7.67	4.89	2.04	N/A	N/A	N/A	N/A
BP2378168	3	6.47	4.08	4.21	2.15	N/A	N/A	N/A	N/A
BP2378241	1	6.37	5.06	4.17	1.88	N/A	N/A	N/A	N/A
BP2378242	2	4.27	9.32	5.35	2.37	N/A	N/A	N/A	N/A
BP2378243	3	9.12	5.63	6.58	3.48	N/A	N/A	N/A	N/A
BP2378090	3	4.79	6.40	5.42	2.52	6.88	6.45	3.92	6.04
BP2378113	2	5.63	6.19	4.75	2.38	2.47	3.42	3.03	3.47
BP2378127	1	3.31	4.23	4.29	2.00	2.02	6.35	2.37	3.97
BP2378129	3	6.91	9.10	3.88	2.67	7.69	6.23	3.83	8.65
BP2378169	1	4.26	4.91	4.49	2.19	6.22	4.21	2.71	3.29
BP2378199	1	6.95	4.04	6.58	2.26	6.57	3.63	2.47	3.22
BP2378200	2	6.16	4.28	4.58	2.02	6.40	5.94	2.35	3.79
BP2378201	3	6.66	9.86	4.55	1.94	6.63	6.75	3.20	5.05

** Forward flow values measured at 15.0 psi air test pressure, wetted with 60:40 (v/v) isopropyl alcohol/water, at ambient temperature. Maximum allowable forward flow = 16.0 mL/min.*

Table 17

Water intrusion* test results (mL/min) after steam-in-place cycles in the forward ('out to in') direction (one-hour cycles at 142 °C (288 °F)) of 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFRPVH4)

Filter Serial Number	Number of One-Hour Cycles							
	25	50	75	100	125	150	175	200
BP2378016	0.19	0.17	N/A	N/A	N/A	N/A	N/A	N/A
BP2378017	0.18	0.17	N/A	N/A	N/A	N/A	N/A	N/A
BP2378018	0.17	0.18	N/A	N/A	N/A	N/A	N/A	N/A
BP2378173	0.18	0.16	N/A	N/A	N/A	N/A	N/A	N/A
BP2378091	0.18	0.17	N/A	N/A	N/A	N/A	N/A	N/A
BP2378279	0.19	0.18	N/A	N/A	N/A	N/A	N/A	N/A
BP2378280	0.19	0.21	N/A	N/A	N/A	N/A	N/A	N/A
BP2378281	0.19	0.19	N/A	N/A	N/A	N/A	N/A	N/A
BP2378054	0.20	0.19	0.17	0.21	N/A	N/A	N/A	N/A
BP2378055	0.17	0.17	0.32	0.19	N/A	N/A	N/A	N/A
BP2378056	0.18	0.19	0.17	0.20	N/A	N/A	N/A	N/A
BP2378241	0.18	0.17	0.15	0.18	N/A	N/A	N/A	N/A
BP2378242	0.17	0.17	0.16	0.21	N/A	N/A	N/A	N/A
BP2378243	0.20	0.21	0.18	0.21	N/A	N/A	N/A	N/A
BP2378146	0.20	0.20	0.16	0.19	N/A	N/A	N/A	N/A
BP2378168	0.18	0.18	0.17	0.19	N/A	N/A	N/A	N/A
BP2378113	0.19	0.18	0.17	0.18	0.20	0.20	0.19	0.17
BP2378127	0.18	0.18	0.17	0.19	0.19	0.18	0.19	0.23
BP2378129	0.18	0.21	0.18	0.21	0.24	0.28	0.21	0.17
BP2378090	0.20	0.17	0.17	0.20	0.20	0.20	0.18	0.19
BP2378169	0.18	0.16	0.16	0.18	0.16	0.18	0.18	0.20
BP2378199	0.18	0.19	0.16	0.18	0.17	0.19	0.18	0.18
BP2378200	0.17	0.18	0.17	0.20	0.19	0.22	0.20	0.19
BP2378201	0.20	0.20	0.17	0.24	0.22	0.20	0.18	0.21

** Water intrusion values at 2500 mbar air test pressure, using deionized water at ambient temperature. Maximum allowable flow = 0.33 mL/min.*

All tested filter cartridges provided a sterile effluent when challenged with *B. diminuta* (ATCC 19146) suspended in water at a minimum challenge level of > 1.0 x 10^7 CFU/cm^2, resulting in a minimum calculated titer reduction of > 1.5 x 10^{11}. This included test filter cartridges that had been exposed to two hundred (200) steam-in-place cycles at 140 °C (288 °F). The results of the challenge testing are shown in Table 18.

Table 18

Results of B. diminuta retention testing (liquid challenge) after steam in place cycles in the forward ('out to in') direction (one-hour cycles at 142 °C (288 °F)) of 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFRPVH4)

Filter Serial Number	No. of One-Hour Cycles	Forward Flow (mL/min) *	Water Intrusion (mL/min) **	Challenge Level per Filter (CFU)	Sterile Effluent	Titer Reduction
PB2378/016	50	6.62	0.19	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2378/017	50	7.58	0.23	2.6×10^{11}	Yes	$> 2.6 \times 10^{11}$
PB2378/018	50	6.67	0.19	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2378/279	50	10.7	0.22	1.8×10^{11}	Yes	$> 1.8 \times 10^{11}$
PB2378/280	50	7.17	0.21	9.7×10^{11}	Yes	$> 9.7 \times 10^{11}$
PB2378/281	50	15.0	0.22	2.4×10^{11}	Yes	$> 2.4 \times 10^{11}$
PB2378/054	100	13.8	0.25	2.2×10^{11}	Yes	$> 2.2 \times 10^{11}$
PB2378/055	100	7.47	0.20	2.0×10^{11}	Yes	$> 2.0 \times 10^{11}$
PB2378/056	100	7.90	0.22	1.5×10^{11}	Yes	$> 1.5 \times 10^{11}$
PB2378/146	100	15.0	0.24	1.9×10^{11}	Yes	$> 1.9 \times 10^{11}$
PB2378/241	100	12.1	0.22	1.6×10^{11}	Yes	$> 1.6 \times 10^{11}$
PB2378/242	100	10.5	0.19	2.0×10^{11}	Yes	$> 2.0 \times 10^{11}$
PB2378/090	200	18.9	0.23	1.8×10^{11}	Yes	$> 1.8 \times 10^{11}$
PB2378/113	200	17.6	0.18	2.0×10^{11}	Yes	$> 2.0 \times 10^{11}$
PB2378/127	200	13.1	0.13	1.5×10^{11}	Yes	$> 1.5 \times 10^{11}$
PB2378/129	200	17.4	0.24	1.7×10^{11}	Yes	$> 1.7 \times 10^{11}$
PB2378/199	200	6.76	0.18	2.8×10^{11}	Yes	$> 2.8 \times 10^{11}$
PB2378/201	200	8.10	0.20	1.7×10^{11}	Yes	$> 1.7 \times 10^{11}$

** Forward flow values measured at 15.0 psi air test pressure, wetted with 60:40 (v/v) isopropyl alcohol/water, at ambient temperature. Maximum allowable forward flow = 16.0 mL/min.*

*** Water intrusion values at 2500 mbar air test pressure, using deionized water at ambient temperature. Maximum allowable flow = 0.33 mL/min.*

The results of effluent quality testing are summarized in Table 19. Testing was performed on six (6) test filter cartridges post-steam exposure (two (2) test filter cartridges from three (3) different sub-batches). All effluent quality acceptance criteria for this part number were met with respect to particulates, pH, oxidizable substances, and conductivity.

Table 19

Results from effluent quality testing of 254 mm (10 in.) Emflon HTPFR filter cartridges

Serial Number	No. of One-Hour Steaming Cycles	Particulates	pH	Oxidizable Substances	Conductivity
PB2378/169	200	Pass	Pass	Pass	Pass
PB2378/200	200	Pass	Pass	Pass	Pass
PB2378/243	100	Pass	Pass	Pass	Pass
PB2378/168	100	Pass	Pass	Pass	Pass
PB2378/091	50	Pass	Pass	Pass	Pass
PB2378/173	50	Pass	Pass	Pass	Pass

6.2.2 Filter Part Number AB05HTPFR2PVH4

After two hundred (200) one-hour steam-in-place cycles at 142 °C (288 °F) in the forward ('out to in') direction all test filter cartridges passed forward flow and water intrusion integrity testing. The results of the integrity tests after steaming are shown in Table 20.

Table 20

Results of forward flow and water intrusion integrity testing after exposure to two hundred (200) one-hour steam in place cycles at 142 °C (288 °F) in the forward ('out to in') direction of 127 mm (5 in.) Emflon HTPFR filter cartridges (p/n AB05HTPFR2PV

Filter Serial Number	Forward Flow (mL/min) *	Water Intrusion Value (mL/min) **
IT4652012	0.76	0.13
IT4652047	0.97	0.12
IT4652020	1.11	0.14
IT4653052	1.18	0.13
IT4651025	1.32	0.13
IT4653024	1.48	0.11
IT4653018	2.03	0.12
IT4652052	2.05	0.12
IT4651062	2.15	0.12
IT4653002	2.29	0.12
IT4651056	2.51	0.11
IT4651019	2.88	0.15

** Forward flow values measured at 15.0 psi air test pressure, wetted with 60:40 (v/v) isopropyl alcohol/water, at ambient temperature. Maximum allowable forward flow = 8.0 mL/min*

*** Water intrusion values at 2500 mbar air test pressure, using deionized water at ambient temperature. Maximum allowable flow = 0.16 mL/min.*

6.3 Conclusions

Emflon HTPFR filter cartridges have been demonstrated to be capable of withstanding multiple steam-in-place sterilization cycles in the forward ('out to in') direction. Exposure to steam-in-place cycles can be considered equally or more challenging regarding exposure to saturated steam and differential pressure than autoclaving, thus also providing the data basis for a respective autoclave exposure claim.

- The filter cartridges have been shown to maintain integrity as demonstrated by passing forward flow and water intrusion testing after steam exposure, maintaining the retention performance of a sterilizing filter as demonstrated by providing sterile effluent in liquid challenge testing with *B. diminuta* and maintaining the ability to pass applicable effluent quality testing.

- The data presented in this section support the following product claim for steam-in-place in the forward direction or autoclaving of Emflon HTPFR filter cartridges with a 100% safety margin.

Table 21

Claim for maximum steam exposure by steam-in-place cycles in the forward ('out to in') direction or autoclaving

Filter Part Number	Steaming Temperature	Cycle Duration	Number of Cycles
AB1HTPFR7PVH4, AB05HTPFR2PVH4	142 °C (288 °F)	One hour	100

7 Resistance to Hot Air

This test aimed to determine the resistance of Emflon HTPFR filter cartridges to exposure to hot temperature air cycles at up to 140 ºC (284 °F) for typical operation periods (up to one (1) year) for this filter type.

7.1 Summary of Methods

Testing was performed by so-called accelerated testing. Accelerated testing is based on the general rule that the rate of a chemical reaction will double for every 10 ºC increase in temperature. The accelerated test temperature of 140 °C (284 °F) was chosen based on the mechanical stability of the filter components at this temperature. The accelerated test time required to create data supporting the target exposure time (12 months) and target exposure temperature (100 °C, 212 °F) were derived from the Arrhenius equation using a reaction rate coefficient of 2.0, reflecting the doubling in reaction speed every 10 °C temperature increase. The test time for exposure at the accelerated test temperature was thus calculated as follows:

$$t_{acc} = \frac{t_{claim}}{Q^{(T_{acc} - T_{claim})/10}}$$

Where:

- t_{acc} = time at accelerated test temperature
- t_{claim} = time at claim temperature
- Q = reaction rate coefficient (2.0)
- T_{acc} = accelerated test temperature
- T_{claim} = claim temperature

Three (3) standard manufacturing 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) from three (3) different sub-batches were used for the tests. Single filters were exposed to hot air at 140 ºC (284 °F) for twenty-three (23) days at a flow rate of approximately 1,500 L/min (53 SCFM, 90 Nm^3/h). Based on the above equation, this is equivalent to twelve (12) months in the air at 100 ºC (212 °F). Before and after exposure to each hot air cycle, filter integrity was determined using the water intrusion test method. On completion of each cycle the filters were also visually examined for physical signs of degradation of the filter hardware components.

7.2 Results

The results of water intrusion testing prior to and after hot air exposure at increasing time intervals are shown in Table 22.

Table 22

Water intrusion values of 254 mm (10 in.) Emflon HTPFR filters (p/n AB1HTPFR7PVH4) prior to and after exposure to hot (140 °C, 284 °F) air at increasing time intervals

	Water Intrusion (mL/min) *			
Filter Serial Number	**T = 0**	**T = 7 days**	**T = 14 days**	**T = 23 days**
IS4934045	0.17	0.15	0.18	0.19
IS4935033	0.17	0.17	0.18	0.17
IS4936071	0.18	0.21	0.17	0.19

**Water intrusion values at 2500 mbar air test pressure, using deionized water at ambient temperature. Maximum allowable flow = 0.33 mL/min.*

At all time intervals the filters passed the water intrusion integrity test and did not display visual signs of degradation.

7.3 Conclusions

The data presented demonstrate that Emflon HTPFR filter cartridges are suitable for use in hot air environments for prolonged time periods. The accelerated aging test results indicate that Emflon HTPFR filter cartridges will retain integrity while being exposed to hot air at temperatures up to 100 °C (212 °F) for one year.

8 Airflow / Differential Pressure Characteristics

These tests aimed to determine the differential pressure characteristics of Emflon HTPFR filter cartridges at different airflow rates in air systems representative of common use conditions: vent use (atmospheric pressure) and compressed air systems running at 1.0 bar, 2.0 bar and 4.0 bar (14.5 psi, 29.0 psi, 58.0 psi) system pressures.

8.1 Summary of Methods

Typical production 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) or 127 mm (5 in.) Emflon HTPFR filter cartridges (p/n AB05HTPFR2PVH4) were installed in stainless steel air filter housings designed for use in compressed gas and vent applications. The differential pressure across the filter assembly (filter housing and filter cartridge) was measured while clean compressed air was directed through the filter assembly, using increasing flow rates up to 360 Nm^3/h (212.4 SCFM), under both atmospheric vent and pressurized operating conditions.

In vent conditions, the downstream side of the filter assembly was open to atmospheric pressure and airflow through the filter was controlled from the upstream side. Under pressurized conditions, predetermined air pressures were maintained upstream of the filter assembly; airflow rate through the filter was controlled by restricting flow using a valve on the downstream side.

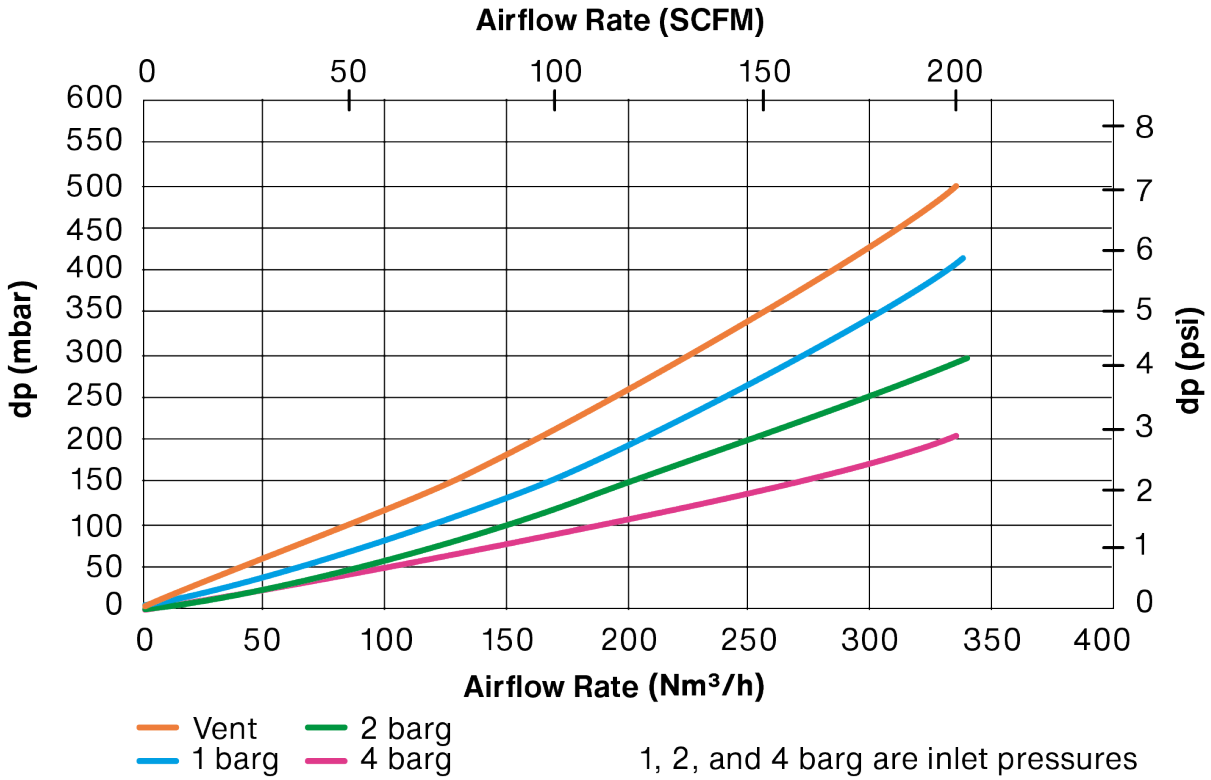
All airflow measurements were corrected to the following normal conditions for gases: atmospheric pressure (1013 mbara, 14.7 psia), 20 °C (68 °F).

8.2 Results

The flow versus differential pressure values at atmospheric pressure and various system pressures (1.0 barg, 2.0 barg, and 4.0 barg (14.5 psig, 29.0 psig, and 58.0 psig)) are shown in Figure 6 for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) and in Figure 7 for 127 mm (5 in.) Emflon HTPFR filter (p/n AB05HTPFR2PVH4). The graphs show that at high air flows the relationship between flow and pressure differential is not fully linear.

Figure 6

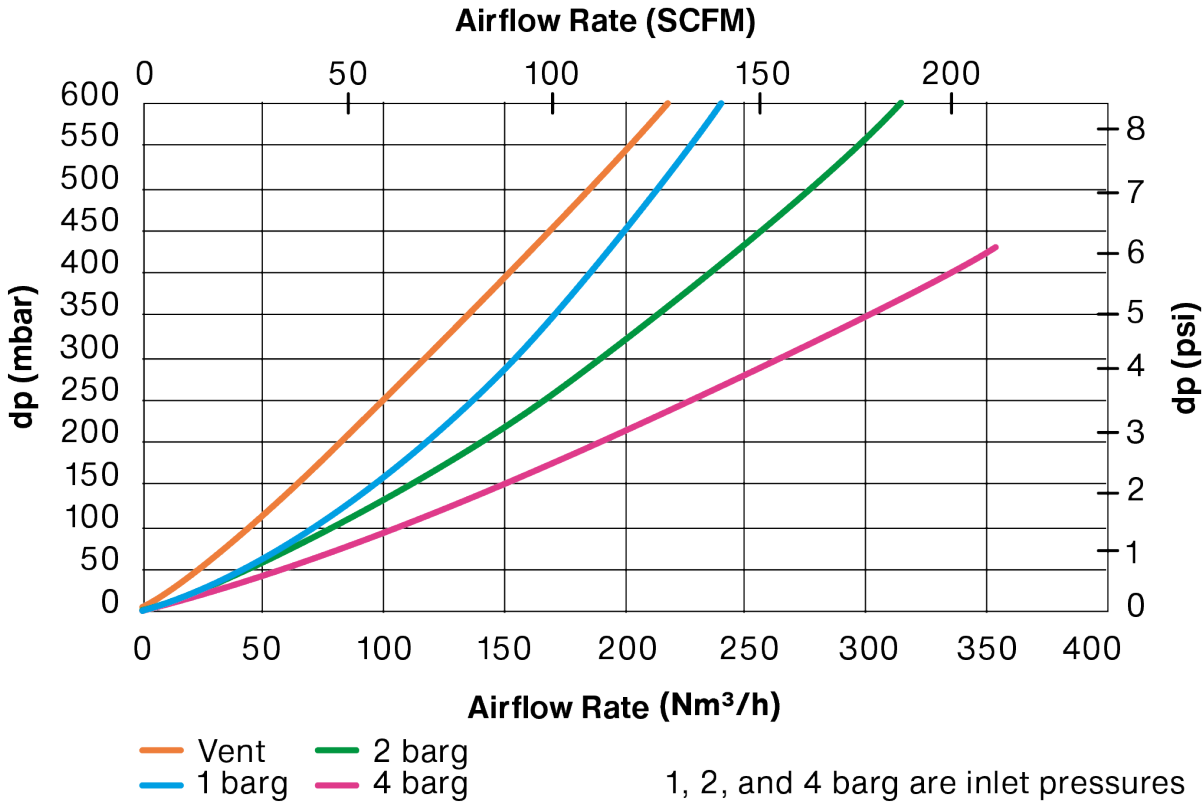
Airflow/differential pressure characteristics of 254 mm (10 in.) Emflon HTPFR filter cartridge / housing system (p/n AB1HTPFR7PVH4)



100 mbar = 1.45 psi, 1 Nm^3/h = 0.59 SCFM

Figure 7

Airflow/differential pressure characteristics (housing losses subtracted) of 127 mm (5 in.) Emflon HTPFR filter cartridge/housing system (p/n AB05HTPFR2PVH4)



100 mbar = 1.45 psi, 1 Nm^3/h = 0.59 SCFM

8.3 Conclusions

The airflow/differential pressure curves presented above can be used to size filter system employing Emflon HTPFR filter cartridges. The results show that at high airflows the relationship between flow and differential pressure is not fully linear. This behavior is expected as at higher gas flow additional pressure losses occur in the smallest flow path diameters, such as filter core and adapter, due to turbulent flow. Therefore, in the case of a high flow filter element such as Emflon HTPFR, these diameters become the restricting factor and their pressure losses need to be considered accordingly for sizing large installations.

9 Extractables Testing

These tests aimed to quantify the material which may be extracted from Emflon HTPFR filter cartridges using water and 50% ethanol (50:50 (v/v) ethanol/water).

9.1 Summary of Methods

Preparation of Filter Samples

Extractables tests were performed on typical 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4), each containing 0.84 m^2 (9.04 ft^2) of effective filtration area (EFA). The filter cartridges were subjected to a single autoclave cycle at 125 ºC (257 ºF) for sixty (60) minutes to maximize the quantity of any extractable material present.

Extraction Procedure

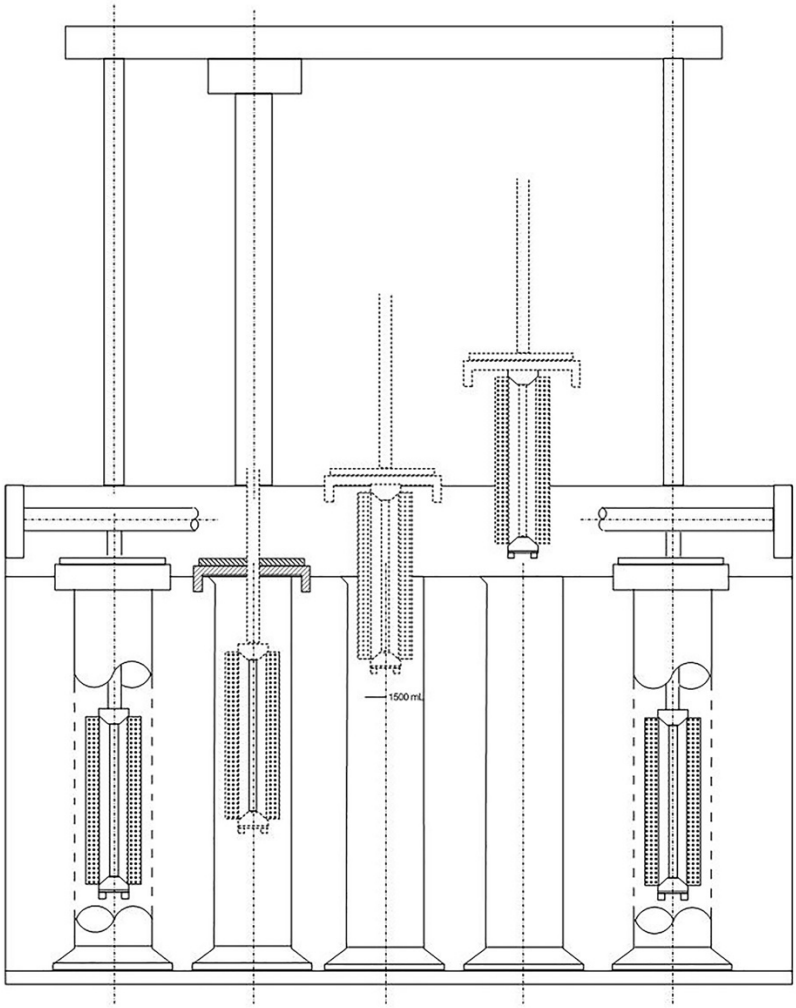
Extraction tests were performed by reciprocation. The test filter cartridges were immersed in 1,500 mL of extraction fluid (water or 50:50 (v/v) ethanol/water) in a clean measuring cylinder. For twenty-four (24) hours, the test filter cartridge was gently reciprocated up and down at 21-24 ºC (69-75 ºF) using an automated test stand.

The reciprocation method allows flow through the filter membrane because of the pressure head that is created each time the element is partially lifted out of the liquid. The extraction apparatus is shown in Figure 8.

After completing the filter extraction for twenty-four (24) hours, the filters were subjected to a second extraction of twenty-four (24) hours using a fresh volume of the same extraction fluid and the same test conditions, to demonstrate depletion of extractables over time.

Figure 8

Apparatus for filter extraction by reciprocation



9.1.1 Analysis of Material Extracted

Following the extraction, a defined volume of the extraction liquid was evaporated to dryness and the non-volatile residue (NVR) was determined gravimetrically. The total NVR for the filter was calculated by correcting for the total extraction volume used.

Qualitative analysis was performed on the NVR by Fourier Transform Infra-Red (FTIR) spectroscopy.

9.2 Results

Levels of extractables in the form of NVR obtained from the tested Emflon HTPFR filter cartridges are shown in Table 23.

Table 23

Non-volatile aqueous extractables (mg) from 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) after autoclaving at 125 ºC (257 ºF), obtained in two (2) consecutive 24-hour extractions at ambient temperature

Filter Serial Number	Extraction Fluid	NVR (mg) From 1st 24-Hour Extraction	NVR (mg) From 2nd 24-Hour Extraction	Total NVR (mg)
BP2378032	Water	2.6	< 1	< 3.6
BP2378093		6.7	1.7	8.4
BP2378172		2.0	< 1	< 3.0
BP2378021	50:50 (v/v) Ethanol/water	12.4	8.6	21.0
BP2378092		18.2	8.2	26.4
BP2378132		15.5	8.9	24.4

Examples of Fourier transform infra-red spectra of the NVR obtained by extraction from water and 50:50 (v/v) ethanol/water are shown in Figure 9 and Figure 10, respectively.

Figure 9

Typical FTIR spectrum of the NVR obtained by water extraction from 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4)

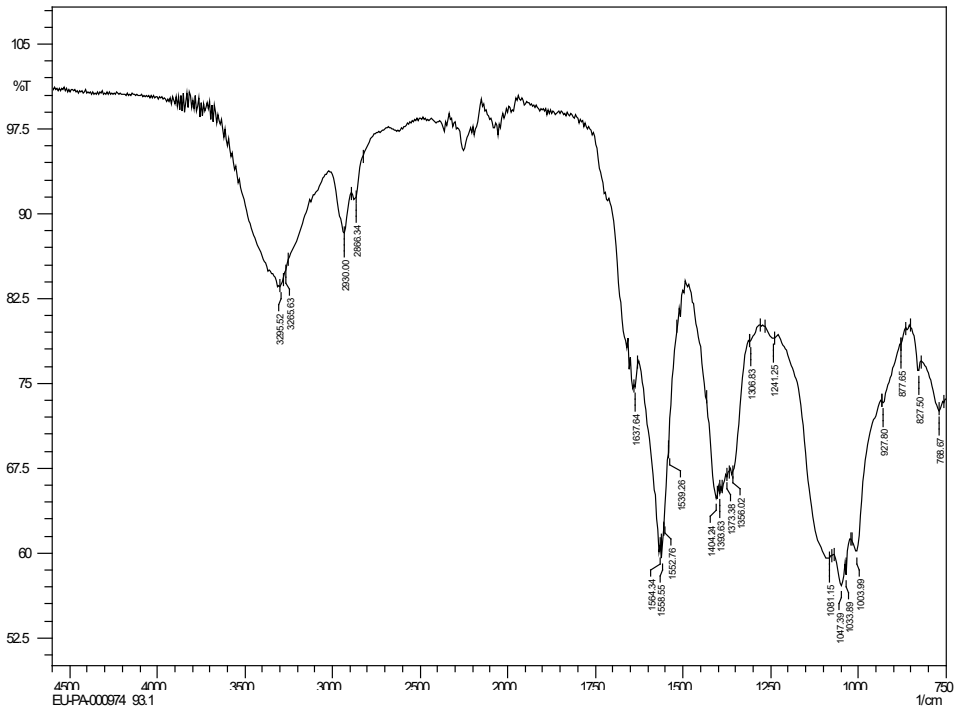
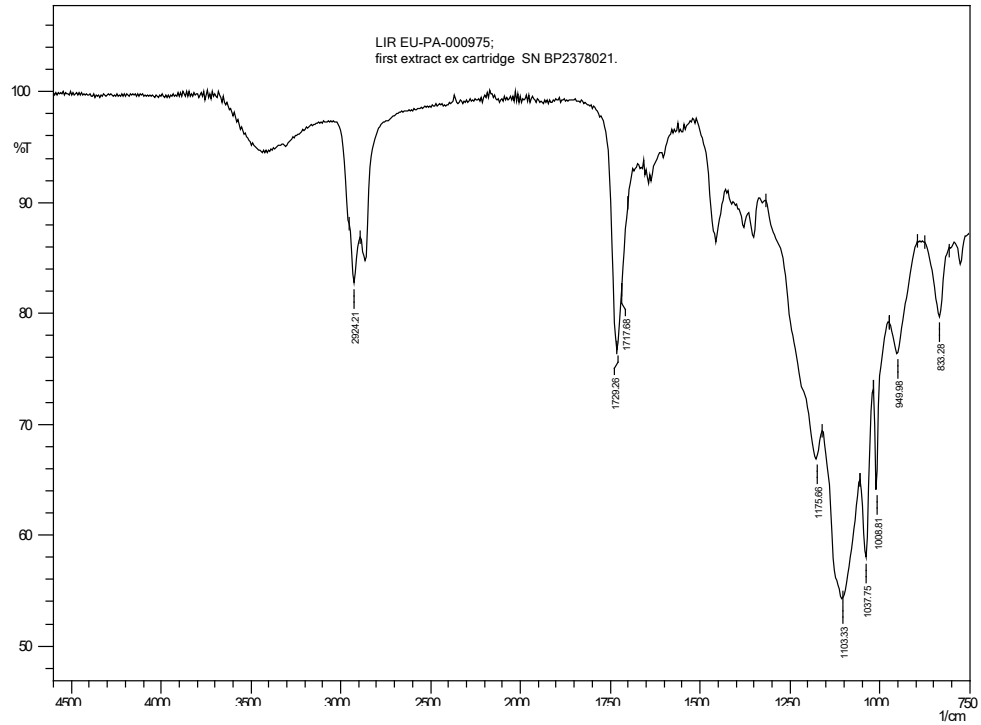


Figure 10

Typical FTIR spectrum of the NVR obtained by 50:50 (v/v) ethanol/water extraction from 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4)



9.3 Conclusions

Typical 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4), each containing 0.84 m^2 (9.04 ft^2) of effective filtration area (EFA), were used for the extractables tests.

The total non-volatile residue from two (2) successive 24-hour extractions in water ranged from < 3 mg to 8.4 mg and thus consistently amounted to less than 9 mg of total NVR per filter cartridge. The total non-volatile residue from two successive 24-hour extractions in 50:50 (v/v) ethanol/water ranged from 21.0 to 26.4 mg per filter cartridge.

The two (2) consecutive extractions of each tested filters in both solvents showed a decrease in non-volatile residue, demonstrating depletion of extractables available to the solvents. Therefore, these results will be valid regardless of the volume of solution processed and for contact times up to and exceeding 48 hours.

The FTIR spectra of the non-volatile residues indicate the presence of extractables typical of soluble oligomers of polypropylene (determined by the signals observed in the 2800–3000 cm^{-1}, 1460 cm^{-1}, and 1370 cm^{-1} region), as well as additives from polypropylene, such as antioxidants and fatty acid derivatives such as stearate salts, amides, or esters commonly used as the processing aids (determined by the signals at 800-1750 cm^{-1}). This composition is consistent with the materials of construction for this filter type.

10 Biological Safety Tests on Components of Emflon HTPFR Filter Cartridges

This study aimed to evaluate the biological safety of the materials of construction of Emflon HTPFR filter cartridges.

Table 24

Materials of construction of Emflon HTPFR filter cartridges

Component	Material
Filter membrane	Two layers of hydrophobic polytetrafluoroethylene (PTFE)
Support/drainage layers	Polyphenylene sulfide (PPS)
Core and endcaps	Polypropylene
Filter cage	Polypropylene filled with titanium dioxide (whitener)
Adapter	Polypropylene with fully encapsulated stainless-steel reinforcing ring
O-rings	Silicone elastomer ('H4' option)

10.1 Summary of Methods

The tests were performed at an independent testing laboratory in accordance with the Biological Reactivity Tests, *In Vivo* for Class VI Plastics-121 °C as described in USP <88> and the Biological Reactivity Tests, *In Vitro*, under USP <87> (performed as Elution Test).

10.1.1 Biological Reactivity Tests, *In Vivo*, for Class VI Plastics-121 °C as per USP <88>

The testing procedures described in the USP <88> include:

- Injection of extracts of plastic materials (Acute Systemic Injection test; Intracutaneous test)
- Implantation of the solid material into animal tissue (Implantation test)

The four extracting media listed in the USP for Acute Systemic Injection test and Intracutaneous test simulate parenteral solutions and body fluids. These include:

- Sodium chloride
- 1 in 20 solution of alcohol in sodium chloride
- Polyethylene glycol 400
- Vegetable oil (sesame or cottonseed oil)

The USP states that extracts may be prepared at one of three standard conditions:

- 50 °C (122 °F) for 72 hours
- 70 °C (158 °F) for 24 hours
- 121 °C (250 °F) for 1 hour

The most stringent condition not resulting in physical changes in the plastic is recommended. Therefore, the filter materials were extracted in the four extraction fluids at 121 °C (250 °F) for one (1) hour.

10.1.1.1 Acute Systemic Injection Test

The Acute Systemic Injection test serves to evaluate the potential of a single injection of an extract to produce systemic toxicity. Sodium chloride extracts and 1 in 20 solution of alcohol in sodium chloride extracts were injected intravenously. Vegetable oil extracts and polyethylene glycol 400 extracts were injected intraperitoneally.

10.1.1.2 Intracutaneous Test

The Intracutaneous test serves to evaluate the potential of a single injection of an extract to produce tissue irritation. All four of the extracts listed above were used for the Intracutaneous test.

10.1.1.3 Implantation Test

The Implantation test serves to observe reactions of live tissue in direct contact with the test specimen. Each of the materials of the Emflon HTPFR filter cartridges was implanted separately.

10.1.2 Biological Reactivity Tests, *In Vitro,* as per USP <87> (Elution Test).

Three test procedures (Agar Diffusion test, Direct Contact test, Elution test) are described in USP <87>. While all of them assess the potential of a test specimen for cytotoxicity, the most applicable test should be selected based on the material of the test specimen, the final product, and its intended use. For the polymeric materials of construction of Emflon HTPFR filter cartridges the Elution test was performed using Minimum Essential Medium (MEM) as extraction fluid. The MEM Elution extracts of the test specimens were brought into contact with mammalian cell cultures and their biological reactivity assessed.

10.2 Results

No biological response was observed in any of the tests performed, therefore the materials used in Emflon HTPFR filter cartridges passed all tests specified.

10.3 Conclusions

The filter material components used in Emflon HTPFR filter cartridges have met the requirements for biological reactivity, *in vivo*, under USP <88> (for Class VI–121 °C plastics) and *in vitro*, under USP <87> (Elution Test). Testing under USP <88> included the Systemic Toxicity Test, the Intracutaneous Test, and the Implantation Test. Testing under USP <87> was performed as Minimum Essential Medium (MEM) Elution Cytotoxicity Test.

11 Shelf-Life Studies

Pall Corporation does not assign specific expiration dates to non-sterile pharmaceutical grade filters. Shelf-life studies using Emflon HTPR filters post five (5) years real time storage were performed to assist our biopharmaceutical customers, who require a defined shelf life. Filters were subjected to all quality control testing in accordance with the applicable Pall "Certificate of Test for Pharmaceutical Grade Filters," to resistance to steam exposure testing, and to airflow/differential pressure measurements, to confirm that relevant product claims continued to be met after the applied storage period.

11.1 Filter Samples for Shelf-Life Studies

Pall recommends the following storage conditions for filters from polymeric materials such as Emflon HTPFR filter cartridges:

- Product is stored in the original packaging at 0-30 °C in a dry environment.
- Direct exposure to sunlight is avoided.
- Care is taken to avoid physical damage during handling and avoidance of thermal shock by quickly changing the temperature, especially from sub-zero conditions.

254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) from three (3) different lots that had passed all relevant Pall quality control tests were stored for five (5) years under defined conditions, aligned with recommended storage conditions above:

- In original bag and box, together in a larger cardboard box.
- Protected against radiation and weather conditions.
- At ambient temperature (15 to 25 °C / 59 to 77 °F).

11.2 Tests for Pall's Certificate of Test for Pharmaceutical Grade Filters for Shelf-Life Studies

Pall pharmaceutical (P)-grade filters undergo a defined set of quality control tests as part of the manufacturing lot release testing. These tests were applied to the stored samples of Emflon HTPFR filter cartridges to confirm that the filter cartridges after five (5) years real time storage continue to meet applicable quality release tests requirements. These tests included fabrication integrity and robustness to autoclaving, bacterial challenge testing, and effluent quality testing (oxidizables, pH, endotoxins).

11.2.1 Fabrication Integrity and Robustness to Autoclaving

Pall's Certificate of Test for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) states under Fabrication Integrity and regarding robustness to autoclaving that

- Each filter element in the respective lot successfully passed a manufacturing forward flow test. The user forward flow limit for this product is 16.0 mL/min using air at a test pressure of 1040 mbar (15.0 psi) when fully wetted with 60:40 (v/v) isopropyl alcohol (IPA):water. The forward flow test limit has been validated for bacterial removal by correlation of the above parameters with microbiological challenge test.
- Samples of the respective lot were subjected to a water intrusion test using air at a test pressure of 2500 mbar (36.0 psi) with a limit of 0.33 mL/min.
- Samples from the respective manufacturing lot also maintained integrity after multiple autoclave cycles.

11.2.2 Summary of Methods to Confirm Meeting "Fabrication Integrity" Acceptance Criteria After Five (5) Years Real Time Storage

Three (3) test filter elements were subjected to forward flow and water intrusion testing to confirm fabrication integrity. Two (2) test filter elements were subjected to six (6) one-hour autoclave cycles at 125 °C (257 °F), forward flow and water intrusion testing performed after autoclaving to confirm robustness to autoclaving.

11.2.3 Results for Fabrication Integrity

The forward flow and water intrusion test results are shown in Table 25. All Emflon HTPFR filter cartridges tested passed the forward flow and the water intrusion tests.

Table 25

Forward flow and water intrusion test results for 254 mm (10 in) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) after five (5) years real time storage

Filter Serial Number	Forward Flow * (mL/min)	Forward Flow (Pass/Fail)	Water Intrusion ** (mL/min)	Water Intrusion (Pass/Fail)
IS4934046	9.60	Pass	0.21	Pass
IS4935027	8.59	Pass	0.19	Pass
IS4936083	14.5	Pass	0.20	Pass

** Forward flow values measured at 15.0 psi air test pressure, wetted with 60:40 (v/v) isopropyl alcohol/water at ambient temperature. Maximum allowable forward flow = 16.0 mL/min.*

*** Water intrusion values at 2500 mbar test pressure, using deionized water at ambient temperature. Maximum allowable flow = 0.33 mL/min.*

11.2.4 Results for Robustness to Autoclaving

The forward flow and water intrusion test results after six (6) autoclave cycles at 125 °C (257 °F) are shown in Table 26. All Emflon HTPFR filter cartridges tested passed the forward flow and the water intrusion tests after six (6) one-hour autoclave cycles.

Table 26

Forward flow and water intrusion test results of 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) after five (5) years real time storage post six (6) one-hour autoclave cycles at 125 °C (257 °F)

Filter Serial Number	Forward Flow * (mL/min)	Forward Flow (Pass/Fail)	Water Intrusion ** (mL/min)	Water Intrusion (Pass/Fail)
IS4935016	5.78	Pass	0.20	Pass
IS4936093	4.40	Pass	0.19	Pass

** Forward flow values measured at 15.0 psi air test pressure, wetted with 60:40 (v/v) isopropyl alcohol/water at ambient temperature. Maximum allowable forward flow = 16.0 mL/min.*

*** Water intrusion values at 2500 mbar, test pressure, using deionized water at ambient temperature. Maximum allowable flow = 0.33 mL/min.*

11.2.5 Bacterial Retention

Pall's Certificate of Test for 254 mm (10 in) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) states under Bacteria Retention that finished product has been sampled and successfully tested for retention of an acceptable challenge microorganism, using procedures described in Pall validation guides and correlated to ASTM Standard Test Method F838-05, in conformance with the applicable requirements of the FDA Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice (September 2004).

11.2.6 Summary of Methods to Confirm Meeting "Bacterial Retention" Acceptance Criteria After Five (5) Years Real Time Storage

The three (3) 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4), that had been confirmed to pass forward flow and water intrusion integrity tests after five (5) years real time storage (see Fabrication Integrity), were subjected to bacterial challenge testing using *B. diminuta* (ATCC 19146) at a challenge level of > 1 x 10^{11} CFU of per filter (> 1 x 10^{7} CFU/cm^2). The detailed method is also described in Section 3.2 of this document.

11.2.7 Results for Bacteria Retention

The forward flow and *B. diminuta* retention results for the tested Emflon HTPFR filter cartridges are shown in Table 27. All filter cartridges yielded a sterile effluent when challenged with > 1 x 10^{11} CFU of *B. diminuta* per filter (> 1 x 10^{7} CFU/cm^2).

Table 27

Bacterial retention testing results of 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) using B. diminuta after five (5) years real time storage

Filter Serial Number	Challenge Level per Filter (CFU)	Sterile Effluent	Titer Reduction *
IS4934046	2.57 x 10^{11}	Yes	> 2.57 x 10^{11}
IS4935027	3.03 x 10^{11}	Yes	> 3.03 x 10^{11}
IS4936083	2.10 x 10^{11}	Yes	> 2.10 x 10^{11}

11.2.8 Effluent Quality

Pall's CoT for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) states under "**Effluent Quality**" that filter elements samples from the respective manufacturing lot underwent the following tests, and the lot was released by quality control when it was verified that their respective criteria were met:

- Filters meet with adequate safety margin after flushing (current limits under USP <788> Particulate Matter in Injections with effluent counts determined microscopically). Counts serve to document conformance with the requirements for a non-fiber-releasing filter per Title 21 of the U.S. Code of Federal Regulations (CFR) parts 211.72 and 210.3(b)(6).
- Filters meet the current USP requirement, after flushing, under Sterile Purified Water, as determined by a potassium permanganate test.
- Filters meet internal specification for pH after flushing when tested in accordance with USP <791>.

- Filters meet internal specifications when an aliquot from a soak solution is tested using the Limulus amoebocyte lysate (LAL) reagent in according with USP <85> Bacterial Endotoxins Test.

11.2.9 Summary of Methods to Confirm Meeting "Effluent Quality" Acceptance Criteria After Five (5) Years Real Time Storage

Two (2) 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) underwent the following tests after five (5) years real storage time as per Pall's respective quality control standard operating procedure. One filter sample was used for cleanliness, oxidizables substances and pH testing, while the second sample was used for endotoxins testing, as the use of the same filter sample for the respective other tests is mutually excluded.

- A defined volume of water was flushed through the test filter and the effluent filtered through an analysis disc. Particles on the disc were counted under a microscope.
- After flushing, effluent samples of the test filter were tested according to USP under sterile purified water by a potassium permanganate test.
- After flushing, effluent samples were tested in accordance with USP <791>.
- The filter was soaked in a defined volume of water for a defined time. An aliquot of this soak solution was tested using Limulus Amoebocyte Lysate (LAL) reagent in accordance with USP <85> Bacterial Endotoxins Test.

11.2.10 Results for Effluent Quality

The results of the tests for Effluent Quality are shown in Table 28. All tests yielded a pass result.

Table 28

Effluent quality testing results for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) after five (5) years real time storage

Filter Serial Number	Cleanliness	Oxidizable Substances	pH	Endotoxins
IS4934113	Pass	Pass	Pass	Not performed
IS4934080	Not performed	Not performed	Not performed	Pass

11.3 Resistance to Steam Exposure for Shelf-Life Studies

Steam exposure tests for the original validation of Emflon HTPFR filter cartridges were performed to represent extreme conditions of use and at the upper limit of the temperature range of these filters (142 °C, (288 °F)). The same test conditions were applied to filter samples post real time storage to evaluate any potential impact of storage on this characteristic. The number of cycles employed (100) reflect the claim for the maximum number of one-hour steam cycles as defined in section 3 above.

11.3.1 Summary of Methods

Three (3) 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) from three (3) different lots after five (5) years real time storage were used for these tests, the filter cartridges were installed in stainless steel housings and steamed in place in the forward ('out to in') direction using saturated steam at constant pressure and flow while ensuring effective condensate drainage. After each steam-in-place cycle the filters were cooled by passing dry compressed air through them. Forward flow and water intrusion testing were performed prior to steaming, after fifty (50) and after hundred (100) steaming cycles.

Table 29 shows the applied steaming and cooling conditions.

Table 29

Steam exposure test conditions in the forward ('out to in') direction for Emflon HTPFR filters

Condition	Test Parameter
Steam temperature	142 ºC (288 ºF)
Duration of steam-in-place cycle	60 minutes
Air cooling time	30 minutes
Maximum differential pressure during steaming and cooling cycles	300 mbar (4.35 psid)

11.3.2 Results

All Emflon HTPFR filter cartridges subjected to these steaming tests post five (5) years real time storage passed forward flow and water intrusion testing at all time intervals.

Table 30

Forward flow and water intrusion testing results for 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) after five (5) years real storage time and after steam exposure at 142 °C (288 °F)

	Forward Flow * (mL/min)				Water Intrusion ** (mL/min)			
Filter Serial Number	0 Cycles	50 Cycles	100 Cycles	Result Assessment (Pass/Fail)	0 Cycles	50 Cycles	100 Cycles	Result Assessment (Pass / Fail)
IS4934083	7.36	6.42	2.72	Pass	0.19	0.19	0.17	Pass
IS4935090	6.56	4.66	4.82	Pass	0.19	0.22	0.22	Pass
IS4936018	4.95	6.82	1.95	Pass	0.15	0.18	0.21	Pass

** Forward flow values measured at 15.0 psi air test pressure, wetted with 60:40 (v/v) isopropyl alcohol/water at ambient temperature. Maximum allowable forward flow = 16.0 mL/min.*

*** Water intrusion values at 2500 mbar air test pressure, using deionized water at ambient temperature. Maximum allowable flow = 0.33 mL/min.*

11.4 Airflow/Differential Pressure Characteristics for Shelf-Life Studies

The differential pressure characteristics in air systems for Emflon HTPFR filter cartridges after five (5) years real time storage were determined in vent use and in a compressed air system running at 2.0 bar (29.0 psi) system pressure.

11.4.1 Summary of Methods

Filters from three (3) different lots of 254 mm (10 in.) Emflon HTPFR filter cartridges (p/n AB1HTPFR7PVH4) were installed in stainless steel gas filter housings. The differential pressure across the filter assembly (filter housing and filter cartridge) was measured while clean compressed air was directed through the filter assembly, using flow rates up to 360 Nm^3/h (212.4 SCFM), under both atmospheric vent and pressurized operating conditions.

In vent conditions, the downstream side of the filter assembly was open to atmospheric pressure, and airflow through the filter was controlled from the upstream side. Under pressurized conditions, predetermined air pressures were maintained upstream of the filter assembly; airflow rate through the filter was controlled by restricting flow using a valve on the downstream side.

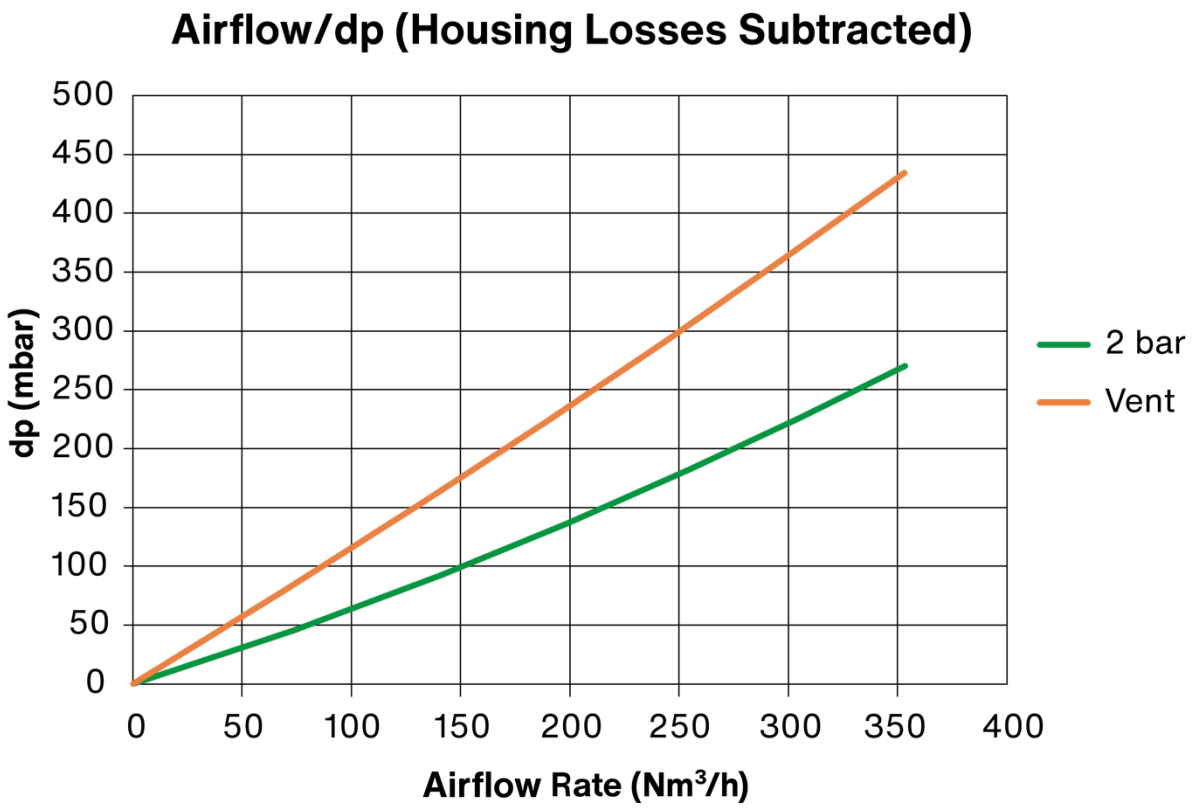
All airflow measurements were corrected to the following normal conditions for gases: atmospheric pressure (1013 mbara, 14.7 psia), 20 °C (68 °F).

11.4.2 Results

The test filters after five (5) years real time storage showed similar results for flow versus differential pressure performance as the filters tested without storage time (see Section 8.2). The airflow/differential pressure values at atmospheric pressure and at 2.0 bar pressure (29.0 psi) are shown in Figure 11.

Figure 11

Airflow/differential pressure characteristics of 254 mm (10 in.) Emflon HTPFR filter/housing system (p/n AB1HTPFR7PVH4) after five (5) years real time storage



100 mbar = 1.45 psi, 1 Nm³/h = 0.59 SCFM

11.5 Conclusion

Emflon HTPFR filters were stored for five (5) years under recommended conditions for filter storage and were subjected to several qualification and performance tests post this real storage time:

- Tests for Pall's Certificate of Test for pharmaceutical grade filters.
  - Fabrication integrity and robustness to autoclaving.
  - Bacterial retention.
  - Effluent quality.
- Resistance to steam exposure.
- Airflow/differential pressure characteristics.

The test filters met the acceptance criteria for all tests and were thus shown to maintain the respective performance characteristics for Emflon HTPFR filters. The data support five (5) years shelf life when stored under recommended storage conditions.

12 Transmissible Spongiform Encephalopathy (TSE) / Bovine Spongiform Encephalopathy (BSE) Statement

Emflon HTPFR filter cartridges do not contain materials of construction that are considered TSE risk materials according to current legislation and guidance in both Europe and the United States:

1. The European CPMP Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathies via human and veterinary medicinal products. (EMA/410/01 Rev.3, applicable from 1st July 2011).
2. The U.S. **Code of Federal Regulations**, Title 9 of part 94.18, which sets forth restrictions on the geographical sourcing of products obtained from ruminants.
3. The U.S. **Code of Federal Regulations**, Title 21 of part 189.5, which defines specified risk materials obtained from cattle.

Pall has an established program with our raw material suppliers to assess whether animal derived products (e.g., bovine/ovine/caprine) are present in the materials employed for our pharmaceutical grade products. We have identified that polypropylene resins, used to manufacture plastic components of the referenced products, contain trace levels of additives, which may be derived from bovine tallow. Tallow derivatives are not considered specified BSE risk materials according to the current revision of Title 21, of the U.S. **Code of Federal Regulations**, part 189.5. Furthermore, the CPMP's Note for guidance (EMA 410/01) gives specific consideration to tallow derivatives and states they are unlikely to be infectious due to the rigorous processing steps used (an example of which is trans-esterification, or hydrolysis, at not less than 200 °C (392 °F) under pressure for not less than 20 minutes). The raw materials we purchase have been processed with these steps. Additionally, during the conversion of polypropylene resin into plastic components further high temperature steps are performed.

13 Sample Pharmaceutical Certificate of Test

Each Emflon HTPFR filter cartridge is supplied with a Certificate of Test confirming pharmaceutical industry requirements for biological safety testing, fabrication integrity, and effluent quality.

We hereby certify that:

Pall EMFLON® HTPFR FILTER

Rated: 0.2 µm

Part Number: AB*HTPFR*PV**

Lot Number: SAMPLE

was manufactured in a controlled environment and subjected to a high velocity flush after undergoing integrity testing. The filter membrane used in the filter element has a quantitative bubble point (i.e., "KL") which met or exceeded 1380 mbar (20.0 psi) in isopropyl alcohol.

These filters are not supplied sterile.

13.1.1 Fabrication Integrity

Each filter element in this lot successfully passed a manufacturing forward flow test. The user forward flow limit for this product is 16.00 mL/min using air at a test pressure of 1040 mbar (15.00 psig) when fully wetted with 60:40 (v/v) IPA:water. The forward flow test limit has been validated for bacterial removal by correlation of the above parameters with microbiological challenge test. Samples from this manufacturing lot also maintained integrity after multiple autoclave cycles. Samples were also subjected to a water intrusion test using air at a test pressure of 2500 mbar (36 psig) with a limit of 0.33 mL/min. Recommended test values for integrity testing of Pall filters as installed must be obtained from Pall.

13.1.2 Bacterial Retention

Finished product has been sampled and successfully tested for retention of an acceptable challenge microorganism, using procedures described in Pall validation guides and correlated to ASTM Standard Test Method F838-05, in conformance with the applicable requirements of the FDA Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice (September 2004).

13.1.3 Materials of Construction

The filter components have met the requirements for biological reactivity, *in vivo*, under USP <88> (for Class VI–121 °C plastics) and *in vitro*, under USP <87> (Elution Test). These filters also are made from materials listed for food contact usage per Title 21 of the U.S. **Code of Federal Regulations** (CFR) parts 170-199. Contact Pall for further information regarding materials of construction.

13.1.4 Effluent Quality

Filter element samples from this manufacturing lot underwent the following tests and the lot was released by quality control when it was verified that their respective criteria were met.

13.1.5 Cleanliness

Meets with adequate safety margin, after flushing, current limits under USP <788> Particulate Matter in Injections, with effluent counts determined microscopically. Counts serve to document conformance with the requirements for a non-fiber-releasing filter per Title 21 of the U.S. **Code of Federal Regulations** (CFR) parts 211.72 and 210.3(b)(6).

13.1.6 Oxidizable Substances

Meets the current USP requirement after flushing under sterile purified water, as determined by a potassium permanganate test.

13.1.7 pH

Meets internal specifications after flushing when tested in accordance with USP <791>.

13.1.8 Pyrogens Endotoxins

Meets internal specifications when an aliquot from a soak solution is tested using the limulus amoebocyte lysate (LAL) reagent in accordance with USP <85> Bacterial Endotoxins Test.

In addition to the above tests, this product met manufacturing inspection standards and requirements for full traceability. Consider only unopened, undamaged packages for use. Further information is available by contacting Pall.

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