

Fluorofil™ Plus 0.2µm

Membrane Cartridge

Validation Guide



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Porvair Filtration Group, has specifically designed Fluorofil™ Plus membrane cartridges, for use in providing sterile air and gases and solvent filtration in the pharma industry and other critical applications. Fluorofil™ Plus cartridges utilize the inherently hydrophobic expanded polytetrafluoroethylene (ePTFE) membrane, which provides the highest levels of biosecurity throughout the process industry. When combined with quality all-polypropylene components and high integrity manufacturing techniques, the Fluorofil™ Plus filter cartridge is ideally suited to the most demanding process conditions.

Porvair Fluorofil™ Plus cartridges are constructed in ISO accredited clean rooms under tightly controlled conditions using advanced, highly specialised machinery. Quality and consistency of product are assured ISO 9001 accredited quality control and manufacturing procedures, which are in place throughout all stages of manufacture.

Porvair Fluorofil™ Plus ePTFE membrane cartridges are 100% integrity tested during manufacture by the forward flow diffusion method. Each module of every cartridge is tested to ensure integrity is not compromised by any single module in a cartridge.



Bacterial Challenge Test

Introduction

This report describes the results of testing Fluorofil™ Plus cartridges under approved protocols for the evaluation of bacterial retention characteristics of membrane filters used to sterilise liquids. This method uses *Brevundimonas diminuta* ATCC 19146 as the challenge organism.

The test filters were challenged with a suspension of *Brevundimonas diminuta* prepared at a concentration of approximately 1×10^7 colony forming units (CFU) per cm^2 of Effective Filtration Area (EFA). The sterility of the complete apparatus was tested before the challenge. The challenge was conducted at a maximum differential pressure of 30psig (206kPa). The effluent was collected and assayed quantitatively on 0.45 micron assay membranes. Integrity testing was performed before and after the bacterial challenge procedure.

Justification

This test method was designed to determine the bacterial retention characteristics of membrane filter cartridges used to sterilise liquids. The selection of *Brevundimonas diminuta* as the challenge organism is based on its historical acceptance within the industry resulting from its very small size when grown under stress or starvation conditions. The test procedure complies in intent and content with the ASTM F838-05 Standard Test Method 'Determining Bacterial Retention of Membrane

Filters Utilised for Liquid Filtration' and the Health Industry Manufacturers Association (HIMA) Test Method 'Microbiological Evaluation of Filters for Sterilising Liquids'. The test protocol, choice of organism, and parameters also meet the advisory information given by the Parenteral Drug Association in 'PDA Technical Report No.26, Sterilizing Filtration of Liquids'.

When grown under carefully controlled conditions, many *Brevundimonas diminuta* will pass through 0.45 micron membranes. Due to the organism's size, *Brevundimonas diminuta* represents a most severe bacterial challenge to the filter. The organism's low pathogenicity also favours the use of *Brevundimonas diminuta* in laboratory studies. The challenge conditions include high pressure, high flow rates and a high bacterial concentration per cm^2 of EFA. The growth parameters, temperatures and media were as detailed in the protocol as specified by ASTM and HIMA.

The log reduction value (LRV) is calculated as below:

$$\text{LRV / Filter} = \log_{10} \frac{\text{Number of Organisms in Challenge}}{\text{Number of Organisms in Filtrate}}$$

When filtrate is sterile, 1 is substituted in the denominator and the LRV is expressed as greater than (>) the calculated value.

Bacterial Retention Results

Fluorofil™ Plus 0.2 micron (*Brevundimonas diminuta* ATCC 19146)

Filter ID	Flow Rate @ 30psig	Total Challenge (CFU)	Challenge/Sq cm^2 (CFU/ cm^2)	Filtrate Count (CFU)	Rinse Count (CFU)	Diffusion Rate (ml/min)*	LRV
F20P AMN 98 001	8L/13sec	7.5×10^{10}	1.1×10^7	<1	<1	3.1 (5.0)	>10.88
F20P AMN 98 004	8L/11sec	7.5×10^{10}	1.1×10^7	<1	<1	2.6 (5.0)	>10.88
F20P AMN 98 007	8L/11sec	7.5×10^{10}	1.1×10^7	<1	<1	2.6 (4.0)	>10.88

* At test pressure of 827mbar – 60% IPA 40% water wetted, prior to sterilisation and bacterial challenge. (l) Diffusion rate post challenge.

Conclusion

Porvair Fluorofil™ Plus 0.2 micron cartridges were effective in retaining the bacterial challenge as demonstrated by zero CFU present on the assay membranes.

Introduction

Hydrophobic membrane filters play a critical role in providing sterile air and gases in pharmaceutical, biotechnology and containment applications. The filters must be proficient in removing airborne viruses (bacteriophage) and spores from large volumes of moist air/gas streams over prolonged periods.

The aim of the following tests are to ensure Fluorofil™ cartridges are capable of retaining any bacteriophages and spores which might be present in moist air/gas streams.

The Fluorofil™ cartridges were challenged with high concentrations of MS-2 coli phage (26nm in diameter) and *Bacillus atrophaeus* spores (typically 1 micron × 0.7 micron), at a flow rate of 650 ± 50 litres per minute with a relative humidity greater than 90% over a short period and an extended period of 7 days. The test protocols, conditions and choice of organisms are in accordance with the advisory information given in the Parenteral Drug Association's 'PDA Technical Report 40, Sterilizing Filtration of Gases'.

Summary of Methods

Fluorofil™ 254mm (10") filter cartridges from a standard production batch were used for the tests listed below. Filter integrity was confirmed using the Diffusion Test method.

Test 1:

Cartridges challenged with aerosolised *Bacillus atrophaeus* spores for a period of 10 minutes at 650 ± 50 litres per minute at a relative humidity greater than 90%.

Test 2:

Cartridges challenged with aerosolised MS-2 coliphage for a period of 10 minutes at 650 ± 50 litres per minute at a relative humidity greater than 90%.

Test 3:

Cartridges challenged daily with aerosolised MS-2 coliphage for a period of 10 minutes at 650 ± 50 litres per minute at a relative humidity greater than 90% over a period of 7 days.

Challenge Apparatus

A schematic diagram of the apparatus used in the filter tests is shown opposite. The challenge suspensions were placed in the Collision sprays in the chamber. The suspensions were nebulized by applying compressed air. The relative humidity (>90%) of the air flowing through the system was checked before and after the challenge. A flow rate of 650 ± 50 litres per minute was maintained during 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 onto the cyclone wall and were collected by the swirling liquid, which was then withdrawn by a syringe. At the end of the challenge period the fluid was measured and then assayed for the challenge organism using an appropriate technique.

The system was operated to determine the challenge concentration with the filter removed and the Collision Spray switched 'on'. Background levels were determined with the filter in-situ and the Collision Spray switched 'off'.

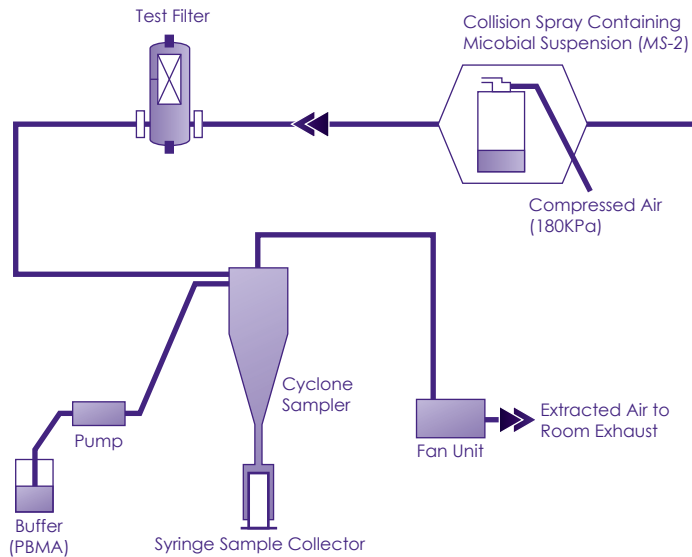
The log reduction value (LRV) is calculated as below:

$$\text{LRV / Filter} = \text{Log}_{10} \frac{\text{Number of Organisms in Challenge}}{\text{Number of Organisms in Filtrate}}$$

When filtrate is sterile, 1 is substituted in the denominator and the LRV is expressed as greater than (>) the calculated value.

Bacteriophage and Spore Retention

Apparatus for aerosol challenge tests



Results

The results of the three challenge tests are presented in Tables 1 to 3.

Table 1 10 minute challenge with aerosolised *Bacillus atrophaeus* spores

Filter Batch Number	Diffusion Rate* (ml/min)	Total Challenge (CFU)	Filtrate Count (CFU)	Titre Reduction
146497017	8.1	6.28×10^8	<1	$>6.28 \times 10^8$
146497013	6.6	6.97×10^8	2	3.49×10^8
146497007	6.6	5.94×10^8	<1	$>5.94 \times 10^8$

Table 2 10 minute challenge with aerosolised MS-2 coliphage

Filter Batch Number	Diffusion Rate* (ml/min)	Total Challenge (CFU)	Filtrate Count (CFU)	Titre Reduction
146497012	8.1	1.37×10^{13}	<15	$>9.13 \times 10^{11}$
146497016	7.3	1.39×10^{13}	138	1.01×10^{11}
146497018	7.3	1.40×10^{13}	436	$>3.21 \times 10^{10}$

* Test pressure 800 mbar: 60% IPA 40% water wetted. Maximum allowable diffusion ≤ 10 ml/min.

Table 3 Long term (7 day) aerosolised MS-2 coliphage challenge*

Day	Filter Batch Number	Total Challenge (PFU)	Filtrate Count (PFU)	Titre Reduction
1	146497014	4.820 x 10 ¹²	196	2.459 x 10 ¹⁰
	146497010	3.980 x 10 ¹²	2168	1.836 x 10 ⁹
	146497015	3.895 x 10 ¹²	n/d**	-
2	146497014	1.040 x 10 ¹²	27	3.852 x 10 ¹⁰
	146497010	1.157 x 10 ¹²	31	3.732 x 10 ¹⁰
	146497015	1.146 x 10 ¹²	986	1.162 x 10 ⁹
3	146497014	1.299 x 10 ¹¹	16	8.116 x 10 ¹⁰
	146497010	5.630 x 10 ¹¹	77	7.311 x 10 ⁹
	146497015	1.132 x 10 ¹¹	71	8.636 x 10 ⁹
4	146497014	3.951 x 10 ¹¹	352	1.122 x 10 ⁹
	146497010	4.759 x 10 ¹¹	704	6.760 x 10 ⁸
	146497015	4.462 x 10 ¹¹	1995	2.237 x 10 ⁸
5	146497014	6.993 x 10 ¹¹	2065	3.386 x 10 ⁸
	146497010	8.680 x 10 ¹¹	1285	6.755 x 10 ⁸
	146497015	8.500 x 10 ¹¹	70	1.214 x 10 ¹⁰
6	146497014	5.685 x 10 ¹¹	21	2.707 x 10 ¹⁰
	146497010	7.861 x 10 ¹¹	558	1.409 x 10 ⁹
	146497015	6.815 x 10 ¹¹	50	1.363 x 10 ¹⁰
7	146497014	6.286 x 10 ¹¹	64	9.822 x 10 ⁹
	146497010	5.574 x 10 ¹¹	<1.5	>3.716 x 10 ¹¹
	146497015	6.346 x 10 ¹¹	641	9.900 x 10 ⁸

* Filters were determined to be integral at the start of the test.

** Data not used.

Conclusion

The test data confirmed that standard production Fluorofil™ filter cartridges will retain very high challenge levels of aerosolised phage, as demonstrated using MS-2 coliphage and aerosols of non-vegetative spores, as demonstrated using spores of *Bacillus atrophaeus*.

Integrity Tests

For critical applications, filter validation requires testing with the bacteria *Brevundimonas diminuta* to confirm the retention characteristics of the filter. Since this is a destructive test, it cannot be performed on all filters. However, by correlating microbial challenge tests with non-destructive integrity tests, filter performance can be assured.

The bubble point, diffusion and pressure hold tests are industry accepted non-destructive methods for verifying the integrity of a membrane filter.

Test Parameters Summary

All Fluorofil™ Plus 0.2 micron filter cartridges are 100% integrity tested (MLP 43) during manufacture.

Test Procedure	Test Pressure (mbar)	Acceptable Flow per 10' Module
Bubble Point Test	1080mbar	Not applicable
Forward Flow Diffusion Test	800mbar	10ml/min
Pressure Hold Test	800mbar	10ml/min
Water Intrusion Test	2500mbar	13ml/10min

All test data are valid for 60% IPA 40% Water, other than the Water Intrusion Test which should be carried out in pure water.

The following detailed integrity test procedures are available on request:

Bubble Point Test (manual)	MLP 49
Forward Flow Diffusion Test (manual)	MLP 43
Pressure Hold Test (manual)	MLP 33
Bubble Point, Forward Flow Diffusion, Pressure Hold Test (automatic)	MLP 67
Water Intrusion Test	MLP 61
Wetting Procedure	MLP 45

Diffusion Test

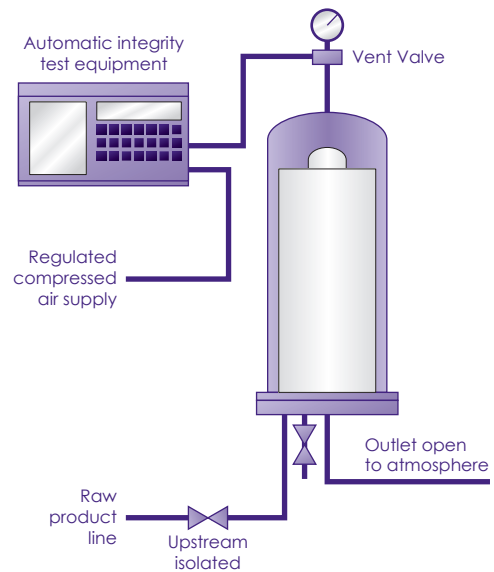
Wetting

Before an integrity test, the hydrophobic ePTFE membrane must be completely wetted with a liquid having a surface tension of <28dyne/cm. Therefore to achieve thorough wetting and to ensure an accurate diffusion rate, a solution of 60% Isopropyl alcohol (IPA) and 40% water is used.

Diffusion Test

The Fluorofil™ Plus cartridge is wetted with 60% IPA 40% water and a test pressure of 827mbar is applied to the upstream side of the filter assembly. After a stabilisation stage, the gas flow through the wetted membrane can be measured manually on the downstream side or on the upstream side using an automatic integrity test equipment, as illustrated below.

Arrangement of filter apparatus for automated integrity testing



Fluorofil™ Plus Materials of Construction

The **Porvair** Fluorofil™ Plus filter cartridge is manufactured using high quality components made from non-toxic and biologically inert raw materials. All components of the Fluorofil™ Plus cartridge are FDA listed for food contact use in the 'Code of Federal Regulations (CFR), Title 21' as listed:

Fluorofil™ Plus components meet latest EC Directives for food contact.

Component	Materials of Manufacture	FDA Number
Membrane	ePTFE	21CFR177.1550
Core	Stainless Steel	-
Sleeve	Polypropylene	21CFR177.1520
Adaptors	Polypropylene	21CFR177.1520
End Caps	Polypropylene	21CFR177.1520
End Caps	Polypropylene	21CFR121.2501
Seals	Typically Silicone	21CFR177.2600
Supporting Materials	Polypropylene	21CFR177.1520
Sealing Method	Thermal Bonding	-

Cartridge Dimensions (Nominal)

Diameter: 70mm (2.8")
Length: 127mm (5")
254mm (10")
508mm (20")
762mm (30")
1016mm (40")

Maximum Differential Pressure

Normal flow direction at:

20°C (68°F): 6.0bar (87lb/in²)
80°C (176°F): 4.0bar (58lb/in²)
100°C (212°F): 3.0bar (43lb/in²)
120°C (248°F): 2.0bar (29lb/in²)
125°C (257°F): 1.5bar (22lb/in²)

Reverse flow direction at:

20°C (68°F): 2.1bar (30lb/in²)
80°C (176°F): 1.0bar (15lb/in²)
100°C (212°F): 0.5bar (7lb/in²)

Based on cyclic exposure to hot air and steam Fluorofil™ Plus cartridges are able to maintain integrity for an extended period of time at 60°C (140°F).

Tests for Biological Safety

USP Toxicity Test (1)

Porvair Fluorofil™ Plus cartridges are manufactured using the unique **Porvair** patented end-capping process. All materials are FDA approved as listed above and components have been tested independently by UBTL Inc., 520 Wakara Way, Salt Lake City, Utah, USA. The results of the biological tests for plastics confirmed that the components of construction were non-toxic.

Laboratory Number	60173
Sample Source	Porvair Filtration Group Ltd.
Test Required	USP Toxicity Class V-121C
Type of Test	Systemic Injection
Mice	ICR Swiss Webster

Extract	Weight	Number	Animals Showing Signs of Toxicity				
			0 hours	4 hours	24 hours	48 hours	72 hours

Controls

Saline	17-23	5	0	0	0	0	0
EtOH 5%	17-23	5	0	0	0	0	0
Oil	17-23	5	0	0	0	0	0
Peg 400	17-23	5	0	0	0	0	0

Test Samples

Saline	17-23	5	0	0	0	0	0
EtOH 5%	17-23	5	0	0	0	0	0
Oil	17-23	5	0	0	0	0	0
Peg 400	17-23	5	0	0	0	0	0

Conclusion

No systemic toxicity: non-toxic.

USP Toxicity Test (2)

Laboratory Number	60173
Sample Source	Porvair Filtration Group Ltd.
Test Requested	USP Toxicity Class V-121C
Type of Test	Type B Intracutaneous
Sterilised by	88/12 Ethylene Oxide

Extract	Test/Control Rabbit #	Sites	Average Score		
			24 hours	48 hours	72 hours
Saline	Test T411	10	0	0	0
	Control T411	10	0	0	0
	Test T432	10	0	0	0
	Control T432	10	0	0	0
EtOH 5%	Test T434	10	0	0	0
	Control T434	10	0	0	0
	Test T435	10	0	0	0
	Control T435	10	0	0	0
Oil	Test T436	10	0	0	0
	Control T436	10	0	0	0
	Test T438	10	0	0	0
	Control T438	10	0	0	0
Peg 400	Test T400	10	0	0	0
	Control T400	10	0	0	0
	Test T439	10	0	0	0
	Control T439	10	0	0	0

Conclusion

No toxicity noted intracutaneously: non-toxic.

MEM Elution

Laboratory Number	60227
Sample Source	Porvair Filtration Group Ltd.
Cell Line	Mouse Heteroploid Connective Tissue (L929)
Incubation Period	24 ± 1 hours at 37°C with 5% CO ₂
Method of Scoring	Cytopathic Effect (0-4)
Extraction Ratio	60cm ² /20ml

Purpose

The Minimal Essential Solution (MEM) elution test is designed to determine the cytotoxicity of extractable substances exposed to cellular monolayers. The appearance of cellular destruction by these extracts is evidence of varying degrees of cytotoxicity.

Justification

The amount of test material to be extracted is based on USP surface area recommendations or by weight (1g/5ml of extracting medium). The prepared sample is normally extracted for 24 ± 1 hours at 37°C in MEM. Other temperatures and appropriate times can be used.

The test extracts are decanted and filtered. To each tissue culture test well (35 x 14mm) with a 70-90% confluent monolayer that has had its normal growth medium aspirated, 3ml of the test extract is added. Appropriate negative and positive control materials are included with each test and the test is performed in triplicate for each test extract. The prepared test wells are incubated at 37°C (99°F) with 5% CO₂ and 95-100% relative humidity for 24 to 72 hours, or longer if appropriate. Microscopic readings are made at 24 hour intervals.

MEM Elution

The cell monolayers are then fixed, stained and examined microscopically. The wells are scored as the degree of discernable morphological cytotoxicity on a relative scale of 0 to 4:

- 0 = No observable cytotoxicity
- 1 = Less than 25% of cells affected
- 2 = 25-50% of cells affected
- 3 = 50-75% of cells affected
- 4 = Greater than 75% of cells affected.

The results from the three wells are averaged to give a final cytopathic effect (CPE).

Identification	Score #1	Score #2	Score #3	Average
(-) Control	0	0	0	0
(+) Control	4	4	4	4
Sample	4	0	0	0

Results

Non-toxic.

Limulus Test

Laboratory Number	60184
Sample Source	Porvair Filtration Group Ltd.
LAL Manufacturer	Associates of Cape Cod
Sensitivity	0.06EU/ml
+ Control	Difco LPS E. coli 055:B5, #715269
- Control	McGaw H ₂ O, #J5H358B
Temperature	37 ± 1°C
Time	1 Hour

Sample	Positive Control (ng/ml)			Negative Control Water
	100pg	50pg	25pg	
-	+	+	+	-

Results

Negative.

Physico-chemical Test

Laboratory Number	60180
Sample Source	Porvair Filtration Group Ltd.

Results

	Pass/Fail	Allowable Limits
Heavy Metals	Pass	0.0001%
Buffering Capacity	Pass	<10ml of titrant
Non-volatile Residue	Pass	<15mg
Residue on Ignition	Waive Pass*	<5mg

* The term 'waive pass' is assigned when the non-volatile residue is less than 5mg, indicating that the residue on ignition will also be less than 5mg.

Fluorofil™ Plus Sterilisation by In-line Steam

Objective

Fluorofil™ Plus filter cartridges have been demonstrated to retain integrity after repeated steam in place (SIP) cycles under the test conditions described below.

Procedure

Cartridges were sampled from a routine production batch; integrity tested by forward flow diffusion method, initially, and after every 12th cycle thereafter. Cartridges were steam sterilised by dynamic in-line steam at 125°C (257°F) and 135°C (275°F) for 20 minutes, whilst maintaining differential pressure below 0.5bar. Upstream and downstream condensate was drained throughout each cycle. The cartridges were air cooled for 10 minutes between steam cycles, to simulate the highest levels of thermal shock likely to be encountered in use.

Results

Extract Cartridge Batch Lot Number	Number of 20 Minute Cycles	Temperature	Number Tested	Number Failed
F20 ABA 89	300	121°C (250°F)	3	0
F20 AAP 90	300	130°C (266°F)	3	0
F20 AET 87	500	135°C (275°F)	2	0

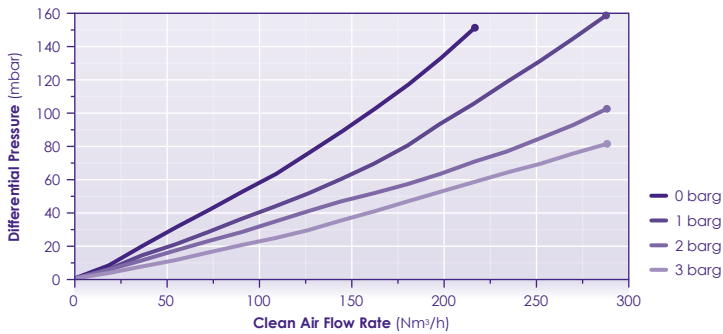
Conclusion

All cartridges tested maintained integrity throughout differing test regimes.

Clean Air Flow Rate

Porvair Fluorofil™ Plus 0.2 micron clean air flow rate, based on a 254mm (10") single cartridge with a effective filtration area of 0.8m² (8.61ft²) in-situ in a **Porvair** housing exhibiting the differential pressure characteristics indicated below.

Fluorofil™ Plus 0.2 micron - Gas Flow Rate (250mm (10") Cartridge)



Test Procedure

Standard production Fluorofil™ Plus cartridges were installed in a stainless steel air filter housing designed for use in compressed gas and vent applications. The differential pressure across the filter assembly was measured while clean compressed air was flowed through the filter assembly, at a range of flow rates and under both 'atmospheric vent' and 'pressurised' operating conditions.

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



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