

# Biofil™ Plus BT 0.45µm

Membrane Cartridge

Validation Guide



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## Introduction

The **Porvair Filtration Group** Biofil™ Plus 0.45 micron microbial rated cartridge has been developed and manufactured for the filtration of liquids in the pharmaceutical, biotechnology and other critical applications. Biofil™ Plus utilises a naturally hydrophilic polyethersulphone (PES) membrane with a mirrored asymmetric pore structure, which provides graded filtration throughout its depth, resulting in higher throughputs. The cartridge's unique built in prefiltration layer allows for longer life, therefore providing increased protection, smaller footprint and more cost effective. When combined with quality all-polypropylene components and high integrity manufacturing techniques, the Biofil™ Plus filter cartridge is ideally suited to the most demanding process conditions.

This validation guide summarises the extensive testing and evaluation program prepared by Porvair, to meet the key demands of the Pharmaceutical Industry.

**Porvair** Biofil™ Plus cartridges are constructed in a clean room under tightly controlled conditions using advanced, highly specialised machinery. Quality and consistency of product is assured by the quality control and manufacturing procedures, which are in place throughout all stages of manufacture.

Biofil™ Plus membrane cartridges are 100% integrity tested during manufacture by the forward flow diffusion test method.



# Bacterial Challenge Test

## Introduction

The objective of the test protocol is to verify the bacterial retention efficiency of Biofil™ Plus cartridges and to determine a correlation between the non-destructive, diffusive flow testing and the filter cartridge's bacterial retention capability in liquid.

## Brief description of challenge procedure

Each test filter was challenged with a suspension of *Serratia marcescens*. A sufficient volume of this challenge suspension, equivalent to at least  $1 \times 10^7$  colony forming units (CFU) per cm<sup>2</sup> of effective filtration area (EFA) was prepared for each filter.

After preparation of the challenge suspension, the filter was integrity tested, and then the filter and test system were sterilised at 121-125°C for 30 minutes using in-line steam. When cool, the sterility of the system was verified and the filter was integrity tested again. The challenge suspension was filtered through the test filter at a delivery pressure of 30psig (2.1bar). The effluent was collected and assayed quantitatively by membrane filtration. Integrity testing was performed before and after the challenge procedure.

## Justification

The selection of *Serratia marcescens* as a challenge organism is based on historical acceptance that the organism is the most suitable for challenging a 0.45 micron pore size membrane filter. The test procedure complies with the recommendations of ASTM F838-05 *Standard Test Method Determining Bacterial Retention of Membrane Filters Utilised For Liquid Filtration*.

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 the filtrate is sterile, 1 is substituted for the number of organisms in the filtrate and the LRV is expressed as greater than (>) the calculated value.

## Results

Table 1 **Bacterial Retention Results (250mm 10<sup>1</sup> cartridge)** (*Serratia marcescens* ATCC #14756)

Filter ID	Flow Rate @ 30psig	Total Challenge (CFU)	Challenge/Sq cm (CFU/cm <sup>2</sup> )	Filtrate Count (CFU)	Rinse Count (CFU)	LRV
BTP45 ICC No.144270 # 002	6L/16sec	1.2 x 10 <sup>11</sup>	2.5 x 10 <sup>7</sup>	<1.0 x 10 <sup>0</sup>	<1	>11.08
BTP45 ICC No.144270 # 006	6L/22sec	1.2 x 10 <sup>11</sup>	2.5 x 10 <sup>7</sup>	<1.0 x 10 <sup>0</sup>	<1	>11.08
BTP45 ICC No.144270 # 007	6L/18sec	1.2 x 10 <sup>11</sup>	2.5 x 10 <sup>7</sup>	<1.0 x 10 <sup>0</sup>	<1	>11.08

Table 2 **Diffusive Flow with Water Wetted Filters**

Filter ID	Test Pressure (mbar)	Diffusion Rate Pre Sterilisation (mL/min)	Diffusion Rate Post Sterilisation (mL/min)	Diffusion Rate Post Challenge (mL/min)
BTP45 ICC No.144270 # 002	2000mbar	19mL/min	21mL/min	3.3mL/min
BTP45 ICC No.144270 # 006	2000mbar	21mL/min	21mL/min	3.3mL/min

## Conclusion

Porvair Biofil™ Plus BTP45 0.45 micron cartridges were effective in retaining the *Serratia marcescens* bacteria as demonstrated by zero CFU present on the assay membranes. The flow rate, total challenge level and the LRV are summarised in Table 1. The diffusion tests and bubble point results are summarised in Table 2.

## Integrity Tests

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Each module of every Biofil™ Plus BTP45 0.45 micron filter cartridges is individually integrity tested during manufacture.

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

The bubble point, diffusion test and pressure hold tests are industry accepted, non-destructive methods for verifying the integrity of a membrane filter. In each method, the test pressure varies directly with surface tension of the wetting fluid. Therefore, the test pressures used in the procedures, listed below, assume the wetting fluid to be water. For test values using wetting fluids other than water, please contact **Porvair Filtration Group**.

The following integrity test procedures are available on request:

<b>Bubble Point Test</b>	MLP 58
<b>Forward Flow Diffusion Test</b>	MLP 57
<b>Pressure Hold Test</b>	MLP 59
<b>Wetting Procedures</b>	MLP 37

## Biofil™ PES Materials of Construction

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

Biofil™ Plus components meet the EEC Directive 2002/72/EC.

Component	Materials of Manufacture	FDA Number
Membrane	Polyethersulfone (PES)	21CFR177.1550 21CFR174.5
Core	Polypropylene	21CFR177.1520
Sleeve	Polypropylene	21CFR177.1520
Adaptors	Polypropylene	21CFR177.152
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/in<sup>2</sup>)  
80°C (176°F): 4.0bar (58lb/in<sup>2</sup>)  
100°C (212°F): 3.0bar (43lb/in<sup>2</sup>)  
120°C (248°F): 2.0bar (29lb/in<sup>2</sup>)  
125°C (257°F): 1.5bar (22lb/in<sup>2</sup>)

Reverse flow direction at:

20°C (68°F): 2.1bar (30lb/in<sup>2</sup>)  
80°C (176°F): 1.0bar (15lb/in<sup>2</sup>)  
100°C (212°F): 0.5bar (7lb/in<sup>2</sup>)

Maximum recommended short term operating temperature: 80°C(180°F).

## Tests for Biological Safety

### USP Toxicity Test (1)

Porvair Biofil™ Plus cartridges are manufactured using FDA approved materials, as listed, and in addition, components have been tested independently by UBTL Inc., 520 Wakara Way, Salt Lake City, Utah, USA. The results of the biological tests for plastics were that the components of construction were non-toxic.

Laboratory Number	60173
Sample Source	Porvair Filtration Group Ltd.
Test Requested	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 toxicity noted systematically: 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 T440	10	0	0	0
	Control T440	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 (1)

Laboratory Number	60227
Sample Source	Porvair Filtration Group Ltd.
Cell Line	Mouse Heteroploid Connective Tissue (L929)
Incubation Period	24 ± 1 hours at 37°C (99°F) w/5% CO <sub>2</sub>
Method of Scoring	Cytopathic Effect (0-4)
Extract Ratio	60cm <sup>2</sup> /20mL

### Purpose

The MEM elution test is designed to determine the cytotoxicity of extractable substances exposed to cellular mono layers. 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 hours ± 1 at 37°C (99°F) 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 (0.55")) 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% CO<sub>2</sub> and 95-100% relative humidity for 24 to 72 hours, or longer if appropriate. Microscopic readings are made at 24 hour intervals.

## MEM Elution (2)

The cell mono layers 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).

### Results

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

### Conclusion

Non-cytotoxic.

## 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 <sub>2</sub> O, #J5H358B
Temperature	37°C (99°F) ± 1°C (2°F)
Time	1 hour

### Results

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

### Conclusion

Negative

## Physicochemical 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.

## Objective

To verify the resistance of Biofil™ Plus filter cartridges to in-line steam sterilisation at a temperature of 125°C (257°F) in the standard flow direction.

## Procedure

Cartridges were sampled from a routine production batch; integrity tested by the forward flow diffusion method (MLP 57) initially, and after every 12th cycle thereafter. Cartridges were steam sterilised by dynamic in-line steam at 125°C (257°F) for 20 minutes, whilst maintaining a differential pressure below 0.5bar. Upstream and downstream condensate was drained throughout each cycle. The cartridges were cold water cooled for 10 minutes between steam cycles, to replicate the highest levels of thermal shock likely to be encountered in use.

The steam sterilisation procedure is summarised as follows:

Steam Preheating → Steam Flow → Cold Water Flow Cooling → Integrity Test Every 12th Cycle

## Results

Cartridge Batch Number	Number of 20 Minute Cycles	Temperature	Number Tested	Number Failed
ICC 200418	112	125°C (257°F)	5	0

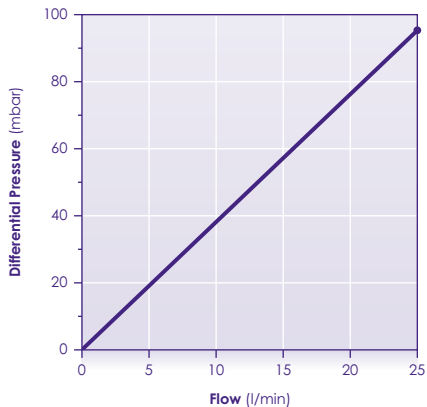
## Conclusion

The Biofil™ Plus cartridges have been shown to withstand in-line sterilisation in the standard flow direction by dynamic steam at a 125°C (257°F) temperature for 112, 20 minute cycles without loss of integrity.

## Flow Rate Characteristics

**Porvair Biofil™ Plus 0.45 micron clean water flow rate**, based on a 250mm (10") single cartridge, with an effective filtration area of 0.67m<sup>2</sup> (7.2ft<sup>2</sup>), in situ in a **Porvair** housing exhibiting the differential pressure characteristics indicated below:

### Clean Water Flow vs Differential Pressure

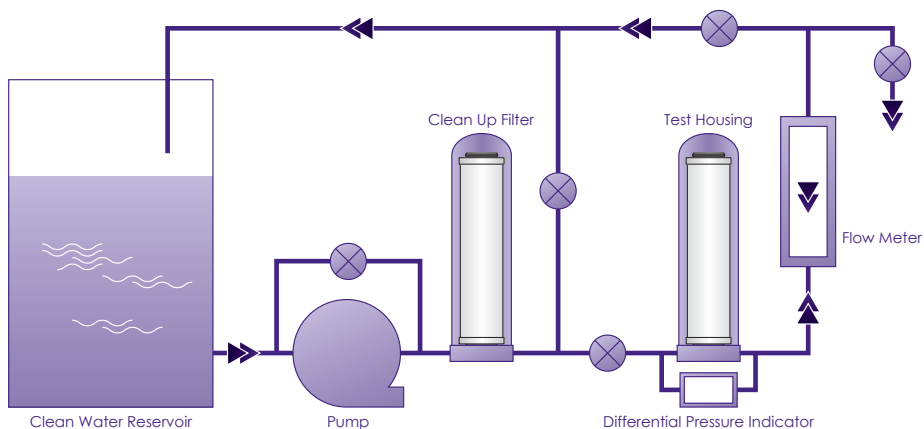


### Test Procedure

**Clean water flow vs  $\Delta p$ :** The test cartridges were immersed in a solution of 60% Isopropyl alcohol (IPA) 40% water for approximately 1 minute.

The water inlet valve was opened and the water allowed to circulate until the pressure differential across the clean up filter stabilised. The test filters were installed and the wetting solution flushed to waste. Water was then allowed to flow through the cartridge for approximately 10 minutes before the differential pressure across the filter/housing, at a flow of 5, 10, 15, 20, and 25 litres/minute, was recorded.

### Schematic of Clean Water Flow Rig







**Porvair Filtration Group Ltd.**

1 Concorde Close  
Segensworth  
Fareham  
Hampshire  
PO15 5RT  
UK

Tel: +44 (0)1489 864330  
Fax: +44 (0)1489 864399  
Email: [info@porvairfiltration.com](mailto:info@porvairfiltration.com)

**Porvair Filtration Group Inc.**

10190 Maple Leaf Court  
Ashland  
Virginia 23005  
USA

Tel: +1 804 550 1600  
Fax: +1 804 550 3262  
Email: [infoUS@porvairfiltration.com](mailto:infoUS@porvairfiltration.com)

[www.porvairfiltration.com](http://www.porvairfiltration.com)

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