porvair
filtration group

Biofil™ II BT 0.2µm

Membrane Cartridge

Validation Guide

World Class Filtration Solutions

Contents

Introduction

The **Porvair Filtration Group** Biofil™ II 0.2 micron microbial rated cartridge has been developed and manufactured for the filtration of liquids in the pharmaceutical, biotechnology and other critical applications. Biofil™ II utilises a naturally hydrophilic polyethersulphone (PES) membrane with a mirrored asymmetric pore structure, which provides graded filtration throughout its depth, resulting in higher throughputs and long service life. When combined with quality all-polypropylene components and high integrity manufacturing techniques, the Biofil™ II 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™ II 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™ II 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™ II 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 *Brevundimonas diminuta* ATCC #19146 that had been cultured to maximise the percentage of organisms capable of passing through a 0.45 micron filter. A sufficient volume of this challenge suspension, equivalent to at least 10^7 CFU per cm^2 of effective filtration area (EFA) was prepared for each filter. The challenge was conducted at a high flow rate and a maximum differential 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 *Brevundimonas diminuta* as a challenge organism is based on literature reports that the organism attains a very small size when grown under starvation conditions. The test procedure complies with the recommendations of the Parenteral Drug Association's Technical Report No. 26 *Sterilizing Filtration of Liquids,* 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 selection of saline lactose broth as a nutrionally-deficient growth medium, results in a significant percentage of *Brevundimonas diminuta* cells that will pass through a 0.45 micron membrane. The growth parameters, temperatures and medium are adapted from PDA TR26, ASTM and HIMA methods.

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

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

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" cartridge)** (*Brevundimonas diminuta* ATCC #19146)

Filter ID	**Flow Rate** @ 30psig	**Total Challenge** (CFU)	**Challenge/Sq cm** (CFU/cm²)	**Filtrate Count** (CFU)	**Rinse Count** (CFU)	**LRV**
BT20 S1BB ICC No.617184 # 18	10L/22sec	7.7×10^{10}	1.1×10^{7}	<1	<1	>10.89
0.45µm Positive Control	50mL/5sec	7.7×10^{7}	8×10^{6}	6.5×10^{5}	N/A	2.07
BT20 S1BB ICC No.617184 # 13	8L/17sec	1×10^{11}	1.5×10^{7}	<1	<1	>11.01
0.45µm Positive Control	50mL/5sec	1.3×10^{8}	9.6×10^{6}	2.3×10^{6}	N/A	1.61

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)
BT20 S1BB ICC No.617184 # 18	1800mbar	8mL/min	7mL/min	7mL/min
Biofil™ II BT20 ICC No.617184 # 13	1800mbar	11mL/min	10mL/min	11mL/min

Conclusion

Porvair Biofil™ II BT20 0.2 micron cartridges were effective in retaining the *Brevundimonas diminuta* bacteria as demonstrated by the absence of bacterial colonies on the assay membranes. The flow rate, total challenge level and the LRV are summarised in table 1. The diffusive flow test results are summarised in table 2.

Integrity Tests

Each module of every Biofil™ II BT20 0.2 micron filter cartridges is individually integrity tested during manufacture.

The detection of defects in the filter's integrity can be carried out by non-destructive integrity testing. The integrity test parameters for **Porvair** 0.2 micron membrane filters are based on correlating the test parameters with the retention of *Brevundimonas diminuta*. **Porvair** Biofil™ II BT20 0.2 micron filter cartridges can be tested by industry standard procedures as recommended in PDA Technical Report 26.

The integrity test pressure is dependent on the surface tension of the liquid to be used. 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.**

The following integrity test procedures are available on request:

Bubble Point Test	MLP 58
Forward Flow Diffusion Test	MLP 57
Pressure Hold Test	MLP 59
Wetting Procedures	MLP 37

Biofil™ PES Materials of Construction

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

Biofil™ II 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^2)
80°C (176°F): 4.0bar (58lb/in^2)
100°C (212°F): 3.0bar (43lb/in^2)
120°C (248°F): 2.0bar (29lb/in^2)
125°C (257°F): 1.5bar (22lb/in^2)

Reverse flow direction at:

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

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

USP Toxicity Test (1)

Porvair Biofil™ II cartridges are manufactured using FDA approved materials, as listed above, 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	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 (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_2
Method of Scoring	Cytopathic Effect (0-4)
Extract Ratio	60cm²/20mL

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_2 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_2O, #J5H358B
Temperature	37°C (99°F) ± 1°C (2°F)
Time	1 hour

Results

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

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™ II 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 39) 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 136823	80	125°C (257°F)	2	0
ICC 136831	80	125°C (257°F)	4	0
ICC 138685	80	125°C (257°F)	2	0

Conclusion

The Biofil™ II 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 80, 20 minute cycles without loss of integrity.

Biofil™ II Sterilisation by Hot Water

Objective

To verify the resistance of Biofil™ II filter cartridges to hot water sanitation at 85-90°C (185-194°F) in the standard flow direction.

Procedure

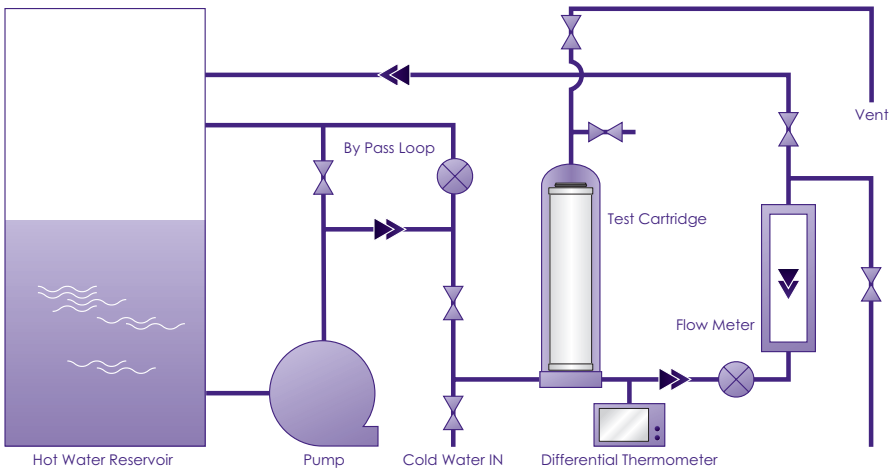
Cartridges were sampled from a routine production batch and integrity tested, by the forward flow diffusion test method, prior to hot water sanitation. Cartridges were subsequently sanitised at a temperature between 85 - 90°C (185-194°F) for 20 minutes, at a flow of 10 litres/minute per 250mm (10").

The hot water sanitation procedure is summarised as follows:

The cartridges were water cooled between each cycle, at a flow of 10 litres/minute per 250mm (10"), to simulate the highest levels of thermal shock likely to be encountered in use. The cartridges were removed after every 17th cycle for integrity testing.

Hot Water Flow → **Cold Water Flow Cooling** → **Integrity Test** Every 17th Cycle

Schematic of Hot Water Sterilising Rig



Results

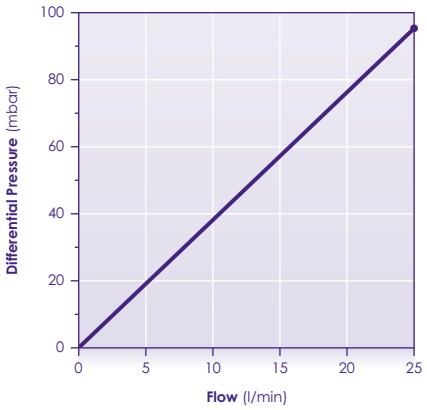
Cartridge Part Number	Cartridge Batch Number	Number of 20 Minute Cycles	Temperature	Number Tested	Number Failed
BT20S3BB	ICC 138671	100	85-90°C (185-194°F)	3	0

Conclusion

The Biofil™ II filter cartridges have been shown to withstand hot water sanitation in the standard flow direction at a temperature of between 85-90°C (185-194°F) for a maximum of 100, 20 minute cycles.

Porvair Biofil™ II 0.2 micron clean water flow rate, based on a 250mm (10") single cartridge, with an effective filtration area of 0.65m² (7.0ft²), in situ in a **Porvair** housing exhibiting the differential pressure characteristics indicated below:

Clean Water Flow vs Differential Pressure

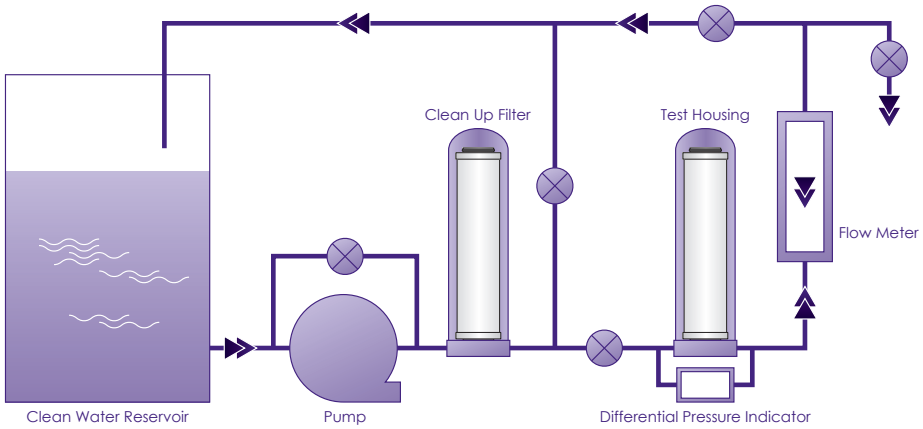


Test Procedure

Clean water flow vs Δp: 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



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