porvair
filtration group

Vinofil™ 0.45µm

Membrane Cartridge

Validation Guide

World Class Filtration Solutions

Contents

Introduction

Porvair Filtration Group has specifically designed Vinofil™ membrane cartridges, for wine and sparkling wine filtration, as a final filter for cold biological stabilisation. Vinofil™ utilises a double layer of 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 Vinofil™ 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 rigorous demands of wine producers.

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

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

Introduction

Scope of the challenge test is to verify the bacterial retention efficiency of Vinofil™ cartridges and to determine a correlation between the non-destructive diffusion and bubble point tests and the bacterial retention capability in liquid.

The Vinofil™ cartridges were subjected to two separate bacteria challenge tests, the organisms being *Serratia marcescens* ATCC #14756 and *Oenococcus oenos* ATCC # 23279. The organisms were prepared at a concentration of at least 1 x 10^7 colony forming units (CFU) per cm^2 of effective filtration area (EFA).

Justification

The test method was designed to determine the bacterial retention characteristics of membrane filters used to filter liquids. The selection of *Serratia marcescens* as the challenge organism is based on the size of the organism, and historical acceptance as defining 0.45µm membranes. *Oenococcus oenos* was selected due to its common occurrence in the beverage industry. The test procedure is adapted from the ASTM F838-83 Standard Test Method *Determining Bacterial Retention of Membrane Filters Utilised For Liquid Filtration* and the Health Industry Manufactures Association (HIMA) Test Method *Microbiological Evaluation of Filters For Sterilizing Liquids*.

Brief description of challenge procedure

After preparation of the challenge suspension, the filter is integrity tested, then the filter and test system are sterilised at 121-125°C for 30 minutes using in-line steam. When cool, sterility of the system is verified and the filter is integrity tested again. The challenge suspension of at least 10^7 CFU/cm^2 of effective filtration area is filtered through the test filter at a delivery pressure of 30 psig and that filtrate is filtered though analytical membrane filter discs.

These assay filters are removed and plated onto SCDA. The plates are incubated at 20-25°C for 18±2 hours. For Oenococcus oenos the assay filters were plated onto ATAG and incubated at 20-25°C for 168 hours.

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 denominated and the LRV is expressed as greater than (>) the calculated value.

Results

Table 1 **Bacterial Retention Results (250 mm 10" cartridge)** (*Serratia marcescens* ATCC # 14756)

Filter ID	**Flow Rate** @ 30 psig	**Total Challenge** (CFU)	**Challenge** (CFU/cm^2)	**Filtrate Count** (CFU)	**Rinse Count** (CFU)	**LRV**
Vinofil™ V45 ICC No.117786 # 49	5 L/18 sec	4.1 x 10^{10}	10^7	1	<1	>10.61
Vinofil™ V45 ICC No.117786 # 15	5 L/18 sec	4.1 x 10^{10}	10^7	3	<1	>10.14
Vinofil™ V45 ICC No.117786 # 22	5 L/23 sec	4.1 x 10^{10}	10^7	2	<1	>10.31
0.8 µm Positive Control	50 mL/21 sec	8.2 x 10^7	8.6 x 10^6	1.4 x 10^8	N/A	-0.22

Table 2 **Diffusion Rate / Bubble Test Results**

Filter ID	**Test Pressure** (mbar)	**Diffusion Rate Pre-sterilisation** (mL/min)	**Diffusion Rate Post Challenge** (mL/min)	**Bubble Point Post Challenge** (mbar)
Vinofil™ V45 ICC No.117786 # 49	1500	7.3	28	1723
Vinofil™ V45 ICC No.117786 # 15	1500	7.4	11	1723
Vinofil™ V45 ICC No.117786 # 22	1500	25	14	1723

Table 3 **Bacterial Retention Results (250 mm 10 inch cartridge)** (*Oenococcus oenos* ATCC # 23279)

Filter ID	**Flow Rate** @ 30 psig	**Total Challenge** (CFU)	**Filtrate Count** (CFU)	**Rinse Count** (CFU)	**LRV**
Vinofil™ V45 ICC No.117786 # 36	4 L/2 min 41 sec	1.2×10^{13}	6.3×10^{7}	<1	5.27
Vinofil™ V45 ICC No.117786 # 40	4 L/3 min 27 sec	1.2×10^{13}	5.3×10^{7}	<1	5.34
Vinofil™ V45 ICC No.117786 # 46	4 L/2 min 32 sec	1.2×10^{13}	4.3×10^{7}	<1	5.43
1.0 µm Positive Control	50 mL/47 sec	2.9×10^{9}	1.3×10^{8}	N/A	1.36

Table 4 **Diffusion Rate / Bubble Test Results**

Filter ID	**Test Pressure** (mbar)	**Diffusion Rate Pre Sterilisation** (mL/min)	**Diffusion Rate Post Challenge** (mL/min)	**Bubble Point Post Challenge** (mbar)
Vinofil™ V45 ICC No.117786 # 36	1500	8.5	14	1949
Vinofil™ V45 ICC No.117786 # 40	1500	7.4	14	1861
Vinofil™ V45 ICC No.117786 # 46	1500	8.4	10	2275

Conclusion

Porvair Vinofil™ 0.45 micron membrane cartridges consistently provided log reduction values (LRV'S) of > 10 for *Serratia marcescens* and > 5 *Oenococcus oenos*. The flow rate, the total challenge level, and the LRV are summarised in tables 1 and 3. The diffusion tests and bubble point results are summarised in tables 2 and 4.

The Bacterial Retention Tests were carried out by Nelson Laboratories Inc., 6280 South Redwood Road, Salt Lake City, UT 84123, United States Of America (report number. 270346).

Integrity Tests

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 microbial 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.

All Vinofil™ 0.45 micron filter cartridges are 100% integrity tested during manufacture.

The following integrity test procedures are available on request:

Bubble Point Test	MLP 38
Forward Flow Diffusion Test	MLP 39
Pressure Hold Test	MLP 40
Wetting Procedures	MLP 37

Vinofil™ Materials of Construction

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

Vinofil™ components meet the EEC Directive 2002/72/EC.

Component		**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
Support Materials:	Polypropylene	21CFR177.1520
Sealing Method:	Thermal Bonding	

Dimensions	
Diameter:	70mm (2.8")
Length:	125mm (5") 250mm (10") 510mm (20") 760mm (30") 1020mm (40")

Operating Characteristics (maximum differential pressure)		
Normal Flow Direction at:	20°C (68°F) 80°C (176°F) 100°C (212°F) 120°C (248°F) 125°C (257°F)	6.0 bar (87lb/in²) 4.0 bar (57lb/in²) 3.0 bar (43lb/in²) 2.0 bar (29lb/in²) 1.5 bar (22lb/in²)
Reverse Flow Direction at:	20°C (68°F) 80°C (176°F) 100°C (212°F)	2.1 bar (30lb/in²) 1.0 bar (15lb/in²) 0.5 bar (7lb/in²)

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

USP Toxicity Test (1)

Porvair Vinofil™ 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

Controls

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

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 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 / 5 mL of extracting medium). The prepared sample is normally extracted for 24 hours ± 1 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 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.

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

Results

Non-cytotoxic

Limulus Test

Laboratory Number	60184
Sample Source	Porvair Filtration Group Ltd.
LAL Manufacturer	Associates of Cape Cod
Sensitivity	0.06 EU / mL
+ Control	Difco LPS E. coli 055:B5, #715269
- Control	McGaw H_2O, #J5H358B
Temperature	37 ± 1°C
Time	1 hour

Results

Sample	**Positive control** (ng / mL) 100 pg	50 pg	25 pg	**Negative control** H_2O
-	+	+	+	-

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 5 mg, indicating that the residue on ignition will also be less than 5 mg.*

Objective

To verify the resistance of Vinofil™ filter cartridges to in-line steam sterilisation at a temperature of 125°C in the standard flow direction.

Procedure

Cartridges were sampled from routine production batches and integrity tested by the forward flow diffusion test method prior to in-line steaming. Each cartridge was subsequently steam sterilised reaching a temperature of 125°C (measured at the outlet of the housing), additionally, the differential pressure was held constant and did not exceed 0.3 bar during steam sterilisation. After the steam sterilising cycle of 20 minutes, the steam pressure was allowed to drop to atmosphere and the system water cooled by filtration of water for 5 minutes to simulate the highest levels of thermal shock. The cartridges were removed from the steam rig after every 10th cycle for integrity testing.

The steam sterilisation procedure is summarised as follows:

Steam Pre-heating → **Steam Flow** → **Cold Water Flow Cooling** → **Integrity Test** Every 10th Cycle

Results

Cartridge Part Number	Cartridge Batch Number	Number of 20 Minute Cycles	Temperature °C	Number Tested	Number Failed
V45S1BB	117786	80	125	5	0

Conclusion

The Vinofil™ cartridges have been shown to withstand in-line sterilisation in the standard flow direction by dynamic steam at a 125°C temperature for 80, 20 minute cycles without loss of integrity.

Objective

To verify the resistance of Vinofil™ filter cartridges to hot water sanitation at 85-90°C in the standard flow direction.

Procedure

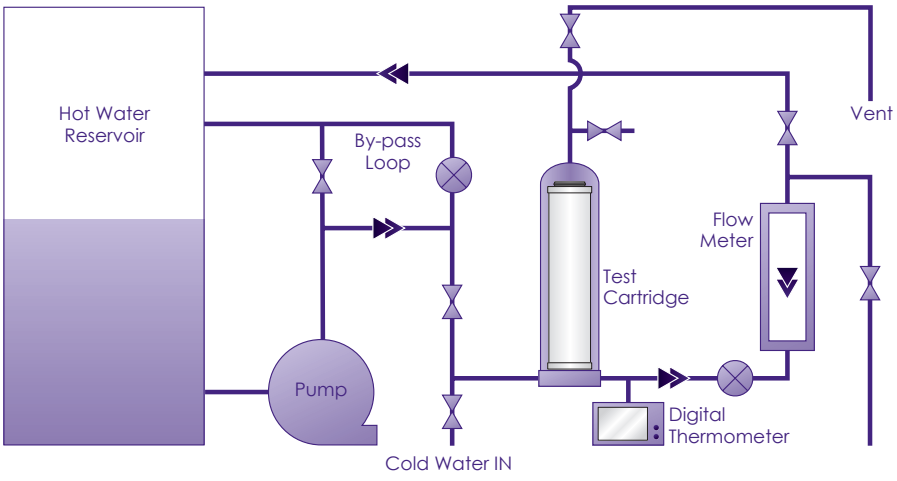
Cartridges were sampled from routine production batches and integrity tested by the forward flow diffusion method prior to hot water sanitation. Cartridges were subsequently sanitised at a temperature between 85-90°C for 20 minutes at a flow of 10 L/min per 250 mm (10").

The hot water sanitation procedure is summarised as follows:

The cartridges were water cooled between each hot water cycle at a flow of 10 L/min per 250 mm (10") cartridge 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

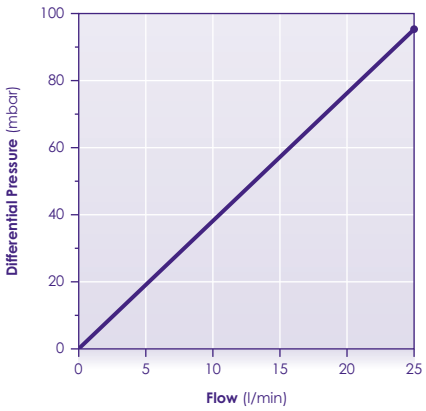
Cartridge Part Number	Cartridge Batch Number	Number of 20 Minute Cycles	Temperature °C	Number Tested	Number Failed
V45S1BB	117786	200	85-90	3	0

Conclusion

The Vinofil™ filter cartridges have been shown to withstand hot water sanitation in the standard flow direction at a temperature of between 85-90°C for a maximum of 200, 20 minute cycles.

Porvair Vinofil™ 0.45 micron clean water flow rate, based on a 250 mm (10") single cartridge, in situ in a **Porvair** housing exhibiting the differential pressure characteristics indicated below:

Clean Water Flow vs Differential Pressure

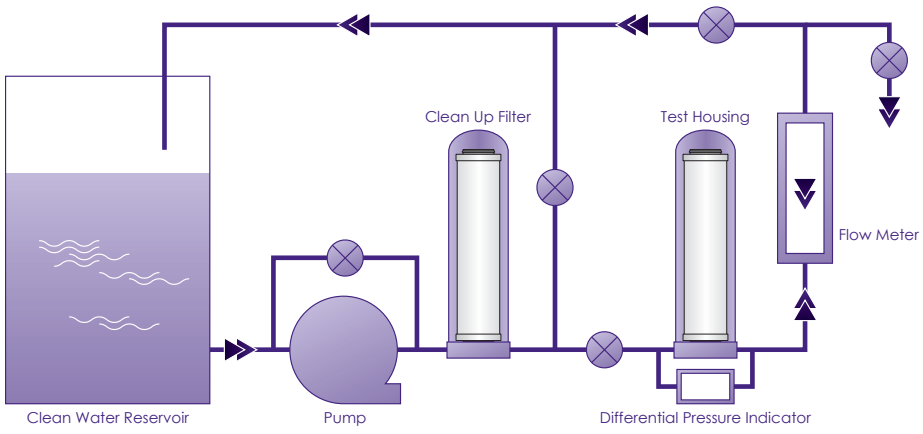


Test Procedure

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 L/min, was recorded.

Schematic of Clean Water Flow Rig



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