Life Sciences

Validation Guide

USTR 2175

Validation Guide for Pall Supor® EBV Filter Cartridges

*Filtration. Separation. Solution.*SM

CONTENTS

1. Overview

1.1 Introduction

Pall Supor® EBV filter cartridges have been designed as 0.2µm-rated liquid sterilizing filters for use within the Pharmaceutical industry. The filter is comprised of two layers of polyethersulfhone membrane. The coarser upstream membrane layer provides built-in prefiltration to the finer downstream membrane layer. The filter is manufactured using the **Pall** Ultipleat® construction. This laid-over pleat configuration maximizes membrane area in order to increase flow rates and maximize filter life.

The purpose of this report is to summarize the tests that were performed to qualify the performance of **Supor** EBV filters under standard test conditions. The qualification program included:

- **Microbial validation tests**
- **Endurance to in-line steam sterilization**
- **Determination of water flow characteristics**
- **Extractables testing using water and ethanol**
- **Biological reactivity tests**
- **Determination of consistency of membrane performance using protein adsorption tests**

1.2 Summary of conclusions

Microbial validation tests

Supor EBV filters were tested using bacterial challenge tests with *Brevundimonas diminuta* (ATCC 19146), in accordance with the FDA guidelines on Sterile Products produced by Aseptic Processing (1987).

The Forward Flow integrity test was shown to be a suitable non-destructive integrity test for **Supor** EBV filters, and test parameters correlated to liquid bacterial challenge tests have been set as follows for 25 cm (10 inch) filters (part number AB1EBV7PH4):

Forward Flow integrity test parameters

Test pressure	2760 mbar (40 psi)
Wetting liquid	Water
Temperature	20°C ± 5°C
Test gas	Air
Maximum allowable Forward Flow limit	23 ml/min

Endurance to in-line steam sterilization

Supor EBV filters (part number AB1EBV7PH4) have been demonstrated to be capable of withstanding multiple in-line steam sterilization cycles. Of the ten filters tested, all were found to retain integrity after exposure to ten one-hour steam cycles at 125°C.

The tests performed also demonstrate that **Supor** EBV filters are robust and capable of withstanding differential pressures that may exceed 300 mbar (4.5 psi) in the forward direction, as demonstrated by exposing filters to differential pressures of up to 1000 mbar (14.5 psi) during steam tests performed at 125°C.

Determination of water flow characteristics

Water flow rates at set differential pressures have been determined. The typical clean water flow was found to be 10 l/min at 100 mbar (1.45 psi) differential pressure at 20°C. This data can be used to assist users in sizing filter systems employing **Supor** EBV filters.

Extractables testing using water and ethanol

The amount of non-volatile residue extracted from typical **Supor** EBV filters has been determined using water and ethanol as the extraction fluids. The levels of extractables determined were extremely low. The aqueous extractables ranged from 8 to 35 mg per 25cm (10 inch) filter element (part number AB1EBV7PH4).

Actual service will impose different conditions, such as different exposure times, temperature, liquid purity etc. Evaluation under process conditions is therefore also recommended.

Biological reactivity tests

Supor EBV filters meet the requirements of the USP Biological Reactivity Tests (***in vivo***) for Class VI-121°C plastics. The tests included the systemic injection test, the intracutaneous test and the implantation test.

Consistency of membrane performance using protein adsorption tests

IgG adsorption was determined using seven sets of **Supor** EBV membrane. In all cases >95% protein transmission was observed. These data demonstrate the reproducibility and low binding characteristics of **Supor** EBV membrane.

2. Microbial validation tests

2.1 Introduction

The FDA guidelines on Sterile Products Produced by Aseptic Processing (1987) state, 'A sterilizing filter is one which, when challenged with the micro-organism *Pseudomonas diminuta (P. diminuta)*, at a minimum concentration of 10^7 organisms per cm^2 of filter surface, will produce a sterile effluent'.

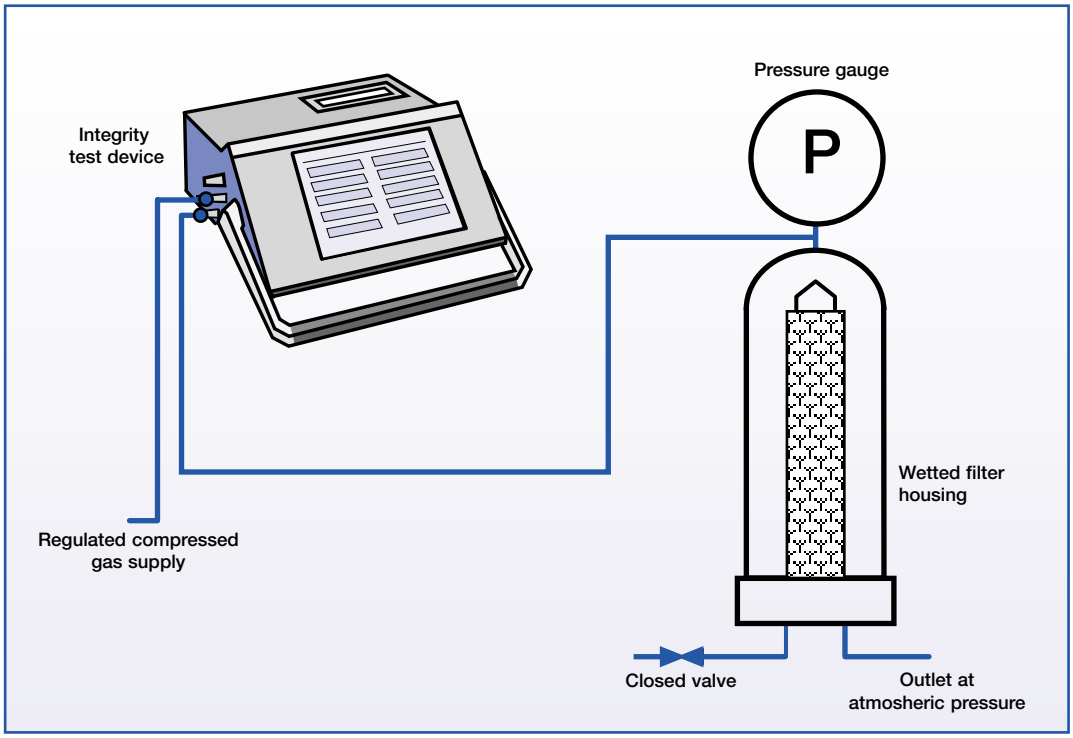
In order to meet the requirements of this guideline, liquid challenge tests using *Brevundimonas (Pseudomonas) diminuta* (ATCC 19146) were performed with **Supor** EBV filter cartridges using a minimum of $1x10^7$ colony forming units (CFU)/cm^2 of effective filtration area.

The correlation between microbial retention and a non-destructive integrity test is also an important aspect of the validation of sterilizing grade filters. The FDA guideline further states, 'After a filtration process is properly validated for a given product, process and filter, it is important to assure that identical filter replacements (membrane or cartridge) used in production runs will perform in the same manner. One way of achieving this is to correlate filter performance data with filter integrity testing data'. The integrity test used during this validation study was the Forward Flow test.

The Forward Flow test

In the Forward Flow test, a filter is wetted with an appropriate test liquid and a 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 downstream or on the upstream side, using sensitive flow measurement equipment such as the Palltronic® 'Flowstar' filter integrity test device, see Figure 2-1.

Figure 2-1. The automated Forward Flow integrity test



The aims of this series of tests were to:

- **Determine the microbial removal efficiency of Supor EBV filters in liquid challenge tests using *Brevundimonas diminuta* (ATCC 19146)**
- **Correlate the non-destructive Forward Flow integrity test with destructive challenge tests**
- **Determine Forward Flow integrity test parameters**

2.2 Summary of methods

Typical **Supor** EBV filters, part number AB1EBV7PH4, from three separate manufacturing batches were subjected to microbial challenge tests using an aqueous suspension of *Brevundimonas diminuta* (ATCC 19146).

Prior to the challenge tests the filters were installed in an appropriate housing and autoclaved at 121°C for 60 minutes. The filters were then flushed with DI water at a flow rate of 4 l/min for 10 minutes with a back pressure of 0.5 bar (7.25 psi). A Forward Flow integrity test was performed using a **Palltronic** integrity test device with an air test pressure of 2760 mbar (40 psi). The filter assembly was then aseptically connected to the pre-sterilized challenge apparatus, as shown in Figure 2-2.

An aqueous suspension of *B. diminuta* was passed through the filter to achieve a challenge level of > $1x10^7$ colony forming units (CFU) per cm^2 of effective filtration area.

During the challenge test the entire filter effluent was passed through a 0.2µm-rated analysis disc on the downstream side of the test filter assembly. The filter disc was incubated on agar and, following incubation, the disc was examined to determine if any colonies had grown, indicating whether or not bacteria had passed through the test filter during the challenge. The titre reduction (T_R) for each filter was determined as follows:

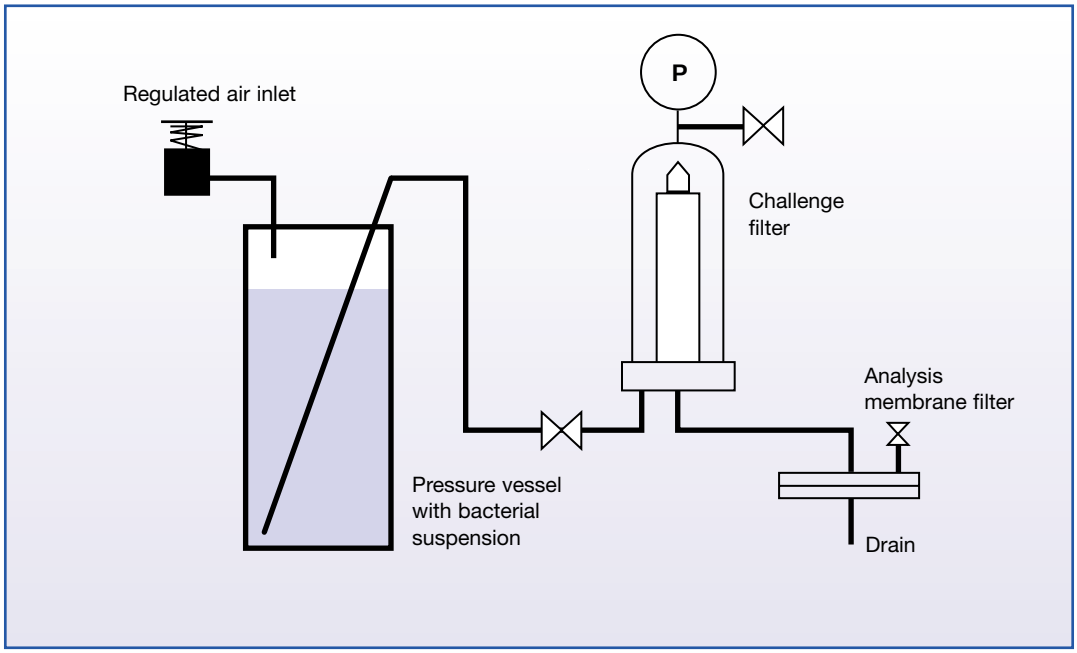
$$T_R = \frac{\text{Total number of organisms influent to the filter}}{\text{Number of colonies recorded on the downstream analysis disc}}$$

When no colonies were detected downstream, the titre reduction was expressed as;

> total number of organisms influent to the filter (e.g. $>1x10^{10}$)

On completion of the challenge test the filter assemblies were autoclaved and then flushed and Forward Flow integrity tested as described previously.

Figure 2-2. Microbial challenge apparatus



2.3 Results

The Forward Flow and *B. diminuta* retention results are shown in Table 2-1. The higher of the two Forward Flow values are presented and the data are arranged in order of increasing Forward Flow value.

All of the filters with Forward Flow values ≤ 35.7 ml/min gave sterile effluent when challenged with > 1 x 10^7 CFU per cm^2 of filtration area using *B. diminuta*.

Table 2-1. Correlation of Forward Flow with B. diminuta retention for Supor EBV filters (part number AB1EBV7PH4)

Pall filter serial number serial number	Forward Flow[1] (ml/min)	Sterile effluent	Titre reduction
IE7323154	6.5	Yes	> 2.02 x 10^{11}
IE7323048	6.9	Yes	> 2.01 x 10^{11}
IE7322192	6.9	Yes	> 3.07 x 10^{11}
IE7323061	7.1	Yes	> 2.02 x 10^{11}
IE7323060	7.1	Yes	> 1.93 x 10^{11}
IE7322149	7.3	Yes	> 3.92 x 10^{11}
IE7322011	7.4	Yes	> 2.09 x 10^{11}
IE7323058	7.5	Yes	> 1.40 x 10^{11}
IE7322129	7.8	Yes	> 2.32 x 10^{11}
IE7322200	7.8	Yes	> 1.50 x 10^{11}
IE7322202	7.9	Yes	> 2.45 x 10^{11}
IE7636012	11.3	Yes	> 8.65 x 10^{11}
IE7323070	11.9	Yes	> 1.40 x 10^{11}
IE7636109	16.0	Yes	> 1.69 x 10^{11}
IE7636110	17.4	Yes	> 1.99 x 10^{11}
IE7636066	18.6	Yes	> 1.54 x 10^{11}
IE7636098	20.0	Yes	> 1.77 x 10^{11}
IE7636052	24.2	Yes	> 1.96 x 10^{11}
IE7636051	24.5	Yes	> 1.75 x 10^{11}
IE7636128	35.7	Yes	> 1.75 x 10^{11}

1 Forward Flow values at 2760 mbar (40 psi) air test pressure, wet with water.

2.4 Conclusions

Based on the results of this study, the Forward Flow integrity test has been demonstrated to be a suitable non-destructive integrity test for **Supor** EBV filters. Integrity test parameters for **Supor** EBV filters, part number AB1EBV7PH4, have been set as follows:

Forward Flow integrity test parameters*

Test pressure	2760 mbar (40 psi)
Wetting liquid	Water
Temperature	20°C ± 5°C
Test gas	Air
Maximum allowable Forward Flow limit	23 ml/min

* See section 2.2 for test procedure.

3. Endurance to in-line steam sterilization

3.1 Introduction

The purpose of these tests was to determine the effects of repeated in-line steam sterilization cycles at 125°C on filter integrity using standard **Supor** EBV filters from production, part number AB1EBV7PH4.

3.2 Summary of methods

Typical **Supor** EBV filters from production (part number AB1EBV7PH4) were used for the tests. The filters were flushed with DI water at a flow rate of 4 l/min with a back pressure of 0.5 bar (7.25 psi) for ten minutes and then Forward Flow integrity tested using an air test pressure of 2760 mbar (40 psi). The filters were then subjected to a one-hour in-line steam cycle at 125°C.

During the initial stages of the steam cycles, the wet filter membrane caused the differential pressure to increase across the filter as steam was introduced. The steam inlet valve was controlled so that the differential pressure across the wetted filter did not exceed 1000 mbar (14.5 psi).

Immediately after the steam cycle had finished, dry compressed air was flushed across the upstream side of the filter surface for 30 minutes in order to replace the steam and cool the assembly. The filters were then flushed with water prior to starting the next steam cycle.

This sequence was repeated until each filter had been exposed to ten steam cycles.

On completion of the ten steam cycles, the filters were flushed with DI water at a flow rate of 4 l/min with a back pressure of 0.5 bar (7.25 psi) for ten minutes and Forward Flow integrity tested again as described above.

3.3 Results

The Forward Flow integrity test results for the **Supor** EBV filters (part number AB1EBV7PH4) before and after exposure to in-line steam cycles are shown in Table 3-1. Filters from three separate manufacturing batches were included in the tests and all the filters retained integrity following exposure to ten one-hour cycles at 125°C.

Table 3-1. Effects of exposure to in-line steam at 125°C on filter integrity for Supor EBV filters (part number AB1EBV7PH4)

Pall filter serial number	Forward Flow[1] (ml/min) values at:	
	Start of test	After 10 steam cycles
IE7323025	7.3	6.7
IE7323063	11.3	6.6
IE7322010	7.1	6.2
IE7322079	7.4	8.2
IE7322198	7.9	8.4
IE7636017	13.0	10.5
IE7636079	13.6	11.9
IE7636181	11.8	11.9
IE7636141	12.7	12.6
IE7636186	13.1	11.5

[1] Forward Flow values at 2760 mbar (40 psi) air test pressure, wet with water, maximum allowable limit value 23 ml/min.

3.4 Conclusions

Supor EBV filters (part number AB1EBV7PH4) have been demonstrated to be capable of withstanding multiple in-line steam sterilization cycles. Of the ten filters tested, all were found to retain integrity after exposure to ten one-hour steam cycles at 125°C.

The tests performed also demonstrate that **Supor** EBV filters are robust and capable of withstanding differential pressures that may exceed 300 mbar in the forward direction, as demonstrated by exposing filters to differential pressures of up to 1000 mbar (14.5 psi) during steaming at 125°C.

4. Determination of water flow characteristics

4.1 Introduction

The aim of these tests was to determine the differential pressures measurements across **Supor** EBV filters, part number AB1EBV7PH4, at set water flow rates.

4.2 Summary of methods

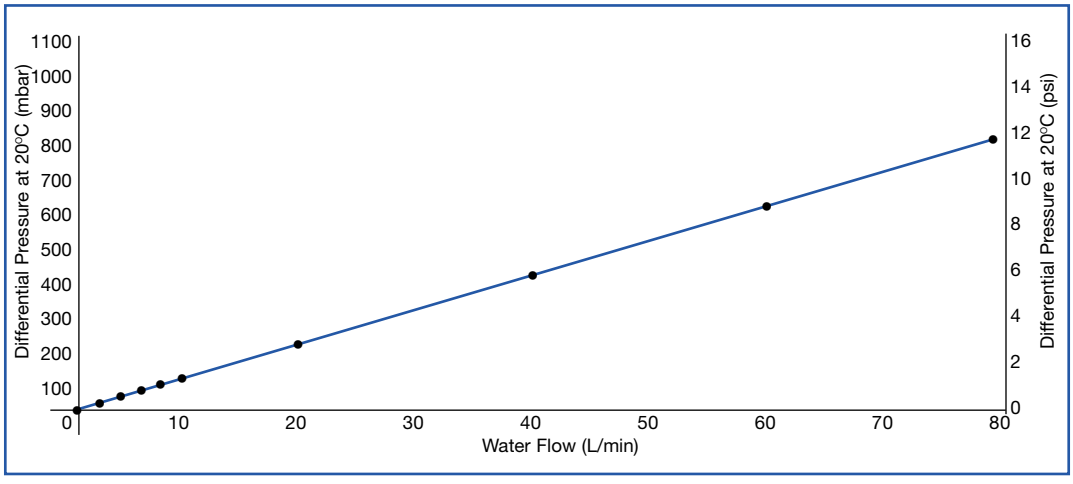
The tests were performed on standard production filters (part number AB1EBV7PH4). Five filters were tested, and these included filters from three different manufacturing batches. The test filters were installed in an appropriate housing and pre-filtered DI water was pumped through the filters in the normal flow ('out to in') direction. Pressure readings from transducers on the upstream and downstream sides of the test assembly were monitored to calculate the differential pressure at set water flow rates.

Further measurements were taken with the filter housing only, with no filter installed. The housing-only results were subtracted from the filter assembly results in order to provide flow/pressure characteristics for the filter only. All data were corrected for a standard temperature of 20°C.

4.3 Results

The water flow / differential pressure measurements obtained using typical **Supor** EBV filters (part number AB1EBV7PH4) are shown in Figure 4-1. The data in the graph represent average values from measurements taken using five different filters.

Figure 4-1. Water flow / differential pressure characteristics of Supor EBV filters, part number AB1EBV7PH4



4.4 Conclusions

Water flow rates at set differential pressures have been determined. The typical clean water flow was found to be 10 l/min at 100 mbar (1.45 psi) differential pressure at 20°C. This data can be used to assist users in sizing filter systems employing **Supor** EBV filters.

5. Extractables testing using water and ethanol

5.1 Introduction

The aim of this series of tests was to quantify and characterize the material that can be extracted from **Supor** EBV filters (part number AB1EBV7PH4) using water and ethanol.

5.2 Summary of methods

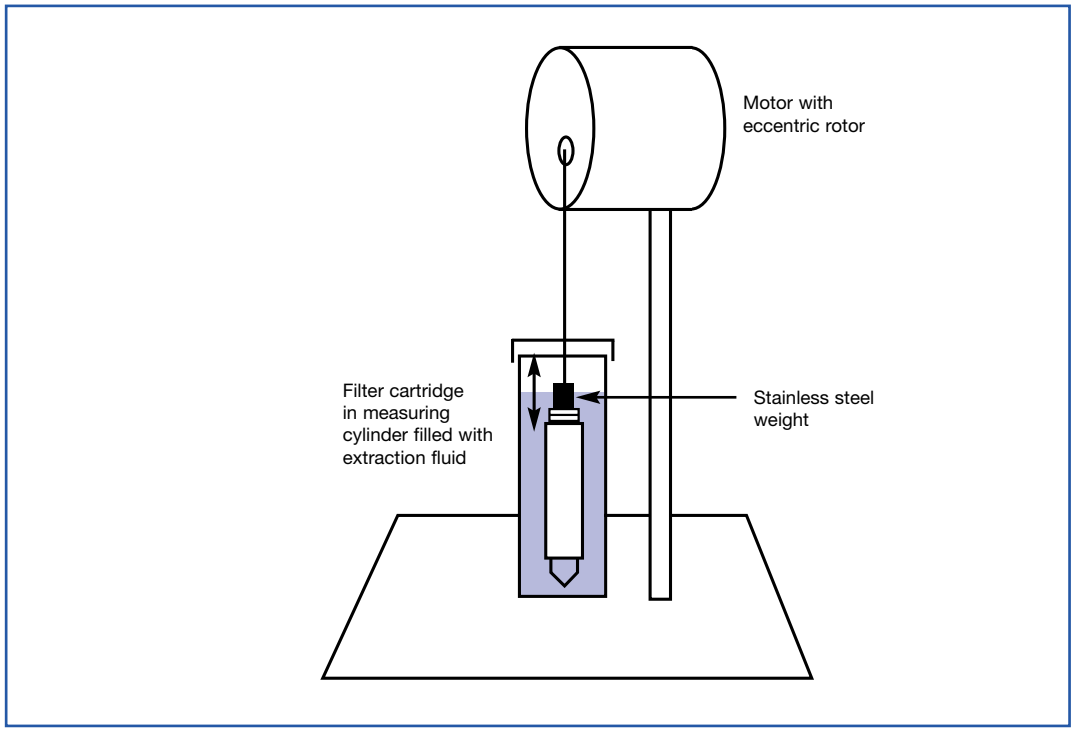
Preparation of filter samples

Tests for extractables were performed on typical production filter cartridges that had been autoclaved in order to maximize the quantity of any extractable material present. The filters were wrapped in aluminum foil and autoclaved for one hour at 121°C, using a slow exhaust cycle. Visible droplets of water remaining on the filter elements were allowed to evaporate at room temperature before the extraction was performed.

Extraction procedure

Dynamic extraction tests were performed in water and ethanol. The test filters were immersed in 1500 ml of extraction fluid in a clean measuring cylinder, as shown in Figure 5-1. For four hours the filter was gently moved up and down. This movement created flow through the filter membrane as a result of the pressure head that was created each time the element was partially lifted out of the liquid.

Figure 5-1. Filter extraction apparatus



Analysis of material extracte

After the extraction, 1000 ml of the extraction liquid was evaporated to dryness and the non-volatile extractables were determined gravimetrically. The ethanol extractables were also analyzed by Fourier Transform Infra Red Spectroscopy (FTIR).

5.3 Results

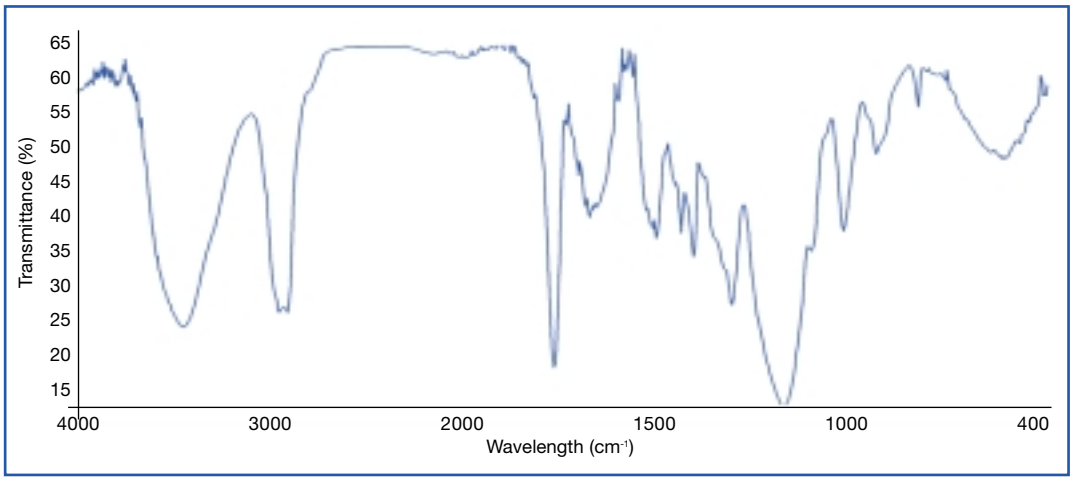
Table 5-1 shows the levels of aqueous and ethanol extractables obtained using typical production **Supor** EBV filters (part number AB1EBV7PH4).

Table 5-1. Non-volatile aqueous and ethanol extractables obtained using Supor EBV filters, part number AB1EBV7PH4

Extraction fluid	Pall filter serial number	Non-volatile residue(mg)
DI Water	IE7232013	14
	IF0694108	15
	IE7322131	8
	IE5793047	12
	IE7636161	33
	IE7636162	35
	IE7322103	28
	IE7322033	20
96% Ethanol	IE7322058	132
	IE7323015	152
	IE5793013	145
	IF0694062	193
	IE7636178	162
	IE7636090	145
	IE7322005	120
	IE7322029	139

An infra red spectrum (Figure 5-2) of the aqueous extracts from **Supor** EBV filters indicate the presence of extractables typical of polyethersulfhone resins and the methacrylate copolymer used to render the membrane hydrophilic.

Figure 5-2. Infra red spectrum of the aqueous extractables from Supor EBV filters



5.4 Conclusions

The levels of aqueous extractables determined for **Supor** EBV filters are extremely low, and the results reported are typical for production elements post autoclave.

Actual service will impose different conditions, such as different exposure times, temperature, liquid purity etc. Evaluation under process conditions is therefore also recommended.

6. Biological reactivity tests on the materials of construction

6.1 Introduction

The aim of this study was to evaluate the biological suitability of the materials of construction of **Supor** EBV filter cartridges. The materials of construction of the filters are as follows:

Membrane:	Pall hydrophilic polyethersulfhone membrane
Membrane support and drainage layers:	Polypropylene
Core and endcaps:	Polypropylene
Filter cage:	Polypropylene with titanium dioxide
O-rings:	Silicone elastomer for 'H4' option

6.2 Summary of methods

The tests were performed in accordance with the Biological Reactivity Tests *in vivo* for Class VI Plastics (121°C) as described in the current *United States Pharmacopeia*. The tests were conducted by Sterilization Technical Services division of STS duoTEK Inc., New York.

The testing procedures described in the USP include:

- **Injection of extracts of plastic materials**
- **Implantation of the solid material into animal tissue.**

The four extracting media listed in the USP simulate parenteral solutions and body fluids. These include:

- **Sodium Chloride Injection**
- **1 in 20 Solution of Alcohol in Sodium Chloride Injection**
- **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 for 72 hours, 70°C for 24 hours, or 121°C for 1 hour. The most stringent condition not resulting in physical changes in the plastic is recommended, therefore the filters were extracted at 121°C.

Acute Systemic Injection Tests

An Acute Systemic Injection Test was performed to evaluate the potential of a single injection of an extract to produce systemic toxicity. Sodium Chloride Injection and 1 in 20 Solution of Alcohol in Sodium Chloride Injection were injected intravenously. Vegetable oil extract and Polyethylene Glycol 400 extract were injected intraperitoneally.

Intracutaneous Tests

An Intracutaneous Test was performed 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 these tests.

Implantation Tests

Implantation tests were also performed, in order to subject the materials of construction to the most stringent conditions included in the USP. Each of the components of the **Supor** EBV filter was implanted separately.

6.3 Results

No biological response was observed in any of the tests performed and therefore the **Supor** EBV filter passed all of the tests specified.

6.4 Conclusions

Supor EBV filters meet the requirements of the USP Biological Reactivity Tests (*in vivo*) for Class VI-121°C plastics. The tests included the systemic injection test, the intracutaneous test and the implantation test.

7. Determination of consistency of membrane performance using protein adsorption tests

7.1 Introduction

The purpose of this series of tests was to demonstrate the reproducibility of **Supor** EBV membrane performance using protein adsorption tests.

7.2 Summary of methods

Typical samples of **Supor** EBV membrane from production were used for the tests.

A solution of ^{125}I-labelled anti-mouse IgG was prepared in phosphate buffered saline (pH 7.2). The protein concentration in the solution was 0.1 mg/ml. This feed solution was passed through discs of filter membrane (13 mm diameter) at a flow rate of 0.5 ml/min until a total of 2.5 ml had been passed through the disc.

Following filtration, excess solution was carefully blotted from the surface of the discs. The amount of ^{125}I-labelled protein retained by each disc was determined using a gamma counter.

Protein transmission was calculated by subtracting the amount of protein on the filter disc from the total in the original feed solution. Four discs of membrane were tested for each membrane sample.

7.3 Results

The results of the protein transmission tests are shown in Table 7-1. In all cases, >95% protein transmission was observed.

Table 7-1. IgG transmission through Supor EBV membrane

Membrane sample reference	Protein transmission			
	Disc 1	Disc 2	Disc 3	Disc 4
IE7323	> 95 %	> 95 %	> 95 %	> 95 %
IE5792	> 95 %	> 95 %	> 95 %	> 95 %
IE5793	> 95 %	> 95 %	> 95 %	> 95 %
IE7636	>95 %	> 95 %	> 95 %	> 95 %
IF0694	> 95 %	> 95 %	> 95 %	> 95 %
IE7322	> 95 %	> 95 %	> 95 %	> 95 %
IF0419	> 95 %	> 95 %	> 95 %	> 95 %

7.4 Conclusions

IgG transmission was determined using seven sets of **Supor** EBV membrane and in all cases >95% transmission was observed. These data demonstrate the reproducibility and low binding characteristics of **Supor** EBV membrane.

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