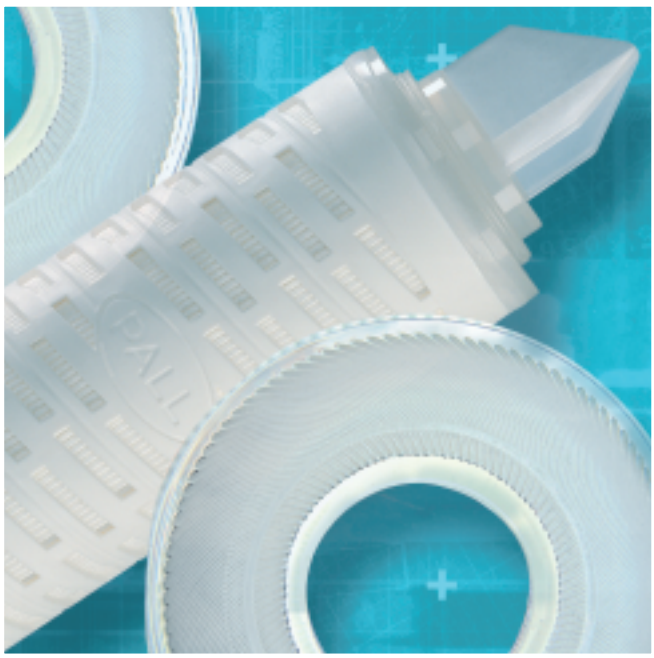
Life Sciences

Validation Guide

USTR 2222a[1]

Pall Supor® EKV Sterilizing Grade Filters



*Filtration. Separation. Solution.*SM

Contents

1. Overview

1.1 Introduction

Supor® EKV filter cartridges and capsules have been designed as 0.2 µm-rated liquid sterilizing filters for use within the pharmaceutical industry. The filter is comprised of two layers of polyethersulfone membrane. The coarser asymmetric upstream membrane layer provides built in prefiltration for the finer downstream sterilizing membrane layer.

The 25 cm (10 in.) AB-style filter is manufactured using 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 EKV filters under standard test conditions. The following Pall filter part numbers have been tested during this study: AB1EKV7PH4, AB05EKV2PH4, MCY4440EKVPH4, KA3EKVP*S/G, KA2EKVP*G, KA1EKVP1 and KA02EKVP2.

The G-designation in the part number indicates that the filter capsules can be sterilized by the user either by autoclave or by gamma-irradiation using doses up to 50 kGy. An S-designation in the product part number indicates that the filter capsule is provided pre-sterilized by gamma irradiation.

The qualification program included:

- Microbial validation tests
- Endurance to in-line steam and autoclave sterilization
- Determination of water flow characteristics
- Extractables testing using water and ethanol
- Biological reactivity tests

Note: The units of pressure quoted in this document are bar and pounds force per square inch (psi). The following figures can be used to convert these units of pressure to Pascals (Pa):

- 1 bar = 1 x 10^5 Pa
- 1 psi = 6.89476 x 10^3 Pa

1.2 Summary of Conclusions

1.2.1 Microbial Validation Tests

Supor EKV 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 EKV filters, and test parameters have been set as follows for 25 cm (10 in.) filters (part number AB1EKV7PH4):

Table 1
Forward Flow Integrity Test Parameters for 25 cm (10 in.) Filter Elements, Part Number AB1EKV7PH4

Test Parameters	
Test pressure	2760 mbar (40 psi)
Wetting liquid	Water
Temperature	20 °C ± 5 °C
Test gas	Air
Maximum allowable Forward Flow limit*	17 mL/min

During the test period the temperature of the filter assembly should not vary more than ± 1 °C.

Forward Flow integrity test values have also been set for other filter styles incorporating Supor EKV filter membrane, as shown in the Table 2: Forward Flow Integrity Test Parameters for Other Styles of Supor EKV Filters. Typical filters from production were subjected to Forward Flow and bacterial challenge tests, demonstrating that filters that pass the Forward Flow test also produce sterile effluent.

Table 2

Forward Flow Integrity Test Parameters for Other Styles of Supor EKV Filters

Pall Filter Part Number	Wetting Liquid*	Air Test Pressure	Maximum Allowable Forward Flow Limit Value**
AB05EKVP	Water	2760 mbar (40 psi)	7.5 mL
MCY4440EKVP	Water	2760 mbar (40 psi)	4.2 mL
KA3EKVP	Water	2760 mbar (40 psi)	4.2 mL
KA2EKVP	Water	2760 mbar (40 psi)	2.1 mL
KA1EKVP	Water	2760 mbar (40 psi)	1.1 mL
KA02EKVP	Water	2760 mbar (40 psi)	0.58 mL

** Temperature 20 °C ± 5 °C*

*** During the test period the temperature of the filter assembly should not vary by more than ± 1 °C.*

1.2.2 Endurance to In-Line Steam Sterilization

Supor EKV filters have been demonstrated to be capable of withstanding multiple in-line steam/autoclave sterilization cycles.

The data presented in this report support the following product claims for in-line steaming/autoclaving of Supor EKV filter cartridges and capsules:

Table 3

Product Claims for In-line Steaming/Autoclaving Supor EKV Filter Cartridges and Capsules

Part Number	Steam/Autoclave Conditions	Maximum Recommended Steam Life Claim
AB1EKV7PH4 AB05EKV2PH4 MCY4440EKVPH4	In-line steam cycles at 125 °C	30 x one-hour cycles
AB1EKV7PH4	In-line steam cycles at 142 °C	5 x one-hour cycles
Non-irradiated G-option Kleenpak™ filters: KA3EKVP*G KA2EKVP*G KA1EKVP*G	One hour, slow exhaust autoclave cycles at 125 °C	5 x one-hour cycles

All of the claims above are supported by data with a 100% safety margin.

Note:
Supor EKV filters must be wetted prior to steam or autoclave exposure.

1.2.3 Determination of Water Flow Characteristics

Differential pressure measurements at set water flow rates have been determined for the following Supor EKV filter part numbers: AB1EKV7PH4, AB05EKV2PH4, MCY4440EKVPH4, KA3EKVP1, KA3EKVP6, KA2EKVP1, KA1EKVP1G and KA02EKVP2.

These data can be used to assist users in sizing filter systems employing Supor EKV filters.

1.2.4 Extractables Testing using Water and 96% Ethanol

The levels of aqueous and ethanol extractables were determined for the following Supor EKV filter part numbers: AB1EKV7PH4, AB05EKV2PH4, MCY4440EKVPH4, irradiated and non-irradiated KA3EKVP1G, KA2EKVP1G and KA1EKVP1G.

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

1.2.5 Biological Reactivity Tests

All of the materials used in Supor EKV filters meet the specifications for Biological Reactivity Tests, *in vivo,* listed in the current revision of the United States Pharmacopeia (USP) for Class VI-121 °C Plastics. The tests included the systemic injection test, the intracutaneous test and the implantation test.

2. Microbial Validation Tests for 25 cm (10 in.) Supor EKV Filter Cartridges

2.1 Introduction

The Food and Drug Administration (FDA) guidelines on Sterile Products Produced by Aseptic Processing (1987) state: "A sterilizing filter is one which, when challenged with the micro-organism *Brevundimonas diminuta* (*B.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 diminuta* (ATCC 19146) were performed with Supor EKV filter cartridges using a minimum of 1 x 10^7 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:

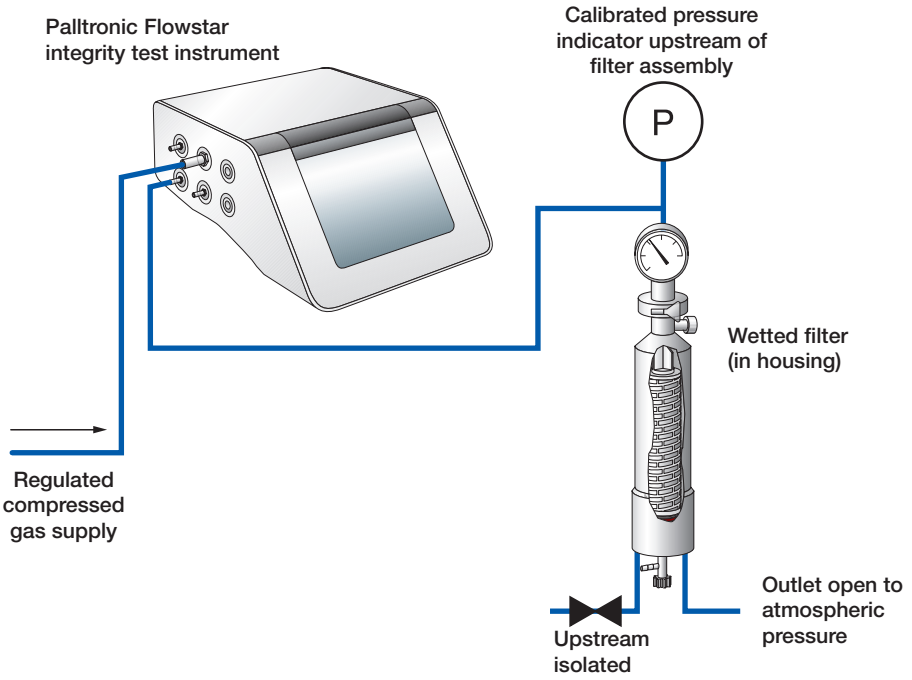
"After a filtration process is properly validated for a given product, process, and filter, it is important to assure that identical filter replacements (membrane or cartridge) used in production runs will perform in the same manner. One way of achieving this is to correlate filter performance data with filter integrity testing data." The integrity test used during this study was the Forward Flow test.

2.1.1 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 upstream side, using sensitive flow measurement equipment such as the Palltronic® Flowstar filter integrity test instrument (Figure 1).

Figure 1

Automated Forward Flow Integrity Test



Forward Flow integrity test parameters have been set for Supor EKV filters based on those set previously for Supor EBV filters as both filter types incorporate the same downstream 0.2 µm rated membrane layer (reference Pall publication USTR 2175: Validation Guide for Supor EBV Filter Cartridges) and other supporting data, as shown in Table 4: Forward Flow Integrity Test Parameters.

Table 4

Forward Flow Integrity Test Parameters

Test Parameters	
Test pressure	2760 mbar (40 psi)
Wetting liquid	Water
Temperature	20 °C ± 5 °C
Test gas	Air
Maximum allowable Forward Flow limit*	17 mL/min

** During the test period the temperature of the filter assembly should not vary more than ± 1 °C.*

The aim of this study was to measure the microbial removal efficiency of typical Supor EKV filters in liquid challenge tests using *Brevundimonas diminuta* (ATCC 19146).

2.2 Summary of Methods

Typical Supor EKV filters, part number AB1EKV7PH4, 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, flushed with DI water at a flow rate of 4 L/min for 10 minutes, and then autoclaved at 121 °C for 60 minutes. A Forward Flow integrity test was then performed using a Palltronic Flowstar integrity test instrument 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: Microbial Challenge Apparatus.

An aqueous suspension of *Brevundimonas diminuta* was passed through the filter to achieve a challenge level of > 1 x 10^7 colony forming units (CFU) per cm^2 of effective filtration area.

During the challenge test the entire filter effluent was passed through a 0.2 µm-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 titer reduction (T_R) for each filter was determined as follows:

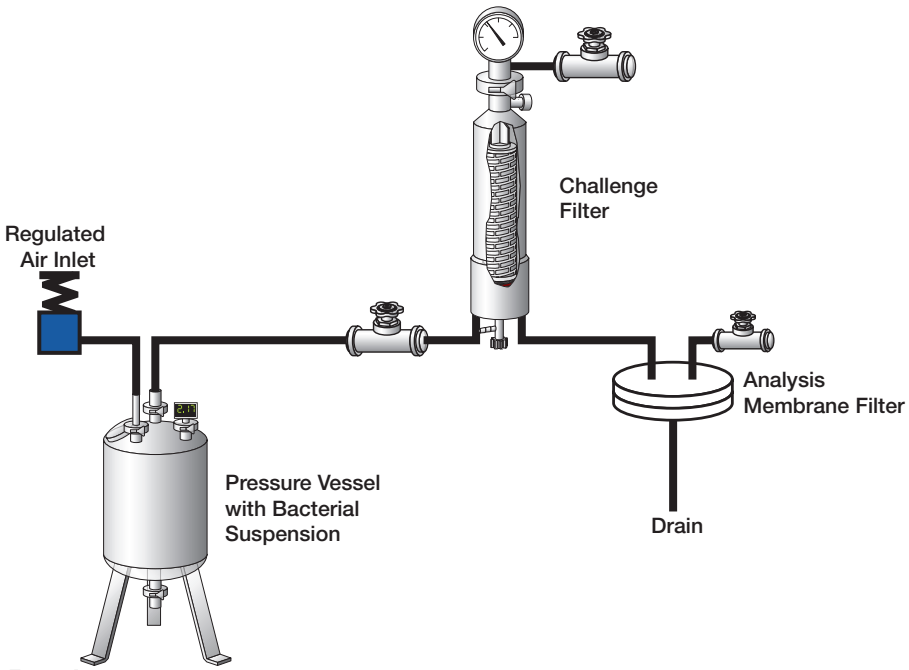
$$T_R = \frac{\text{Total number of organisms influent to the filter}}{\text{Number of colonies recorded on the downstream analysis disc}}$$

When no colonies were detected downstream, the titer reduction was expressed as:

> Total number of organisms influent to the filter (e.g. >1 x 10^{10})

on completion of the challenge test, the filter assemblies were autoclaved and then flushed and Forward Flow integrity tested as described in Section 2.1.

Figure 2
Microbial Challenge Apparatus



2.3 Results

The Forward Flow and *Brevundimonas diminuta* retention results are shown in Table 5. 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 < 14.2 mL/min gave sterile effluent when challenged with > 1 x 10^7 CFU per cm^2 of filtration area using *Brevundimonas diminuta*.

Table 5

Results of Forward Flow and Brevundimonas diminuta Retention for Typical Supor EKV Filters (Part Number AB1EKV7PH4)

Serial Number	Forward Flow (mL/min)*	Sterile Effluent	Titer Reduction
PB972042	8.9	Yes	$> 1.87 \times 10^{11}$
PB972029	9.2	Yes	$> 1.99 \times 10^{11}$
PB971048	9.4	Yes	$> 1.58 \times 10^{11}$
PB972057	9.7	Yes	$> 4.29 \times 10^{11}$
PB986046	9.8	Yes	$> 2.45 \times 10^{11}$
PB986050	9.8	Yes	$> 2.88 \times 10^{11}$
PB971060	10.0	Yes	$> 1.97 \times 10^{11}$
PB971028	10.0	Yes	$> 2.23 \times 10^{11}$
PB972008	10.0	Yes	$> 1.54 \times 10^{11}$
PB971052	10.1	Yes	$> 2.15 \times 10^{11}$
PB972018	10.1	Yes	$> 1.96 \times 10^{11}$
PB972035	10.1	Yes	$> 2.49 \times 10^{11}$
PB971040	10.5	Yes	$> 1.80 \times 10^{11}$
PB986006	10.7	Yes	$> 1.86 \times 10^{11}$
PB971011	10.8	Yes	$> 1.85 \times 10^{11}$
PB986030	10.8	Yes	$> 1.78 \times 10^{11}$
PB986012	12.2	Yes	$> 2.97 \times 10^{11}$
PB971015	12.2	Yes	$> 1.85 \times 10^{11}$
PB971056	12.4	Yes	$> 1.68 \times 10^{11}$
PB986028	14.2	Yes	$> 1.79 \times 10^{11}$

** Forward Flow values at 2760 mbar (40 psi) air test pressure, wet with water, temperature 20 °C ± 5 °C, maximum allowable limit value 17 mL/min.*

2.4 Conclusions

Typical Supor EKV filters from production, part number AB1EKV7PH4 were found to pass the Forward Flow integrity test, and all 20 of the filters tested provided sterile effluent when subjected to aqueous microbial challenge tests using *Brevundimonas diminuta* at a challenge level of $> 1 \times 10^7$ CFU/cm^2.

3. Microbial Validation of Other Styles of Supor EKV Filters

3.1 Introduction

Based on the integrity test parameters set for 254 mm (10 in.) Supor EKV filters (Table 4), Forward Flow integrity test parameters were set for other filter styles incorporating Supor EKV filter medium. The purpose of this series of tests was to perform Forward Flow and bacterial challenge tests on typical filters from production to demonstrate that filters passing the Forward Flow test provided sterile filtrate during challenge tests.

3.2 Summary of Methods

Forward Flow integrity test parameters were set for other filter styles incorporating Supor EKV filter medium as shown in Table 6.

Table 6

Forward Flow Integrity Test Parameters for Other Styles of Supor EKV Filters

Part Number	Wetting Liquid*	Air Test Pressure	Maximum allowable Forward Flow Limit Value**
AB05EKVP	Water	2760 mbar (40 psi)	7.5 mL
MCY4440EKVP	Water	2760 mbar (40 psi)	4.2 mL
KA3EKVP	Water	2760 mbar (40 psi)	4.2 mL
KA2EKVP	Water	2760 mbar (40 psi)	2.1 mL
KA1EKVP	Water	2760 mbar (40 psi)	1.1 mL
KA02EKVP	Water	2760 mbar (40 psi)	0.58 mL

** Temperature 20 °C +5 °C*

*** During the test period, the temperature of the filter assembly should not vary by more than ± 1 °C.*

In order to validate the integrity test limit values, production samples of these filter styles were subjected to Forward Flow and liquid bacterial challenge tests using *Brevundimonas diminuta* according to the procedures described previously in Section 2: Microbial Validation Tests for 25 cm (10 in.) Supor EKV Filter Cartridges.

3.3 Results

The bacterial challenge and Forward Flow results are shown in Table 7. The Forward Flow values that are presented are the higher of the pre- and post-challenge measurements. All of the filters that were tested gave sterile filtrate when challenged.

Table 7

Results of Forward Flow and Brevundimonas diminuta Retention for Typical Supor EKV Filters

Part Number	Serial Number	Forward Flow* (mL/min)	Sterile Effluent	Titer Reduction
AB05EKV2PH4	IG5779099	4.3	Yes	$> 1.13 \times 10^{11}$
	IG5779010	4.3	Yes	$> 1.00 \times 10^{11}$
	IG5779023	4.4	Yes	$> 1.10 \times 10^{11}$
	IG5779050	4.4	Yes	$> 1.26 \times 10^{11}$
	IG5779034	4.6	Yes	$> 1.22 \times 10^{11}$
	IG5779056	4.8	Yes	$> 1.19 \times 10^{11}$
	IG5779066	5.2	Yes	$> 1.11 \times 10^{11}$
	IG5779108	5.2	Yes	$> 1.13 \times 10^{11}$
	IG7030024	5.4	Yes	$> 3.59 \times 10^{10}$
	IG7030032	5.8	Yes	$> 1.09 \times 10^{11}$
	IG7030052	6.1	Yes	$> 1.43 \times 10^{11}$
	IG7030091	6.1	Yes	$> 1.58 \times 10^{11}$
	IG7030043	6.2	Yes	$> 1.27 \times 10^{11}$
	IG7030049	6.9	Yes	$> 1.27 \times 10^{11}$
	IG7030033	7.0	Yes	$> 1.52 \times 10^{11}$
	IG7030035	7.0	Yes	$> 1.56 \times 10^{11}$
	IG7030050	7.1	Yes	$> 1.53 \times 10^{11}$
	IG5779089	7.3	Yes	$> 1.08 \times 10^{11}$
	IG7030014	7.3	Yes	$> 3.54 \times 10^{10}$
	IG5779044	7.4	Yes	$> 9.85 \times 10^{10}$

Table 7 *Continued*

Results of Forward Flow and Brevundimonas diminuta Retention for Typical Supor EKV Filters

Part Number	Serial Number	Forward Flow* (mL/min)	Sterile Effluent	Titer Reduction
MCY4440EKVPH4	IG1088006	1.7	Yes	$> 1.27 \times 10^{11}$
	IG1088008	2.1	Yes	$> 9.07 \times 10^{10}$
	IG1088007	2.2	Yes	$> 1.20 \times 10^{11}$
	IG1088004	2.4	Yes	$> 3.90 \times 10^{10}$
	IG1088003	2.8	Yes	$> 7.84 \times 10^{10}$
	IG1088001	4.5	Yes	$> 6.67 \times 10^{10}$
KA3EKVP6G	IG51870031	2.8	Yes	$> 6.85 \times 10^{10}$
	IG51870004	2.9	Yes	$> 8.30 \times 10^{10}$
	IG51870060	2.9	Yes	$> 1.08 \times 10^{11}$
	IG51870010	3.0	Yes	$> 8.95 \times 10^{10}$
	IG51870012	3.0	Yes	$> 9.25 \times 10^{10}$
KA3EKVP6G	IG51870064	3.1	Yes	$> 8.50 \times 10^{10}$
	IG51870036	3.1	Yes	$> 9.70 \times 10^{10}$
	IG51870019	3.1	Yes	$> 9.90 \times 10^{10}$
	IG51870052	3.5	Yes	$> 7.70 \times 10^{10}$
	IG51870009	3.6	Yes	$> 9.75 \times 10^{10}$
KA3EKVP1G	IG47000054	2.7	Yes	$> 6.18 \times 10^{10}$
	IG47000010	2.8	Yes	$> 3.59 \times 10^{10}$
	IG47000063	2.8	Yes	$> 6.17 \times 10^{10}$
	IG47000035	2.9	Yes	$> 5.50 \times 10^{10}$
	IG47000057	3.1	Yes	$> 6.34 \times 10^{10}$
	IG47000031	3.2	Yes	$> 3.47 \times 10^{10}$
	IG47000083	3.2	Yes	$> 7.85 \times 10^{10}$
	IG47000034	3.7	Yes	$> 4.37 \times 10^{10}$
	IG47000095	4.0	Yes	$> 7.95 \times 10^{10}$
	IG47000108	4.0	Yes	$> 8.10 \times 10^{10}$
KA3EKVP1S	IG46990067	2.6	Yes	$> 8.05 \times 10^{10}$
	IG46990005	2.8	Yes	$> 9.05 \times 10^{10}$
	IG46990008	2.8	Yes	$> 7.40 \times 10^{10}$
	IG46990035	2.8	Yes	$> 7.60 \times 10^{10}$
	IG46990001	2.9	Yes	$> 7.65 \times 10^{10}$
	IG46990011	2.9	Yes	$> 7.90 \times 10^{10}$
	IG46990108	3.0	Yes	$> 6.75 \times 10^{10}$
	IG46990070	3.1	Yes	$> 8.60 \times 10^{10}$
	IG46990059	3.2	Yes	$> 8.05 \times 10^{10}$
	IG46990088	3.9	Yes	$> 8.70 \times 10^{10}$
	IG46990014	2.6	Yes	$> 7.65 \times 10^{10}$
	IG46990038	2.7	Yes	$> 7.20 \times 10^{10}$
	IG46990002	2.8	Yes	$> 6.67 \times 10^{10}$
	IG46990063	2.8	Yes	$> 7.85 \times 10^{10}$
	IG46990077	2.8	Yes	$> 7.90 \times 10^{10}$

Table 7 *Continued*

Results of Forward Flow and Brevundimonas diminuta Retention for Typical Supor EKV Filters

Part Number	Serial Number	Forward Flow* (mL/min)	Sterile Effluent	Titer Reduction
KAEKVP1S	IG46990097	2.8	Yes	$> 8.30 \times 10^{10}$
	IG46990049	2.9	Yes	$> 8.35 \times 10^{10}$
	IG46990040	3.1	Yes	$> 7.80 \times 10^{10}$
	IG46990050	3.1	Yes	$> 7.00 \times 10^{10}$
	IG46990053	3.6	Yes	$> 7.10 \times 10^{10}$
KA2EKVP16G	IH18030012	1.4	Yes	$> 2.22 \times 10^{10}$
	IH18030017	1.4	Yes	$> 1.61 \times 10^{10}$
	IH18030059	1.4	Yes	$> 2.10 \times 10^{10}$
	IH18030080	1.4	Yes	$> 2.17 \times 10^{10}$
	IH18030101	1.4	Yes	$> 2.37 \times 10^{10}$
	IH18030119	1.4	Yes	$> 2.90 \times 10^{10}$
	IH18030053	1.5	Yes	$> 1.77 \times 10^{10}$
	IH18030125	1.5	Yes	$> 2.84 \times 10^{10}$
	IH18030070	1.6	Yes	$> 2.77 \times 10^{10}$
	IH18030092	1.6	Yes	$> 1.94 \times 10^{10}$
KA2EKVP1G	IH18020077	1.4	Yes	$> 2.75 \times 10^{10}$
	IH18020049	1.4	Yes	$> 2.80 \times 10^{10}$
	IH18020111	1.4	Yes	$> 3.62 \times 10^{10}$
	IH18020114	1.4	Yes	$> 2.44 \times 10^{10}$
	IH18020122	1.4	Yes	$> 2.77 \times 10^{10}$
	IH18020020	1.5	Yes	$> 2.89 \times 10^{10}$
	IH18020071	1.5	Yes	$> 3.10 \times 10^{10}$
	IH18020072	1.5	Yes	$> 2.75 \times 10^{10}$
	IH18020099	1.5	Yes	$> 4.15 \times 10^{10}$
	IH18020076	1.9	Yes	$> 3.10 \times 10^{10}$
KA1EKVP1G	IJ74410052	0.7	Yes	$> 2.49 \times 10^{10}$
	IJ74410048	0.7	Yes	$> 2.32 \times 10^{10}$
	IG2350151	0.7	Yes	$> 2.65 \times 10^{10}$
	IG2350068	0.7	Yes	$> 2.52 \times 10^{10}$
	IJ74410062	0.7	Yes	$> 2.49 \times 10^{10}$
	IJ74410203	0.7	Yes	$> 2.57 \times 10^{10}$
	IJ74410011	0.7	Yes	$> 2.32 \times 10^{10}$
	IJ74410147	0.7	Yes	$> 2.73 \times 10^{10}$
KA1EKVP1G	IJ74410079	0.7	Yes	$> 2.44 \times 10^{10}$
	IJ74410125	0.8	Yes	$> 2.44 \times 10^{10}$
	IJ74410161	0.8	Yes	$> 2.73 \times 10^{10}$
	IJ74410185	0.8	Yes	$> 2.57 \times 10^{10}$
	IG2349022	0.8	Yes	$> 2.94 \times 10^{10}$
	IG2349081	0.8	Yes	$> 2.42 \times 10^{10}$
	IG2349098	0.8	Yes	$> 3.02 \times 10^{10}$
	IG2349110	0.8	Yes	$> 2.90 \times 10^{10}$

Table 7 *Continued*

Results of Forward Flow and Brevundimonas diminuta Retention for Typical Supor EKV Filters

Part Number	Serial Number	Forward Flow* (mL/min)	Sterile Effluent	Titer Reduction
KA1EKVP1G	IG2349127	0.8	Yes	$> 2.79 \times 10^{10}$
	IG2350008	0.8	Yes	$> 2.55 \times 10^{10}$
	IG2358079	0.8	Yes	$> 3.04 \times 10^{10}$
	IG2350090	0.8	Yes	$> 2.97 \times 10^{10}$
	IJ74410057	1.5	Yes	$> 1.89 \times 10^{10}$
KA02EKVP2	IF9496024	0.4	Yes	$> 4.30 \times 10^{10}$
	IF9496084	0.4	Yes	$> 4.25 \times 10^{10}$
	IF9496027	0.5	Yes	$> 5.35 \times 10^{10}$
	IF9496033	0.5	Yes	$> 5.35 \times 10^{10}$
	IF9496044	0.6	Yes	$> 5.45 \times 10^{10}$
	IF9496017	0.5	Yes	$> 4.30 \times 10^{10}$
	IF9496088	0.5	Yes	$> 4.25 \times 10^{10}$
	IF9496068	0.5	Yes	$> 5.45 \times 10^{10}$
	IF9496078	0.6	Yes	$> 4.90 \times 10^{10}$

** Filters wet with water and Forward Flow measured using an air test pressure of 2760 mbar (40 psi), temperature 20 °C ± 5 °C.*

3.4 Conclusions

Based on the validation of the 25 cm (10 in.) Supor EBV filter cartridges, part number AB1EKV7PH4, Forward Flow integrity test values have been set for various other filter styles incorporating Supor EKV filter membrane. Typical filters from production were subjected to Forward Flow and bacterial challenge tests, demonstrating that filters that pass the Forward Flow test also produce sterile effluent.

4. Endurance to In-line Steam and Autoclave Sterilization

4.1 Introduction

The purpose of these tests was to determine the effects of repeated exposure to in-line steam or autoclave cycles on filter integrity using standard Supor EKV filters from production. The tests were used to qualify the steam/autoclave exposure claims for Supor EKV filter cartridges and capsules.

4.2 Summary of Methods

4.2.1 In-line Steam Resistance Testing of Supor EKV Filter Cartridges

Steaming at 125 °C

Typical Supor EKV filters from production (part numbers AB1EKV7PH4, AB05EKV2PH4 and MCY4440EKVPH4) were used for the tests. The filters were flushed with DI water for 10 – 20 minutes with a back pressure of 2 bar (30 psi) 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 1 bar (14.5 psi).

Immediately after each one-hour 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 60 one-hour steam cycles.

On completion of the sixty steam cycles, the filters were flushed with DI water and Forward Flow integrity tested again as described in Section 2.1: Introduction.

Steaming at 142 °C

In addition to the tests performed at 125 °C, typical Supor EKV filters from production (part number AB1EKV7PH4) were also exposed to in-line steam cycles at 142 °C. The filters were wetted and tested prior to steam exposure as described above and then the filters were subjected to a total of ten one-hour steam cycles. The filters were air-cooled for 30 minutes between each steam cycle and the filters were re-wetted prior to the start of each new cycle.

4.2.2 Autoclave Resistance Testing of Supor EKV Filter Capsules

Typical G-option Supor EKV filter capsules from production (part numbers KA3EKVP*G, KA2EKVP*G and KA1EKVP1G) were Forward Flow integrity tested water-wet at 2760 mbar (40 psi) and then exposed to ten slow-exhaust one-hour autoclave cycles at 125 °C. On completion of the autoclave cycles, the filter capsules were integrity tested again.

4.3 Results

4.3.1 Results of In-line Steam Resistance Testing of Supor EKV Filter Cartridges

Steaming at 125 °C

The Forward Flow integrity test results for Supor EKV filters (part numbers AB1EKV7PH4, AB05EKV2PH4 and MCY4440EKVPH4) before and after exposure to one-hour in-line steam cycles are shown in Table 8: Effects of In-Line Steam Exposure at 125 °C on Filter Integrity for Supor EKV Filters. All of the filters retained integrity following exposure to 60 one-hour cycles at 125 °C.

Table 8

Effects of In-Line Steam Exposure at 125 °C on Filter Integrity for Supor EKV Filters

Part Number	Number	Forward Flow* (mL/min) after exposure to following number of steam cycles at 125 °C			
		0 cycles	20 cycles	40 cycles	60 cycles
AB1EKV7PH4	PB972002	13.4	ND**	ND	10.5
	PB972009	9.8	ND	ND	11.3
	PB972019	15.4	ND	ND	9.6
	PB972046	9.5	ND	ND	12.9
	PB971043	11.8	ND	ND	12.2
	PB971038	12.8	ND	ND	11.2
	PB971014	11.2	ND	ND	11.2
	PB986014	12.8	ND	ND	10.6
	PB986043	10.2	ND	ND	9.7
	PB971054	11.5	ND	ND	12.1

Table 8 *Continued*

Effects of In-Line Steam Exposure at 125 °C on Filter Integrity for Supor EKV Filters

Part Number	Serial Number	Forward Flow* (mL/min) after exposure to following number of steam cycles at 125 °C			
		0 cycles	20 cycles	40 cycles	60 cycles
AB05EKV2PH4	IG5779030	4.9	6.5	4.2	7.2
	IG7030002	4.4	6.9	5.0	4.9
	IG7030042	4.2	4.0	5.5	4.4
	IG7030003	3.1	5.6	6.4	4.6
	IG5779015	5.0	5.8	4.9	4.8
MCY4440EKVPH4	IG5487105	2.8	3.9	3.5	3.6
	IG5487039	4.1	3.4	3.3	3.9
	IG5487112	3.5	3.6	3.8	3.8
	IG5487074	3.1	3.6	3.4	3.7
	IG5487103	3.0	3.4	3.6	3.5

** Forward Flow values determined at 2760 mbar (40 psi) air test pressure, wet with water, temperature 20 °C ± 5 °C.*

*** ND = Not Determined.*

Steaming at 142 °C

The Forward Flow integrity test results for Supor EKV filters (part number AB1EKV7PH4) before and after exposure to one-hour in-line steam cycles are shown in Table 9. All of the filters retained integrity following exposure to ten one-hour cycles at 142 °C.

Table 9

Effects of In-Line Steam Exposure at 142 °C on Filter Integrity for Supor EKV Filters, Part Number AB1EKV7PH4

Serial Number	Forward Flow* (mL/min) after following number of steam cycles at 142 °C	
	0 cycles	10 cycles
PB971020	9.5	11.3
PB971023	10.5	12.1
PB972001	11.1	11.3
PB972012	14.5	10.5
PB986044	15.8	10.9

** Forward Flow values determined at 2760 mbar (40 psi) air test pressure, wet with water, temperature 20 °C ± 5, maximum allowable limit value is 17 mL/min.*

4.3.2 Results of Autoclave Resistance Testing of Supor EKV Filter Capsules

The Forward Flow integrity test results for G-option Supor EKV filters (part numbers KA3EKVP*G, KA2EKVP*G and KA1EKVP1G) before and after exposure to one-hour slow-exhaust autoclave cycles are shown in Table 10: Effects of Autoclave Exposure at 125 °C on Filter Integrity for non-irradiated G-option Supor EKV Kleenpak Filter Capsules. All of the filters retained integrity following exposure to ten one-hour cycles at 125 °C.

Table 10

Effects of Autoclave Exposure at 125 °C on Filter Integrity for non-irradiated G-option Supor EKV Kleenpak Filter Capsules

		Forward Flow* (mL/min) after following number of autoclave cycles at 125 °C	
Part Number	**Serial Number**	**0 cycles**	**10 cycles**
KA3EKVP6G	IG47000011	2.4	2.3
	IG47000016	2.3	2.5
	IG47000025	2.4	2.5
	IG47000041	2.7	2.7
	IG47000068	2.5	3.1
	IG47000069	2.4	2.4
	IG47000070	2.5	3.0
KA3EKVP6G	IG47000073	2.6	2.9
	IG47000104	2.4	2.7
	IG47000106	2.6	3.0
	IG51870007	2.5	2.8
	IG51870018	2.5	2.8
	IG51870027	2.7	2.7
	IG51870029	2.9	2.7
	IG51870038	2.7	2.8
	IG51870039	3.2	2.8
	IG51870042	2.4	2.7
	IG51870051	2.8	3.1
	IG51870063	3.0	3.0
	IG51870065	2.8	2.7
KA2EKVP1G	IH18020093	1.3	1.2
	IH18020048	1.4	1.4
	IH18020063	1.3	1.2
	IH18020064	1.4	1.2
	IH18020051	1.3	1.4
	IH18020126	1.3	1.0
	IH18020012	1.1	1.3
	IH18020110	1.4	1.2
	IH18020091	1.4	1.2
	IH18020060	1.1	1.2
	IH18030110	1.3	1.4
	IH18030115	1.3	1.2
	IH18030062	1.4	1.3
	IH18030034	1.3	1.3
	IH18030105	1.8	1.4
	IH18030107	1.2	1.0
	IH18030014	1.3	1.4
	IH18030050	1.1	1.3

Table 10 *Continued*

Effects of Autoclave Exposure at 125 °C on Filter Integrity for non-irradiated G-option Supor EKV Kleenpak Filter Capsules

		Forward Flow* (mL/min) after following number of autoclave cycles at 125 °C	
Part Number	**Serial Number**	**0 cycles**	**10 cycles**
KA2EKVP16G	IH18030031	1.5	1.4
	IH18030100	1.2	1.3
KA1EKVP1G	IJ74410063	0.8	0.6
	IJ74410085	0.7	0.7
	IJ74410087	1.0	0.6
	IJ74410091	0.8	0.7
	IJ74410129	0.8	1.0
	IJ74410133	0.8	0.7
	IJ74410143	0.8	0.7
	IJ74410168	0.8	0.7
	IJ74410186	0.8	0.7
	IJ74410243	0.8	0.6

** Forward Flow values determined at 2760 mbar (40 psi) air test pressure, wet with water, temperature 20 °C ± 5 °C.*

4.4 Conclusions

Supor EKV filters have been demonstrated to be capable of withstanding multiple in-line steam/autoclave sterilization cycles.

The data presented in this section support the following product claims for in-line steaming/autoclaving Supor EKV filter cartridges and capsules:

Table 11

In-line Steaming and Autoclaving

Part Number	**Steam/Autoclave Conditions**	**Maximum Recommended Steam Life Claim**
AB1EKV7PH4 AB05EKV2PH4 MCY4440EKVPH4	In-line steam cycles at 125 °C	30 x one-hour cycles
AB1EKV7PH4	In-line steam cycles at 142 °C	5 x one-hour cycles
Non-irradiated G-option Kleenpak filters		
KA3EKVP*G KA2EKVP*G KA1EKVP*G	One hour, slow exhaust autoclave cycles at 125 °C	5 x one-hour cycles

All of the claims above are supported by data with a 100% safety margin.

Note:
Supor EKV filters must be wetted prior to steaming.

5. Determination of Water Flow Characteristics

5.1 Introduction

The aim of these tests was to determine the typical differential pressure measurements across Supor EKV filter cartridges and capsules at set water flow rates.

5.2 Summary of Methods

The tests were performed on standard production filters. The following Pall filter part numbers were tested: AB1EKV7PH4, AB05EKV2PH4, MCY4440EKVPH4, KA3EKVP1, KA3EKVP6, KA2EKVP1, KA1EKVP1 and KA02EKVP2. A minimum of two samples of each filter type was tested.

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 flow measurements were taken with the test rig with no filter cartridge or capsule installed so that the pipe work/housing losses could be measured and then subtracted from the filter assembly results.

All data were corrected for a standard temperature of 20 °C.

5.3 Results

The water flow/differential pressure measurements obtained using typical Supor EKV filter cartridges and capsules are shown in Figures 3 to 5.

Figure 3

Water Flow/Differential Pressure Characteristics of Supor EKV Filters, Part Numbers AB1EKV7PH4 and AB05EKV2PH4

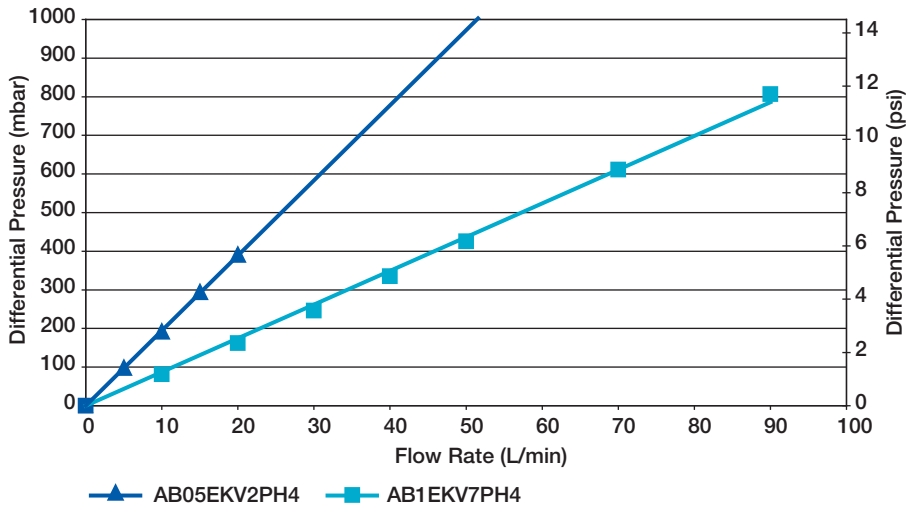


Figure 4

Water Flow/Differential Pressure Characteristics of Supor EKV Filters, Part Numbers MCY4440EKVP, KA3EKVP1, KA3EKVP6, KA2EKVP1 and KA1EKVP1

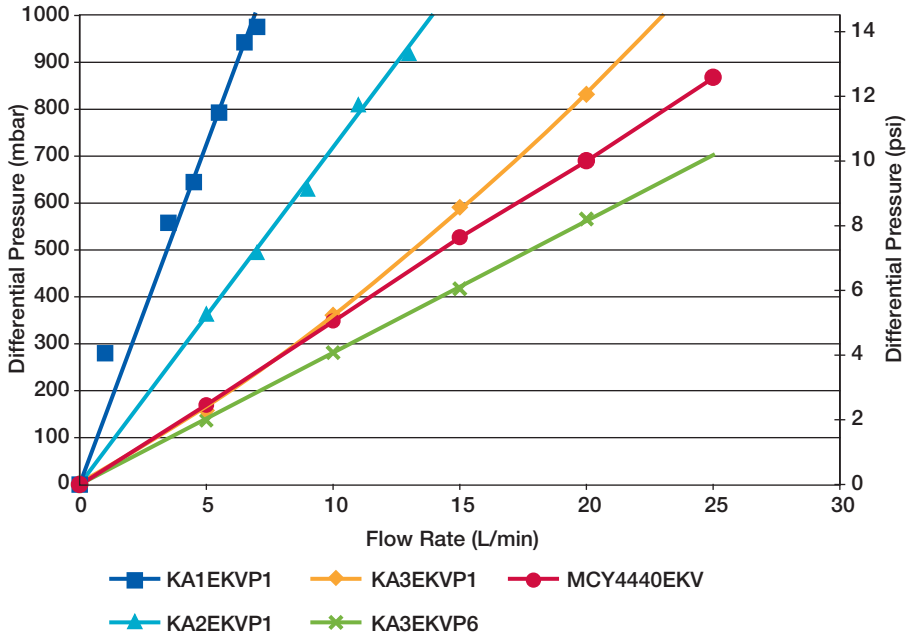
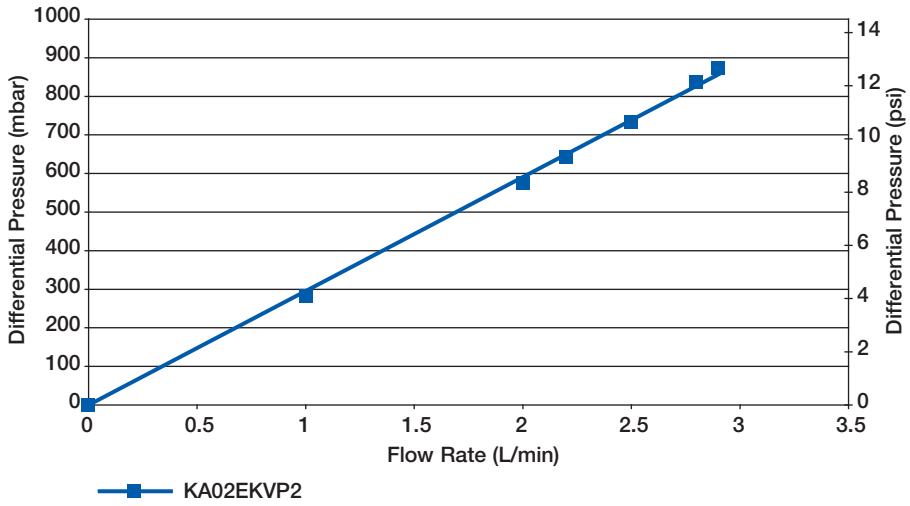


Figure 5

Water Flow/Differential Pressure Characteristics of Supor EKV Filters, Part Number KA02EKVP2



5.4 Conclusions

Differential pressure measurements at set water flow rates have been determined for the following Supor EKV filter part numbers: AB1EKV7PH4, AB05EKV2PH4, MCY4440EKVPH4, KA3EKVP1, KA3EKVP6, KA2EKVP1, KA1EKVP1 and KA02EKVP2.

These data can be used to assist users in sizing filter systems that utilize Supor EKV filters.

6. Extractables Testing Using Water and Ethanol

6.1 Introduction

The aim of this series of tests was to quantify and characterize the material that can be extracted from Supor EKV filter cartridges and capsules using water and 96% ethanol.

6.2 Summary of Methods

Typical production filters were used for the tests and the following Pall filter part numbers were tested: AB1EKV7PH4, AB05EKV2PH4, MCY4440EKVPH4, KA3EKVP1G/S, KA2EKVP1G and KA1EKVP1G.

6.2.1 Preparation of Filter Samples

Prior to the extraction test the filter samples were 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 125 °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.

The pre-sterilized capsules were not autoclaved but tested straight out of their packaging.

6.2.2 Extraction Procedure for Filter Cartridges

For the filter cartridges dynamic extraction tests were performed in a known volume of water or 96% ethanol. The test filters were immersed in the extraction fluid in a clean measuring cylinder, as shown in Figure 6. 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.

Following the extraction period, a measured volume of the extraction liquid was evaporated to dryness and the non-volatile extractables were determined gravimetrically. A correction was made to the NVR value to account for the total extraction volume used.

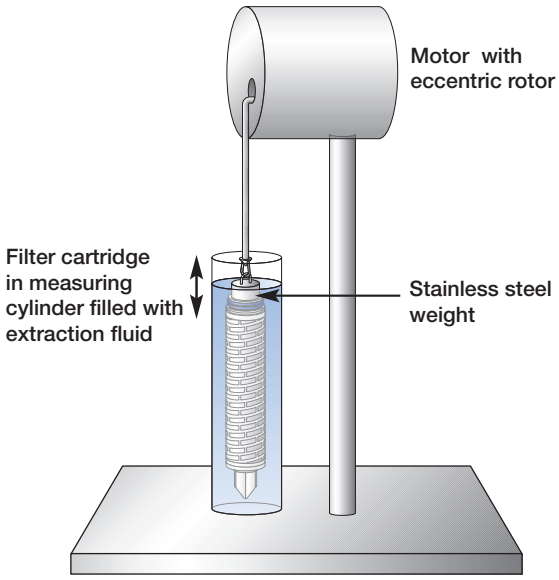
The ethanol extractables were also analyzed by Fourier Transform Infrared Spectroscopy (FTIR).

6.2.3 Extraction Procedure for Filter Capsules

For the filter capsules a known volume of deionized water was re-circulated through each filter for four hours. A measured volume of the extraction fluid was then evaporated to dryness and the weight of non-volatile residue (NVR) extracted from each capsule was calculated. A correction was made to the NVR value to account for the total extraction volume used. The values obtained were then corrected against control water samples that had been recirculated through an identical system but without a filter capsule in place.

Figure 6

Filter Extraction Apparatus



6.3 Results

Table 12 shows the typical levels of aqueous and 96% ethanol extractables obtained from the Supor EKV filters that were tested.

Table 12

Non-volatile Aqueous and Ethanol Extractables Obtained using Supor EKV Filters

Part Number	Extraction Fluid	Serial Number	Non-Volatile Residue (mg)
AB1EKV7PH4	DI water	PB971009	6
		PB971013	4
		PB986010	3
		PB972031	3
	96% ethanol	PB971026	197
		PB971046	197
		PB986053	192
		PB972011	194
AB05EKV2PH4	DI water	IG0030021	16
		IG5779003	24
MCY4440EKVPH4	DI water	Sample 1	7
	96% ethanol	Sample 2	34
KA3EKVP1G	DI water	IG0741179	7
		IG0741004	7
		IG1571072	5
		IG1572111	4
	96% ethanol	IG0741118	44
		IG0741119	47
		IG1571023	59
		IG1572089	60
KA3EKVP1G*	DI water	IG0741111	0
		IG0741108	1

Table 12 *Continued*

Non-volatile Aqueous and Ethanol Extractables Obtained using Supor EKV Filters

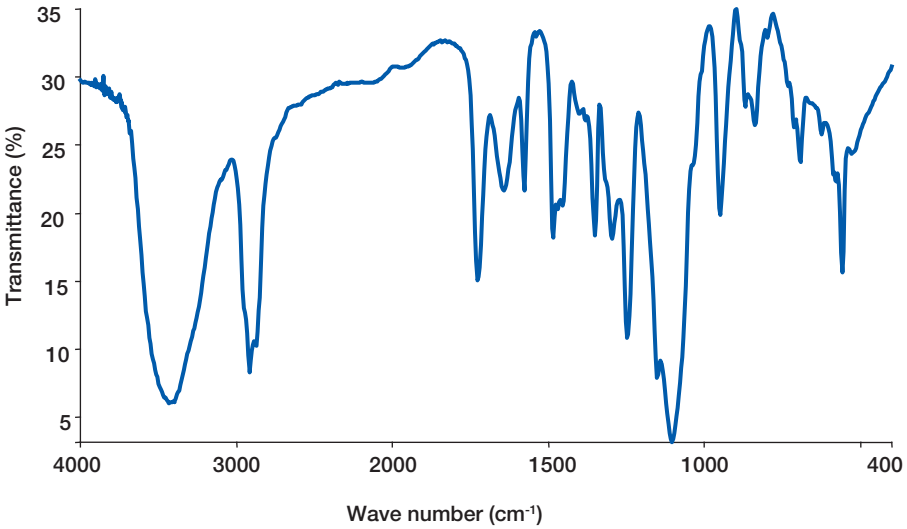
Part Number	Extraction Fluid	Serial Number	Non-Volatile Residue (mg)
KA2EKVP1G	DI water	IH18020088	2
		IH18020016	2
	96% ethanol	IH18030033	2
		IH18030015	2
KA1EKVP1G	DI water	IJ74410149	2.2
		IJ74410174	2.1

** (Samples irradiated to represent 'S' option)*

A typical infrared spectrum (Figure 7) of one of the 96% ethanol extracts from a Supor EKV filter (part number AB1EKV7PH4) indicates the presence of extractables typical of polyethersulfone resins and the copolymer used to render the membrane hydrophilic. The biological reactivity tests performed on the materials used in the construction of Supor EKV filters is described in Section 7: Biological Reactivity Tests on the Materials of Construction of this report.

Figure 7

Infrared Spectrum of the Ethanol Extractables from Supor EKV Filters



6.4 Conclusions

The levels of aqueous and ethanol extractables were determined for the following Supor EKV filter part numbers: AB1EKV7PH4, AB05EKV2PH4, MCY4440EKVPH4, irradiated and non-irradiated KA3EKVP1G, KA2EKVP1G and KA1EKVP1G.

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

7. Biological Reactivity Tests on the Materials of Construction

7.1 Introduction

The aim of this study was to evaluate the biological suitability of the materials of construction of Supor EKV filter cartridges and capsules. The materials of construction of the filters are detailed in Table 13:

Table 13
Materials of Construction

Materials	
Membrane	Pall hydrophilic polyethersulfone membrane
Membrane support and drainage layers	Polypropylene
Core and endcaps	Polypropylene
Filter cage	Polypropylene with titanium dioxide
O-rings	Silicone elastomer for H4 option
Capsule housing (for Kleenpak and Mini Kleenpak filter capsules)	Polypropylene

7.2 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 Pharmacopoeia.

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
- Polyethylene Glycol 400
- 1 in 20 Solution of Alcohol in Sodium Chloride Injection
- 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 filter materials were extracted at 121 °C.

7.2.1 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.

7.2.2 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.

7.2.3 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 materials of the Supor EKV filter was implanted separately.

7.3 Results

No biological response was observed in any of the tests performed and therefore the materials used in Supor EKV filters passed all of the tests specified.

7.4 Conclusions

The materials used in Supor EKV filters met the specifications for Biological Reactivity Tests, *in vivo,* listed in the current revision of the United States Pharmacopeia (USP) for Class VI – 121 °C Plastics.

The tests included the systemic injection test, the intracutaneous test and the implantation test.

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