Life Sciences

Validation Guide

USTR 2237

Validation Guide for Pall® Mini Kleenpak™ Filter Capsules with Emflon® PFR membrane

Filtration. Separation. Solution.SM

CONTENTS

1. Overview

1.1 Introduction

This report contains validation data applicable to **Pall** Mini **Kleenpak** filter capsules incorporating **Emflon** PFR-grade membrane, part number KA02PFRP2.

The validation program included:

- **Correlation of Forward Flow integrity test values with microbial removal using *Brevundimonas diminuta* (ATCC 19146) for filters incorporating Emflon PFR membrane**
- **Liquid microbial challenge tests with typical production Mini Kleenpak PFR filter capsules**
- **Resistance to autoclave sterilization**
- **Determination of air flow/differential pressure characteristics**
- **Biological reactivity tests on the materials of construction**

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 Pascal :

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

1.2 Summary of Conclusions

Microbial Validation Studies

- **A correlation between Forward Flow values and microbial removal has been demonstrated for Emflon PFR grade filters, part number AB1PFR7PVH4.**
- **Forward Flow limit values have been set for Mini Kleenpak PFR filter capsules. These values incorporate a safety margin.**
- **Typical production Mini Kleenpak PFR filters have been subjected to microbial challenge tests using *Brevundimonas diminuta* and all samples tested gave sterile filtrate.**

Resistance to Autoclave Sterilization

Forward Flow integrity tests performed on Mini **Kleenpak** PFR filters demonstrated that the capsules retained integrity after exposure to thirty 30-minute autoclave cycles at 125°C (257°F) and twenty 30-minute autoclave cycles at 135°C (275°F).

Air Flow/Differential Pressure Characteristics

Air flow/differential pressure characteristics of Mini **Kleenpak** PFR filters have been measured on unused filters under atmospheric vent conditions and pressurized air conditions at 0.68 barg (10 psig) and 2 barg (30 psig).

At 100 mbar (1.47 psi) differential pressure, the typical measured air flows were:

- **50 NL/min (1.7 cfm) under vent conditions**
- **60 NL/min (2.1 cfm) at 0.68 barg (10 psig) inlet pressure**
- **90 NL/min (2.8 cfm) at 2 barg (30 psig) inlet pressure**

Biological Reactivity Tests on the Materials of Construction

The components of Mini **Kleenpak** PFR capsules meet the requirements of the USP for Class VI (121°C) Plastics *(in vivo)*. The tests covered the systemic injection test, the intracutaneous test and the implantation test.

2. Microbial Validation Studies

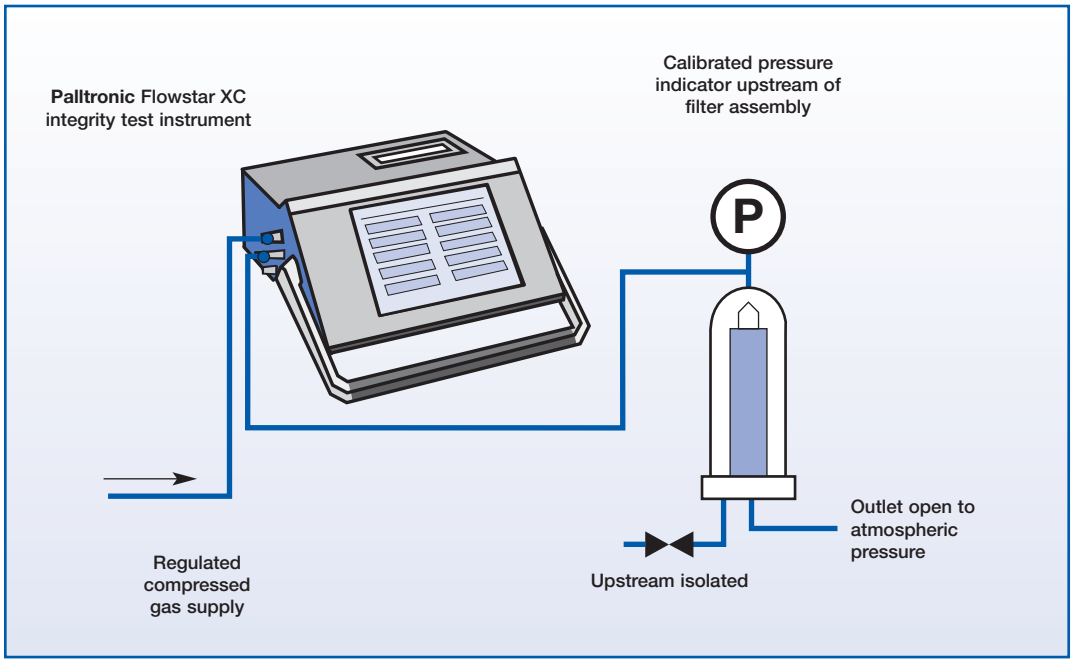
2.1 Correlation Study

2.1.1 Introduction

The correlation between microbial retention and a non-destructive integrity test is an important aspect of the validation of sterilizing grade filters. The FDA Guidelines for Sterile Products Produced by Aseptic Processing (1987) state, "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.

In order to perform the Forward Flow test, a filter is wetted with a suitable 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 XC filter integrity test instrument, as shown in Figure 2-1.

Figure 2-1 The Automated Forward Flow Integrity Test



Forward Flow and liquid challenge tests using *Brevundimonas diminuta* ATCC 19146 have been performed with a range of **Emflon** PFR grade filters, part number AB1PFR7PVH4, using a minimum of 1 x 10^7 colony forming units (CFU) per cm^2 of effective filtration area.

2.1.2 Summary of Results

A correlation between liquid microbial retention and Forward Flow integrity testing has been demonstrated for **Emflon** PFR grade filters, part number AB1PFR7PVH4. This correlation study, performed using liquid microbial challenge tests using *Brevundimonas diminuta* (ATCC 19146) is fully described in Pall publication 'Validation Guide for Pall Emflon PFR Filter Cartridges', contact Pall for details.

The data demonstrating the correlation between microbial challenge and Forward Flow integrity testing are shown in Table 2-1. The data are arranged in order of increasing Forward Flow value.

Table 2-1. Correlation of Forward Flow with *B. diminuta* retention for Emflon PFR filter cartridges (part number AB1PFR7PVH4)

Filter Serial Number	Forward Flow* (mL/min)	Sterile Effluent	Titer Reduction
PILF309054	3.3	Yes	> 1.69×10^{11}
PILF309016	3.3	Yes	> 1.69×10^{11}
PILF309029	3.3	Yes	> 1.69×10^{11}
IA1516018	3.4	Yes	> 1.10×10^{11}
PILF309001	3.4	Yes	> 1.33×10^{11}
PILF309026	3.5	Yes	> 1.69×10^{11}
PILF309022	3.5	Yes	> 1.33×10^{11}
PILF309056	3.5	Yes	> 1.93×10^{11}
PILF309052	3.6	Yes	> 1.69×10^{11}
IA1516067	3.6	Yes	> 1.10×10^{11}
PILF309018	3.6	Yes	> 1.93×10^{11}
IA1516006	3.6	Yes	> 1.10×10^{11}
PILF309015	3.6	Yes	> 1.93×10^{11}
PILF309053	3.7	Yes	> 1.93×10^{11}
PILF309021	3.7	Yes	> 1.33×10^{11}
PILF309030	3.8	Yes	> 1.33×10^{11}
PILF309028	3.8	Yes	> 1.33×10^{11}
IA1516052	3.8	Yes	> 1.10×10^{11}
PILF309050	3.9	Yes	> 1.33×10^{11}
PILF309055	3.9	Yes	> 1.69×10^{11}
PILF309006	4.0	Yes	> 1.93×10^{11}
PILF309002	4.1	Yes	> 1.44×10^{11}
PILF296002	4.1	Yes	> 1.44×10^{11}
PILF309013	4.5	Yes	> 1.93×10^{11}
PILF296035	4.7	Yes	> 1.44×10^{11}
IA1516015	5.7	Yes	> 1.10×10^{11}
PILF309003	5.7	Yes	> 1.44×10^{11}
IA1516002	7.0	No	1×10^{5}
IA2417003	7.5	No	1×10^{5}
PILF296012	10.0	Yes	> 1.51×10^{11}
PILF296013	36.0	No	1.51×10^{6}

* Forward Flow values at 1100 mbar (16 psi) air test pressure, wet with 25% (v/v) tertiary butyl alcohol in water, temperature 20°C ± 5°C (68°F ± 9°F).

Based on the correlation study, Forward Flow test parameters were set for **Pall** AB1-style 254 mm (10") filter cartridges incorporating **Emflon** PFR sterilizing grade membrane. Forward Flow test parameters for Mini **Kleenpak** PFR filter capsules were set based on the difference in membrane area between the AB1-style and the Mini **Kleenpak** configuration. The integrity test parameters are shown in Table 2-2. These Forward Flow values incorporate a safety margin.

Table 2-2 Forward Flow Integrity Test Parameters for AB1 and Mini Kleenpak Filter Styles Incorporating Emflon PFR Filter Medium

Pall Filter Part Number	Wetting Liquid*	Air Test Pressure	Forward Flow Limit**
AB1PFR7PVH4	25% (v/v) tertiary butyl alcohol in water	1100 mbar (16 psi)	5.5 mL/min
KA02PFRP2	25% (v/v) tertiary butyl alcohol in water	1100 mbar (16 psi)	0.21 mL/min
	60% (v/v) isopropanol in water***	1040 mbar (15 psi)	0.57 mL/min

* Temperature 20° ± 5°C (68°F ± 9°F).
** During the test period the temperature of the filter assembly should not vary more than ± 1°C (2°F).
*** Forward Flow test parameters were set for 60% (v/v) isopropanol in water based on the differences in the physical properties of this wetting liquid with 25% (v/v) tertiary butyl alcohol in water.

2.1.3 Conclusions

- **A correlation between Forward Flow integrity test values and microbial removal has been demonstrated for Emflon PFR grade filters**
- **Forward Flow limit values have been set for Mini Kleenpak PFR filter capsules. These values incorporate a safety margin.**

2.2 Microbial Challenge Tests Performed on Typical Mini Kleenpak PFR Filter Capsules

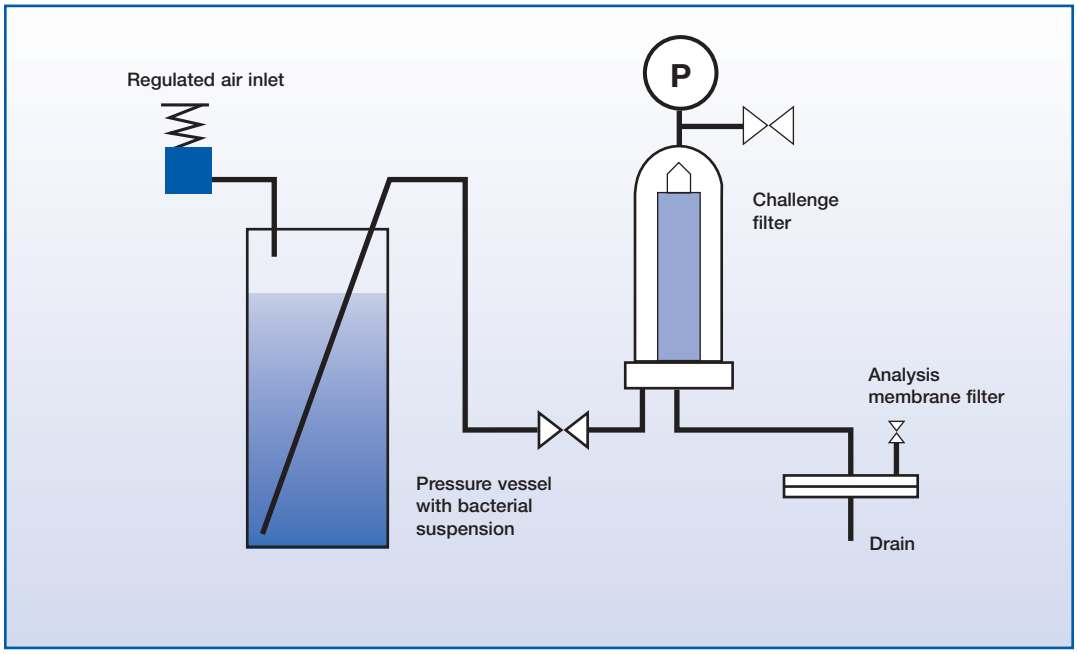
2.2.1 Introduction

The aim of these tests was to confirm that Mini **Kleenpak** filters incorporating **Emflon** PFR membrane that had Forward Flow values below the maximum allowable limit values gave sterile filtrate when subjected to liquid challenge tests using *B. diminuta.*

2.2.2 Summary of Methods

Mini **Kleenpak** PFR filter capsules (part number KA02PFRP2) were selected from standard manufacturing lots and Forward Flow integrity tested, prior to being autoclaved at 121°C (250°F) for 60 minutes. The filter capsules were then aseptically connected to a pre-sterilized challenge apparatus, shown schematically in Figure 2-2, and subjected to microbial challenge tests using an aqueous suspension of *Brevundimonas diminuta* (ATCC 19146). The challenge level used during the tests was > 1 x 10^7 CFU per cm^2 of membrane area.

Figure 2-2 Microbial Challenge Apparatus



During the challenge test the entire filtrate was passed through a 0.2 µm-rated analysis disc installed 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 capsule was determined as follows:

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

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

> Total number of bacteria influent to the filter.

Following the challenge test, the filter capsules were autoclaved at 121°C (250°F) for 60 minutes. The filter capsules were then flushed with water and Forward Flow integrity tested.

2.2.3 Results

The Forward Flow and microbial challenge results for typical Mini **Kleenpak** PFR filter capsules are shown in Table 2-3. The higher of the two Forward Flow values measured on each filter capsule are presented.

Table 2-3 Bacterial Challenge and Forward Flow Results obtained using Mini Kleenpak PFR Filter Capsules (Part Number KA02PFRP2)

Pall Capsule Serial Number	Forward Flow* (mL/min)	Sterile Effluent	Titer Reduction
AA0000044	0.03	Yes	> 9.35×10^9
AA0000060	0.05	Yes	> 8.40×10^9
AA0000085	0.04	Yes	> 7.05×10^9
AA0000081	0.05	Yes	> 9.50×10^9
AA0000031	0.07	Yes	> 6.55×10^9
IE7677002	0.08	Yes	> 9.95×10^9
IE7677006	0.10	Yes	> 1.31×10^{10}
IE7677009	0.10	Yes	> 8.85×10^9
IE7677003	0.11	Yes	> 1.32×10^{10}
IE7677041	0.11	Yes	> 1.09×10^{10}
AA0000086	0.11	Yes	> 6.70×10^9
IE7677011	0.12	Yes	> 1.51×10^{10}
IE7677016	0.12	Yes	> 9.30×10^9
IE7677005	0.13	Yes	> 1.16×10^{10}
IE7677015	0.14	Yes	> 1.10×10^{10}
IE7677022	0.14	Yes	> 1.20×10^{10}
AA0000035	0.21	Yes	> 8.95×10^9
AA0000012	0.28	Yes	> 1.19×10^{10}
AA0000023	0.29	Yes	> 1.18×10^{10}
AA0000043	0.30	Yes	> 1.15×10^{10}

* Air test pressure 1040 mbar (15 psi), wetting liquid 60% (v/v) isopropanol in water, temperature 20°C ± 5°C (68°F ± 9°F), maximum allowable limit value 0.57 mL/min.

2.2.4 Conclusions

A total of 20 Mini **Kleenpak** PFR filter capsules were tested (part number KA02PFRP2), including samples from three separate manufacturing batches. All of the samples passed the Forward Flow integrity test and gave sterile effluent when challenge tested with *B. diminuta.*

3. Resistance to Autoclave Sterilization

3.1 Introduction

The aim of this series of tests was to demonstrate that Mini **Kleenpak** PFR filter capsules retain integrity after exposure to repeated autoclave sterilization cycles performed at either 125°C (257°F) or 135°C (275°F).

3.2 Summary of Methods

Typical Mini **Kleenpak** PFR filter capsules from two separate production batches were used for the tests (part number KA02PFRP2). One set of capsules was subjected to thirty 30-minute autoclave cycles at 125°C (257°F), and another set of capsules was subjected to twenty 30-minute cycles at 135°C (275°F). Before autoclaving and on completion of the final autoclave cycle the integrity of the filter capsules was confirmed using the Forward Flow test method.

3.3 Results

The Forward Flow results before and after exposure to the autoclave cycles are shown in Table 3-1. After flushing in isopropanol, all of the filters passed the Forward Flow integrity test following exposure to the autoclave cycles at 125°C (257°F) and 135°C (275°F).

Table 3-1. Effect of Repeat Autoclave Cycles on Forward Flow Results for Mini Kleenpak PFR Filter Capsules

Autoclave Condition	Pall Filter Capsule Serial Number	Forward Flow* (mL/min)	
		Before autoclave treatment	After autoclave treatment
30 x 30 minute cycles at 125°C (257°F)	IE7677017	0.17	0.10
	IE7677023	0.11	0.16
	IE7677024	0.06	0.13
	IE7677027	0.03	0.14
	IE7677032	0.06	0.12
	AA0000005	0.06	0.09
	AA0000017	0.11	0.16
	AA0000022	0.01	0.04
	AA0000040	0.02	0.13
20 x 30 minute cycles at 135°C (275°F)	IE7677034	0.15	0.16
	IE7677035	0.09	0.15
	IE7677037	0.12	0.14
	IE7677039	0.10	0.05
	IE7677047	0.10	0.12
	AA0000062	0.09	0.05
	AA0000074	0.07	0.06
	AA0000079	0.13	0.09
	AA0000082	0.12	0.17
	AA0000096	0.03	0.12

* Air test pressure 1040 mbar (15 psi), wetting liquid 60% (v/v) isopropanol in water, temperature 20°C ± 5°C (68°F ± 9°F), maximum allowable limit value 0.57 mL/min.

3.4 Conclusions

Forward Flow integrity tests performed on Mini **Kleenpak** PFR filters demonstrated that the capsules retained integrity after exposure to either thirty 30-minute autoclave cycles at 125°C (257°F) or twenty 30-minute cycles at 135°C (275°F).

4. Air Flow/Differential Pressure Characteristics

4.1 Introduction

The aim of these tests was to determine the differential pressure characteristics of typical Mini **Kleenpak** PFR filters when subjected to different air flow rates at different inlet pressures.

4.2 Summary of Methods

Typical filter capsules from production were used for the tests (part number KA02PFRP2). The differential pressure across the filter capsules was measured while clean compressed air was directed through the filter assemblies at a range of flow rates and under both 'atmospheric vent' and 'pressurized' operating conditions.

In 'vent' conditions, the downstream side of the filter capsule was open to atmospheric pressure and air through the filter was controlled from the upstream side. Under 'pressurized' conditions, predetermined air pressures were maintained upstream of the filter capsules and the air flow rates through the filter capsules were controlled by restricting flow on the downstream side.

All air flow measurements were corrected to standard conditions (1013.25 mbar/14.7 psi, 20°C/68°F).

4.3 Results

The air flow versus differential pressure values under atmospheric vent conditions and 0.68 bar g (10 psi g) and 2 bar g (29.4 psi g) applied pressure conditions are shown in Figures 4-1 to 4-3.

These data can be used to confirm the sizing of filter systems that employ Mini **Kleenpak** filter capsules incorporating **Emflon** PFR membrane.

Figure 4-1 Air Flow/Differential Pressure Characteristics for Mini Kleenpak PFR Filters under Atmospheric Vent Conditions

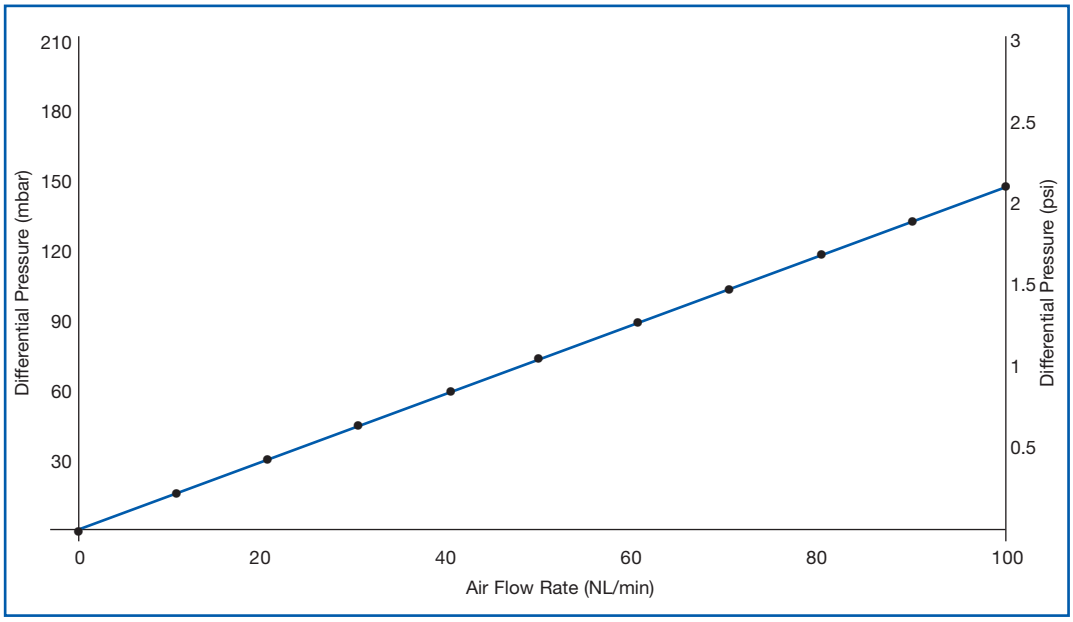


Figure 4-2 Air Flow/Differential Pressure Characteristics for Mini Kleenpak PFR Filters at 0.68 bar g (10 psi g) Inlet Pressure

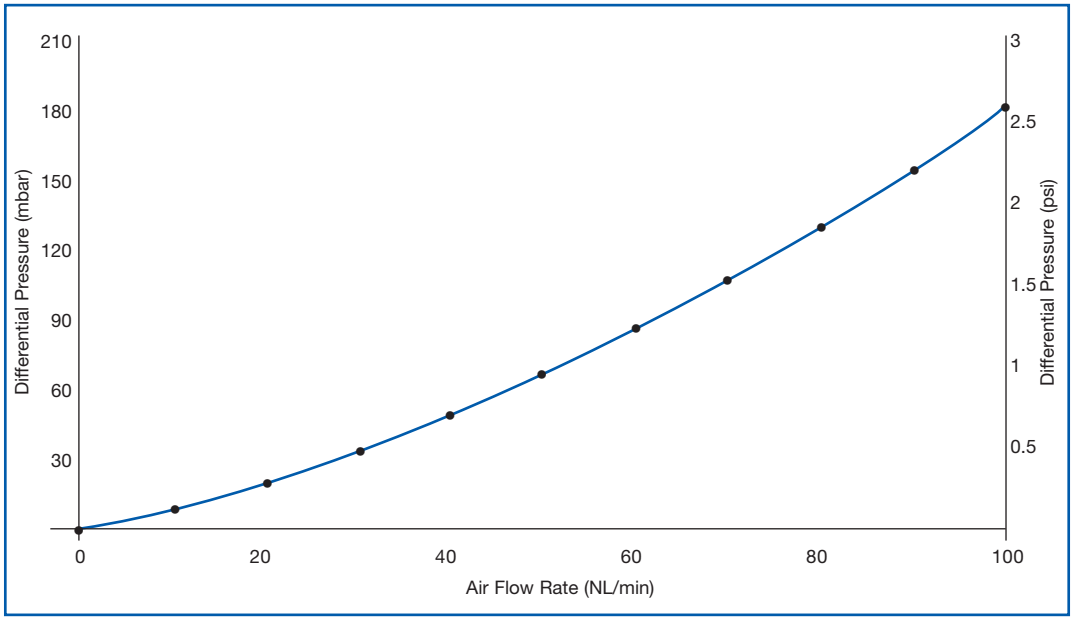
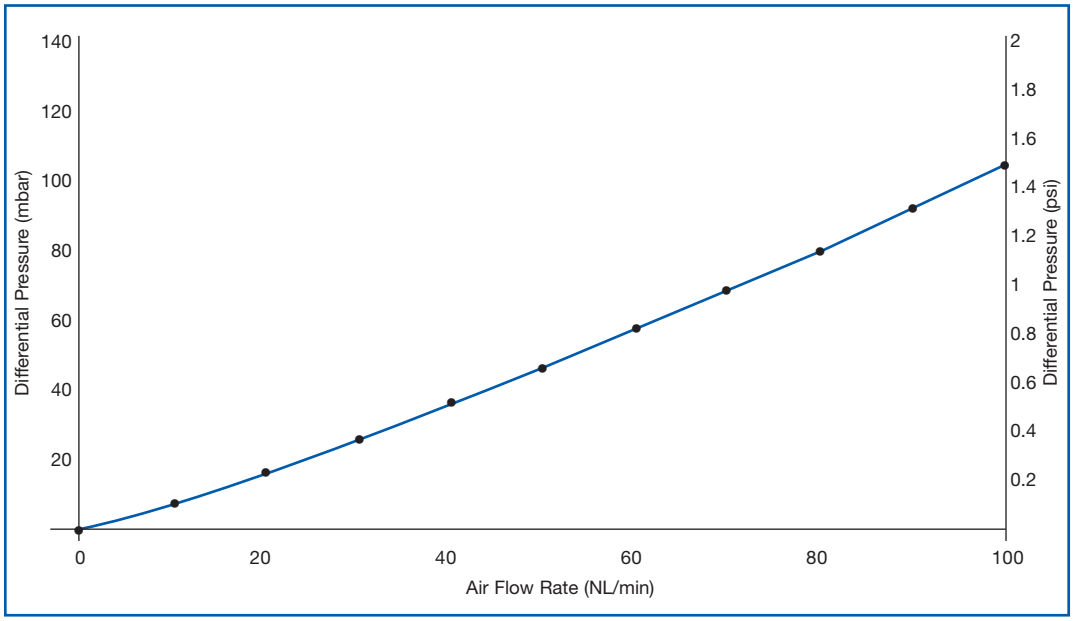


Figure 4-3 Air Flow/Differential Pressure Characteristics for Mini Kleenpak PFR Filters at 2 bar g (29.4 psi g) Inlet Pressure



4.4 Conclusions

The data provided can assist users in sizing systems using filters incorporating **Emflon** PFR filter membrane.

At 100 mbar (1.47 psi) differential pressure, the typical measured air flows were:

- **50 NL/min (1.7 cfm) under vent conditions**
- **60 NL/min (2.1 cfm) at 0.68 barg (10 psig) inlet pressure**
- **90 NL/min (2.8 cfm) at 2 barg (30 psig) inlet pressure**

5. Biological Reactivity Tests on the Materials of Construction

5.1 Introduction

The purpose of this study was to evaluate the biological suitability of the materials of construction of Mini **Kleenpak** PFR capsules. The materials of construction are as follows:

Filter membrane:	Double layer PTFE membrane
Membrane support and drainage layers:	Polypropylene
Core, cage and endcaps:	Polypropylene
Capsule shell:	Polypropylene

5.2 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 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 (122°F) for 72 hours, 70°C (158°F) for 24 hours, or 121°C (250°F) 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 (250°F).

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 materials used in the Mini **Kleenpak** PFR capsule was implanted separately.

5.3 Results

No biological response was observed in any of the tests performed and therefore the materials used in the Mini **Kleenpak** PFR filters passed all of the tests specified.

5.4 Conclusions*

The materials used in Mini **Kleenpak** PFR filters met the requirements of the USP Biological Reactivity Tests *(in vivo)* for Class VI-121°C plastics. The tests covered the systemic injection test, the intracutaneous test and the implantation test.

* Copies of test certificates are available upon request. Please contact Pall for details.

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