BioPharmaceuticals

Validation Guide

USTR 1623(1)

Validation Guide for Pall Emflon PFA AB Style Filter Cartridges

*Filtration. Separation. Solution.*SM

Table of Contents

This guide contains validation data applicable to the 'Pall' microbially-rated **Emflon** PFA AB style filter cartridges.

The validation program included:

- Correlation of Forward Flow integrity tests with bacterial retention tests
- Extended steam sterilisation tests
- Determination of differential pressure at different air flow rates and inlet pressures
- Extractables tests
- Biological reactivity tests

Materials of construction and performance parameters of the **Emflon** PFA AB style filters are described in detail in Pall publication USD 1573 which is intended to be used in conjunction with this guide.

Pall can supply additional information you may require.

1.1 Summary of Conclusions

A. Bacterial Removal and Integrity Test Parameters

Emflon PFA filter cartridges were demonstrated to provide absolute removal of *Brevundimonas (Pseudomonas) diminuta* aerosol at levels greater than 10^7 colony forming units (cfu) per 254 mm filter as determined by aerosol challenge tests.

Integrity test parameters for a 254 mm filter cartridge were set as follows:

Forward Flow Test Parameters:

Wetting liquid:	30:70 (v/v) tertiary butyl alcohol in water
Test gas:	Air
Test pressure:	830 mbar (12 psi)
Test temperature:	20°C ± 5°C
Maximum allowable Forward Flow limit:	20 ml/min

Wetting liquid:	70:30 (v/v) isopropyl alcohol in water
Test gas:	Air
Test pressure:	760 mbar (11 psi)
Test temperature:	20°C ± 5°C
Maximum allowable Forward Flow limit:	30 ml/min

B. Extended Steam Sterilisation Tests

All **Emflon** PFA filter cartridges tested produced sterile effluent after exposure to 100 one hour cycles of *in-situ* steaming at 125°C, in the forward direction. Post-steaming Forward Flow values of the filters tested remained within the validated test limit. Following this, filter cartridges were further subjected to an additional 45 one hour cycles of *in-situ* steaming at 140°C. All filters retained their integrity, as per the Forward Flow test, after extended steaming for up to 145 hours.

C. Air Flow/Differential Pressure Measurements

See Figure 4 for typical flow versus differential pressure data. The non-linear relationship between flow and pressure differential demonstrated that turbulent flow was present due to pressure losses in the filter core, adaptor, housing inlet/outlet ports etc. The filter membrane component, however, would be expected to give a linear relationship due to laminar flow through the filter matrix. These properties must be considered when sizing **Emflon** PFA filter systems.

D. Extractables Tests

The amount of material typically extracted from **Emflon** PFA filter cartridges is listed below:

Solvent	Non-Volatile Residue (mg/254 mm
Water for Injection	< 1
95% Ethanol	< 30

E. Biological Reactivity Tests

Emflon PFA filter cartridges were found to meet the requirements of the *United States Pharmacopeia* for Class VI (121°C) Plastics.

2.1 Introduction

The aims of these tests were to:

- Determine the microbial removal efficiency of **Emflon** PFA filter cartridges and filter membrane by bacterial challenge tests.
- Correlate a non-destructive integrity test with bacterial challenge test.
- Determine integrity test parameters.

2.2 Outline of Test Procedure

2.2.1 Bacterial Challenge

Bacterial challenge tests using aerosolised *Brevundimonas (Pseudomonas) diminuta* (ATCC 19146) at levels of 10^7 cfu per 254 mm filter were performed with standard production **Emflon** PFA filter cartridges.

Additionally, flat sheet samples of **Emflon** PFA filter medium were challenged with $>10^7/cm^2$ cfu of *Brevundimonas (Pseudomonas) diminuta* in aerosol form at a face velocity equivalent to 185 Nm^3/h (110 scfm) per 254 mm filter cartridge.

Detailed test methods are described in Appendix 1.

2.2.2 Forward Flow Test

The correlation between bacterial retention and a non-destructive integrity test is extremely important for validating filter performance. The non-destructive integrity test for hydrophobic filters used during this validation was the Forward Flow test.

In 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 stabilisation period, the gas flow through the wetted membrane is measured, either:

- Directly on the upstream or downstream side using flow measurement equipment, or
- Indirectly by isolating the upstream gas supply and measuring the pressure decay which is a function of the gas flow through the membrane.

2.2.3 Validation Study

Emflon PFA filter cartridges (part number AB1PFA7PH4) with a range of Forward Flow values at the pre-determined test pressure, were selected from standard production lots. The filters were subjected to bacterial challenge tests using *Brevundimonas (Pseudomonas) diminuta* (ATCC 19146) at a challenge level per 254 mm filter of $>1x10^7$ cfu. It was determined that a Forward Flow test pressure of 830 mbar (12 psi) was suitable for integrity testing **Emflon** PFA filter cartridges wetted with a solution of 30% by volume tertiary butyl alcohol in water. The Forward Flow integrity test was performed as per the schematic shown in Figure 1.

An outline of the procedures used are shown in Figure 2.

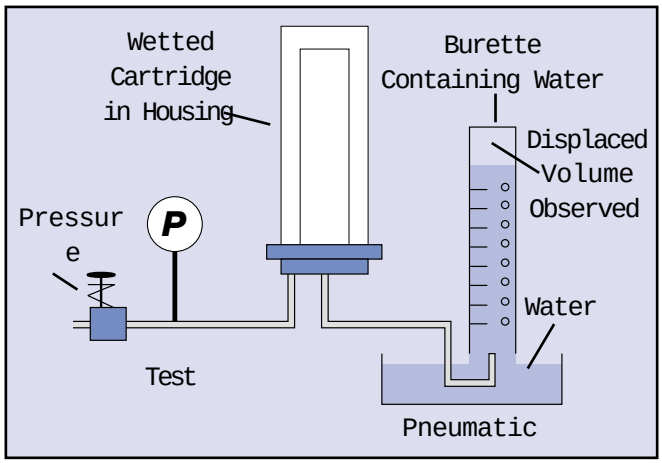
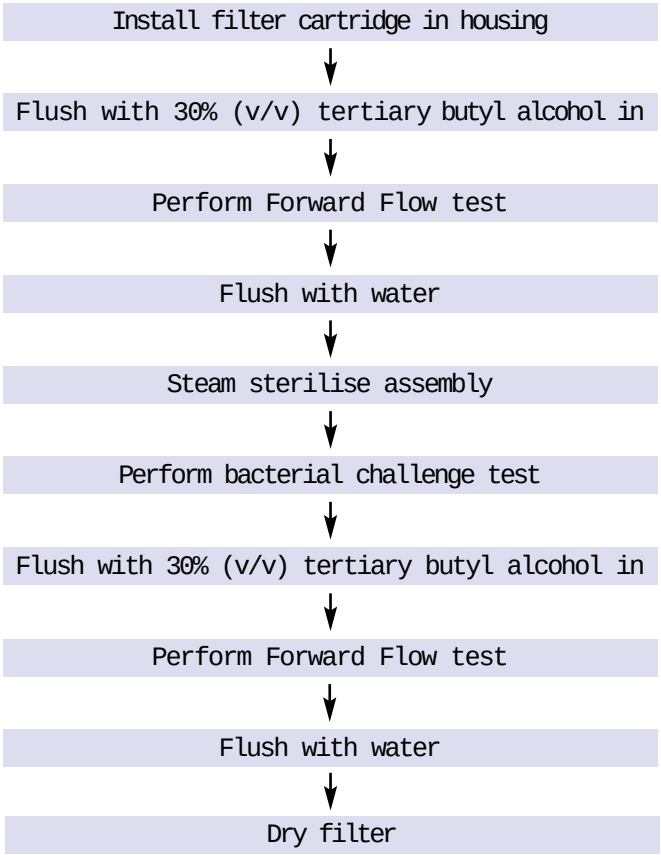


Figure 1: *Test Set-Up for Forward Flow Test*

Figure 2

Schematic of Bacterial Challenge Test Procedure



2.3 Bacterial Challenge and Correlation with Forward Flow Integrity Test Values

2.3.1 Results

The data obtained from this validation study are shown in Table 1 and presented graphically in Figure 3. The data are presented in order of increasing Forward Flow values. It was found that all filters with Forward Flow values ≤40 ml/min gave sterile effluent when challenged with >1x10^{7} cfu of *Brevundimonas (Pseudomonas) diminuta* per filter. Of the 5 filters with Forward Flow values between 44 and 70 ml/min, 3 gave sterile effluent.

The data obtained from aerosol bacterial challenge tests using flat sheet filter media samples from four different lots are shown in Table 2. In each case, no passage of *Brevundimonas (Pseudomonas) diminuta* was observed.

TABLE 1: Correlation of Forward Flow with Brevundimonas (Pseudomonas) aerosol retention for Emflon PFA filter cartridges (part number AB1PFA7PH4)

Filter Cartridge Serial Number	Forward Flow (ml/min)	Sterile Effluent
EG0170077	2.1	Yes
EG0170212	2.2	Yes
EG0170039	2.4	Yes
EG0170130	2.5	Yes
EG0170048	2.8	Yes
EG0170057	3.2	Yes
EG0170218	3.3	Yes
EG1070214	3.7	Yes
EG0170220	4.4	Yes
EF1400146	4.5	Yes
EG0170224	4.6	Yes
EG0170216	4.7	Yes
EG0170149	5.2	Yes
EG0170222	5.9	Yes
EF1400088	6.0	Yes
EG0170159	9.0	Yes
EG0170012	9.3	Yes
EG0170108	10.3	Yes
EG0170006	10.8	Yes
EF1400067	11.5	Yes
EG0170128	12.5	Yes
EG0170078	15.0	Yes
EG0170024	17.0	Yes
EG0170217	17.0	Yes
EG0170219	17.7	Yes
EG0170215	18.2	Yes
EF1400121	21.0	Yes
EG0170009	29.0	Yes
EG0170210	40.0	Yes
EG0170112	44.0	No
EG0170156	45.0	Yes
EG0170223	46.5	Yes
EG0170211	68.0	No
EG0170221	70.0	Yes

Total challenge:	>1x10^7 per 254 mm filter
Test bacterium:	Brevundimonas (Pseudomonas)
Forward Flow test	830 mbar (12 psi)
Wetting fluid:	30:70 (v/v) tertiary butyl alcohol

Figure 3. Representation of Forward Flow with *Brevundimonas (Pseudomonas) diminuta* Retention for **Emflon** PFA Filter Cartridges (part number AB1PFA7PH4)

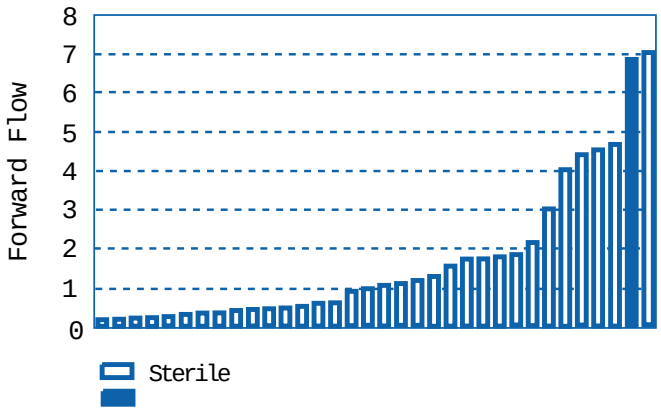


TABLE 2: Retention of Brevundimonas (Pseudomonas) diminuta by aerosol challenge for Emflon PFA filter membrane flat sheet sample

Membrane Lot Number	Challenge Level (cfu/cm^2)	Sterile Effluent
32242	1.3 x 10^7	Yes
03051	1.5 x 10^7	Yes
15852	1.5 x 10^7	Yes
32642	1.1 x 10^7	Yes

Challenge	>1x10^7 per cm^2
Test bacterium:	Brevundimonas (Pseudomonas) diminuta
Forward Flow test pressure:	830 mbar (12 psi)
Wetting fluid:	30:70 (v/v) tertiary butyl alcohol in

2.3.2 Conclusions

Based on the test results, the Forward Flow test was shown to be a suitable non-destructive integrity test for **Emflon** PFA filters. AB1 style 254 mm **Emflon** PFA filters which have Forward Flow values equal to or lower than 20 ml/min (wet with 30% by volume tertiary butyl alcohol in water) will provide absolute removal of incident *Brevundimonas (Pseudomonas) diminuta* aerosol at levels of 1x10^7 cfu. Foward Flow test parameters were set as follows:

Test pressure:	830 mbar (12 psi)
Wetting	30:70 (v/v) tertiary butyl alcohol in
Temperature:	20°C ± 5°C
Test gas:	Air
Maximum allowable Forward Flow:	20 ml/min

NOTE: For the validation study, Forward Flow tests were conducted using a solution of 30:70 (v/v) Tertiary butyl alcohol (TBA)/water as a wetting fluid. (The volumes should be measured separately and then mixed). The use of such a solution is advantageous as it enables a sensitive discrimination between filters that would pass or fail the test. **Emflon** PFA filters can also be Forward Flow tested with adequate sensitivity using a 70:30 (v/v) isopropyl alcohol (IPA)/water mixture. This fluid is generally more readily available than TBA, but is more flammable. Forward Flow test parameters have been derived for a 254 mm filter by correlation of Forward Flow in 30% TBA with that in 70% IPA:

Test pressure:	758 mbar (11 psi)
Wetting liquid:	70:30 (v/v) isopropyl alcohol in
Temperature:	20°C ± 5°C
Test gas:	Air
Maximum allowable Forward Flow:	30 ml/min

These Forward Flow integrity test parameters:

- Incorporate a safety margin, and
- Provide a high level of assurance of retention of *Brevundimonas (Pseudomonas) diminuta* when challenged with >1 x 10^7 cfu/per 254 mm filter.

For values for other **Emflon** PFA filter styles or for values in other test fluids, please contact Pall.

3. THE EFFECTS OF EXTENDED STEAM EXPOSURE ON FILTER INTEGRITY

3.1 Introduction

The aim of these validation tests was to determine the effects of repeated *in-situ* steam exposure on the integrity of **Emflon** PFA filter cartridges.

3.2 Summary of Methodology

The procedure for these tests are generalised industry protocols and follow the recommended instructions for steam sterilisation described in Pall Publication USD 805 *'Steam Sterilisation of **Pall** Filter Assemblies Which Utilise Replaceable Filter Cartridges.'*

A direct flow of steam through the filter assembly was used. After confirming the integrity of **Emflon** PFA filters from routine production, each filter was dried and installed in a stainless steel housing. Steam at 125°C was then introduced into the system upstream of the filter. The steam pressure and flow were held constant during a one hour period, after which the filter was allowed to cool. After 100 cycles of steaming and cooling the **Emflon** PFA filter cartridge was dried, re-wet with 30% tertiary butyl alcohol, and integrity tested using the Forward Flow method.

After the Forward Flow test, each filter was subjected to challenge with an aerosol of *Brevundimonas (Pseudomonas) diminuta* as described in Section 2 to confirm filter performance.

Additionally, two filter cartridges were subjected to multiple one-hour steaming cycles at 140°C after being steamed *in-situ* for 100 one hour cycles. These filters were then integrity tested using the Forward Flow method.

3.3 Results

Table 3 shows the Forward Flow values (30% TBA-wet) of fourteen filter cartridges tested in this validation study. The Forward Flow value of each filter cartridge remained below the maximum allowable Forward Flow limit of 20 ml/min and retained *Brevundimonas (Pseudomonas) diminuta* aerosol after 100 one hour periods of *in-situ* steaming.

Table 4 shows the Forward Flow values (70% IPA-wet) of the two filter cartridges subjected to additional extended steaming at 140°C. The Forward Flow values of both of these filter cartridges remained below the maximum allowable Forward Flow limit of 30 ml/min.

TABLE 3: Performance of Filters After 100 x 1 Hour Periods of *in-situ* Steaming at 125°C

Filter cartridge serial number	Steam exposure	Forward Flow* 30% TBA (ml/min)	Sterile Effluent
EG0170159	100 hours	9.0	Yes
EG0170006	100 hours	10.8	Yes
EF1400060	100 hours	11.0	Yes
EF1400040	100 hours	12.2	Yes
EG0170128	100 hours	12.5	Yes
EF1400028	100 hours	12.8	Yes
EF1400143	100 hours	13.1	Yes
EG0170078	100 hours	15.0	Yes
EF1400024	100 hours	16.8	Yes
EG0170024	100 hours	17.0	Yes
EF1400118	100 hours	17.5	Yes
EF1400127	100 hours	17.8	Yes
EG0170215	100 hours	18.2	Yes
EF1400132	100 hours	18.2	Yes

Forward Flow Test Parameters:

Wetting liquid:	30:70 (v/v) tertiary butyl alcohol in water
Test gas:	Air
Test pressure:	830 mbar (12 psi)
Test temperature:	20°C± 5°C
Maximum allowable Forward Flow limit:	20 ml/min

TABLE 4: Performance of Filters Tested After Exposure to 100 x 1 Hour Cycles of *in-situ* Steaming at 125°C and Subsequent Extended *in-situ* Steaming at 140°C

Filter cartridge serial number	Forward Flow (ml/min) after 100 hours at 125°C	Forward Flow (ml/min) after the following number of		
		5 hours 5 x 1 hour cyles	25 hours 25 x 1 hour	45 hours 45 x 1 hour
EG1890238 EG1890243	11.2 14.7	9.7 14.0	12.3 17.0	12.4 20.0

Forward Flow Test Parameters:

Wetting liquid:	70:30 (v/v) isopropyl alcohol in water
Test gas:	Air
Test pressure:	758 mbar (11 psi)
Test temperature:	20°C ± 5°C
Maximum allowable Forward Flow limit:	30 ml/min

3.4 Conclusion

The integrity of **Emflon** PFA filter cartridges is not affected by exposure to multiple cycles of typical steaming for sterilisation.

Since the service life of the filter will be affected by process conditions (such as exposure to oxidisers, cleaning agents or solvents), the actual service life will vary with the specific conditions of use.

4.1 Introduction

The aim of these tests was to determine the pressure differential characteristics of the filter when subjected to different air flow rates at different inlet pressures.

4.2 Summary of Methodology

Standard production filters were installed in a stainless steel air filter housing designed for use in compressed gas and vent applications. The differential pressure across the filter assembly (filter housing and filter cartridge) was measured while clean compressed air was directed through the filter assembly, at a range of flow rates and under both "atmospheric vent" and "pressurised" operating conditions.

During "vent" conditions, the downstream side of the filter assembly was open to atmospheric pressure and air flow through the filter was controlled from the upstream side. Under "pressurised" conditions, predetermined air pressures were maintained upstream of the filter assembly; air flow rate through the filter was controlled by restricting flow on the downstream side.

All air flow measurements were corrected to standard conditions of 1013 mbar and 20°C.

Further detailed test procedures are described in Appendix 2.

4.3 Results

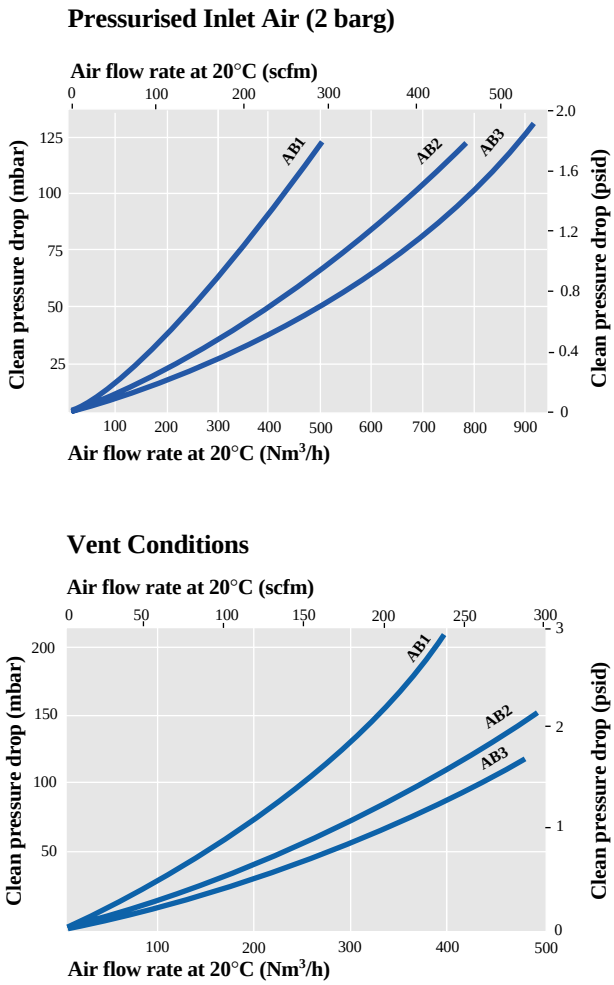
The flow versus differential pressure values of 254, 508 and 762 mm filter cartridges at atmospheric pressure and at an upstream pressure of 2 bar are shown in Figure 4. These data show that there is a non-linear relationship between flow and pressure differential. These data form the basis for sizing filter systems using **Emflon** PFA filter cartridges.

4.4 Conclusions

The flow curves indicate that **Emflon** PFA filter cartridges will provide very high air flow rates with very low pressure drops.

The non-linear relationship between flow and pressure differential demonstrates that turbulent flow is present due to pressure losses in the filter core, adaptor, housing inlet/outlet ports etc. The filter membrane component, however, would be expected to give a linear relationship due to laminar flow through this filter matrix. These properties must be considered when sizing **Emflon** PFA filter systems.

Figure 4: *Typical Air Flow Rates*



5.1 Introduction

The aim of these tests was to determine the amount of material which can be extracted from the **Emflon** PFA membrane filter cartridges by water and by alcohol, both being liquids commonly found in fermentors, food processing and holding tanks and other equipment typically protected by these filters.

5.2 Summary of Methodology

254 mm AB-style filter cartridges from typical production lots were autoclaved for one hour at 125°C using a slow exhaust cycle. Upon cooling to ambient temperature, the filters were extracted as indicated in water or in alcohol.

Each extraction was performed in a manner designed to simulate realistic contact of the filter with the fluid for extended time. In the case of water, the filter was immersed in 1500 ml Water For Injection (WFI) with only the filter outlet exposed. Vacuum was applied to the downstream side of the filter, evacuating any air from the drainage materials and forcing the water against the filter membrane. The filter was gently reciprocated up and down in the water for 4 hours. (There was no flow through the filter. The **Emflon** PFA filter membrane is extremely hydrophobic and differential pressures as high as 5 bar are required to initiate bulk flow of water through the filter material). In the case of alcohol, the autoclaved filter was completely immersed in the liquid. The filter was then reciprocated up and down such that the open end of the filter just emerged above the level of the liquid so that gentle flow of alcohol was obtained through the filter itself. After each extraction 1000 ml of the eluate was evaporated to dryness and the non-volatile extractables were determined gravimetrically.

Detailed tests methods are described in Appendix 3.

5.3 Results

The extractable material found for each filter tested is listed in Table 5 below in milligrams per 254 mm filter cartridge.

TABLE 5: Non-Volatile Residues Extracted From Emflon PFA Filters Part Number AB1PFA7PH4

Filter Cartridge Serial	Solvent	Non-Volatile Residue (mg/254 mm Cartridge)
EG1340071	WFI	0.6
EG1340086	WFI	0.9
EG1340057	95% ethanol	25.5
EG1340099	95% ethanol	23.4

5.4 Conclusions

The levels of extractables found for **Emflon** PFA membrane filter cartridges are extremely low.

The levels of extractables reported here are typical of those from **Emflon** PFA filters; some variations from cartridge to cartridge may occur.

Actual service will impose different conditions, such as, different exposure times, temperatures and solvent purity. Evaluation under actual process conditions is recommended.

6.1 Introduction

The purpose of these tests was to evaluate the biological suitability of the materials of construction of the **Pall Emflon** PFA filter. This was done by performing the Biological Reactivity Tests, *in-vivo*, for Plastics, as described in the *United States Pharmacopeia (USP)*. The materials of construction of **Emflon** PFA filters are as follows:

Filter medium:	Pall polytetrafluoroethylene (PTFE) membrane
Support/drainage	Stabilised polypropylene homopolymer
Endcap/adaptor	Stabilised polypropylene
Core/cage:	Stabilised polypropylene

6.2 Summary of Methodology

The tests were conducted by South Mountain Laboratories, 380 Lackawanna Place, South Orange, New Jersey 07079.

The testing procedures described in the USP include:

- Injection of extracts of plastic materials into animal tissue
- 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. Since **Emflon** PFA membrane filters will be steamed during use, and since the most stringent condition not resulting in physical changes in the plastic is recommended, they 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 (USP) and a 1 in 20 solution of alcohol in sodium chloride injection extracts 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 extracts were used.

Implantation Tests

Implantation was also performed, in order to subject the materials of construction to the most stringent conditions included in the USP. Each component of the filter cartridge was implanted separately.

6.3 Results and Conclusions

Emflon PFA Filters were found to meet the requirements of the USP for Class VI-121°C Plastics. A copy of the successful test results is shown in Appendix 4.

7. CERTIFICATION OF PHARMACEUTICAL-GRADE FILTERS

Pharmaceutical-grade **Emflon** PFA filters, are tested for use in pharmaceutical service, are manufactured in controlled environments, and are subject to stringent quality control, including in-process controls and testing of the elements as follows:

1. Integrity test, on a 100% basis,
2. Oxidisables (USP) and pH tests,
3. Effluent cleanliness test,
4. Limulus amebocyte lysate (LAL) test, and
5. Microbial challenge tests, on a sampling basis.

These tests are described on a certificate which is provided with each **Emflon** PFA filter (see Figure 5). Each certificate carries a facsimile of the signature of the designated Quality Manager.

PALL

Certificate of Test for 'Emflon' PFA Air Filters

We hereby certify that Pall Emflon PFA Filter
Part Number AB1PFA7PVH4
Lot Number SAMPLE

has been manufactured in a controlled environment and subjected to a high velocity flush after undergoing integrity testing.

*** Fabrication Integrity**
This product successfully passed a forward flow test. The diffusional air flow rate did not exceed 20.00 ml/minute at a test pressure of 830 mbar/ 12.00 psig when fully wetted with a solution of 30% (v/v) tertiary butyl alcohol in water. The forward flow test limit has been validated for bacterial removal by correlation of the above parameters with microbiological challenge test. Actual test values for integrity testing of PALL filters as installed must be obtained from Pall. Samples from this manufacturing lot also maintained integrity after multiple steam cycles.

*** Bacterial Retention**
Finished product from this lot has been sampled for microbiological challenge tests and produced sterile air when challenged with an aerosol of **Brevundimonas diminuta** (ATCC 19146) at a challenge level $\geq 10^7$ CFU per 254mm element. Samples of filter medium used in these filters produced sterile effluent when challenged with an aerosol of **Brevundimonas diminuta** at a level $\geq 10^7$ CFU/cm^2 at a flow rate comparable to 171 Nm^3/hr(100 SCFM) per 254mm element.

*** Materials of Construction**
The filter components have met the specifications for biological tests listed in the current revision of the United States Pharmacopeia (USP) for Class VI plastics at 121°C. These filters also are made from materials listed for food contact usage per Title 21 of the U.S. Code of Federal Regulations (CFR) parts 170-199.Contact Pall for further information regarding materials of construction.

*** Effluent Quality**
Filter samples from this manufacturing lot underwent the following tests and the lot was released by Quality Control when it was verified that their respective criteria were met:

Cleanliness:
Meets with adequate safety margin the current USP limits under Particulate Matter in Injections with effluent counts determined microscopically. Counts serve to document conformance with the requirements for a non-fibre-releasing filter per Title 21 of the U.S. Code of Federal Regulations (CFR) parts 211.72 and 210.3 (b) (6).

Oxidizable substances:
Meets the current USP requirements under Purified Water after flushing.

pH:
Meets the current USP requirements under Purified Water after flushing.

Pyrogens:
Meets with adequate safety margin the current USP requirements under Bacterial Endotoxins Test as determined using the Limulus Amoebocyte Lysate (LAL) reagent with an aliquot from a soak solution.

In addition to the above tests, this product has met manufacturing inspection standards and stringent requirements for full traceability. Further information is available by contacting Pall.
The filters are not supplied sterile.

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96/1/A Manager of Quality Assurance and Quality Control Date 29/03/96

Emflon is a Trade Mark of Pall Corporation

APPENDIX 1
BACTERIAL CHALLENGE PROCEDURE

1. Aerosol Validation Bacterial Challenge

1.1 Procedure for Filter Cartridges

1.1.1 Filter membrane was wetted by vacuum drawing 5 l of a 70:30 (v/v) IPA in water solution through the filter.

1.1.2 Forward Flow integrity test was performed at 760 mbar (11 psi) and the tested value was recorded.

1.1.3 The filter was then placed in a housing (P/N VNPTL1G723F13) and flushed with deionised water for 20 minutes at 7.6 l/min to remove the alcohol.

1.1.4 The filter was then removed from the housing and excess water was removed by vacuum draw.

1.1.5 The filter was then loosely wrapped with autoclave paper and sterilised by autoclaving at 121°C for 60 minutes, slow exhaust.

1.1.6 The filter was placed in an oven to dry at 96°C for 12 hours.

1.1.7 The test stand was sterilised for use.

1.1.8 The test filter was then aseptically installed in the test system which consisted of a nebuliser, a separate line for dry make-up air, and split stream impingers to sample the aerosol bacterial challenge with and without the test filter.

1.1.9 The test procedure consisted of generating a microbial aerosol with a nebuliser, introducing the aerosol to the test filter at a given flow rate, and collecting the filter effluent in dual liquid impingers. Controls were performed simultaneously via a split stream by using a two channel timer to direct air flow, on an alternating basis, from the test side filter impingers to the unfiltered control side impingers for recovery.

1.1.10 The stock solutions of *Brevundimonas (Pseudomonas) diminuta* were prepared and titred to concentration of 1 x 10^8 and 1 x 10^{10} microorganisms per ml. Each neubiliser was then loaded with 5.0 ml of challenge solution.

1.1.11 The challenge flow rate was maintained at 16.8 Nm^3/h. Samples were taken at 16.8 Nm^3/h at each pair of impingers by maintaining a vacuum of at least 15 inches Hg downstream of the impingers. Excess air flow of about 15.1 Nm^3/h was discharged through a separate filtered exhaust vent. Air intake was maintained by adjusting the neubiliser pressure to about 655 mbar and setting the make-up air to 16.8 Nm^3/h using a flowmeter.

1.1.12 Each test procedure was performed for 8 minutes with the impingers being changed after each run. The final 2 minutes of each 8 minute run was an air flush to clear the system of aerosol.

1.1.13 Each filter was challenged twice, first with a challenge concentration of 1 x 10^8/ml and then 1 x 10^{10}/ml.

1.1.14 Each impinger contained 20 ml sterile phosphate buffer at the outset. The buffer solutions from impingers on the control side were pooled and assayed. The buffer from the impingers on the test side were kept separate and filtered through separate 0.2µm sterile analysis discs.

The analysis membranes were aseptically removed from the filter funnel, plated on Mueller-Hinton Agar, and incubated for 48 hours at 32°C.

1.1.15 After completion of the test, the test filters were again Forward Flow tested and the value recorded.

1.2 Procedure for Flat Sheet Membranes

1.2.1 The filter membrane was placed in a 92.9 cm^2 effective filtration area housing with conical inlet and outlet.

1.2.2 The filter housing was then loosely wrapped with autoclave paper and sterilised by autoclaving at 121°C for 60 minutes, slow exhaust.

1.2.3 The test stand was sterilised for use.

1.2.4 The test membrane was then aseptically installed in the test system which consisted of a nebuliser loaded, a separate line for dry make-up air and split stream impingers to sample the aerosol bacterial challenge with and without the test membrane.

1.2.5 The test procedure consisted of generating a bacterial aerosol with the nebuliser, introducing the aerosol to the test membrane at a given flow rate and collecting the filter effluent in dual liquid impingers. Controls were performed simultaneously via a split stream by using a two channel timer to direct air flow, on an alternating basis, from the test side filter impingers to the unfiltered control side impingers for recovery.

1.2.6 The stock solutions of *Brevundimonas (Pseudomonas) diminuta* were prepared and titred to concentrations of 1 x 10^8 and 1 x 10^{10} microorganisms per ml. Each nebuliser was then loaded with 5.0 ml of challenge solution.

1.2.7 The challenge flow rate was maintained at 2.2 Nm^3/h. Samples were taken at 1.68 Nm^3/h at each pair of impingers by maintaining a vacuum of at least 15 inches Hg downstream of the impingers. Excess air flow of about 0.5 Nm^3/h was discharged through a separate filtered exhaust vent. Air intake was maintained by adjusting the nebuliser pressure to about 655 mbar and setting the make-up air to 2.2 Nm^3/h using a flowmeter.

1.2.8 Each test procedure was performed for 8 minutes with the impingers being changed after each run. The final 2 minutes of each 8 minute run was an air flush to clear the system of aerosol.

1.2.9 Each membrane was challenged twice, first with a challenge concentration of 1 x 10^8/ml and then 1 x 10^{10}/ml.

1.2.10 Each impinger contained 20 ml sterile phosphate buffer at the outset. The buffer solutions from impingers on the control side were pooled and titered. The buffer from the impingers on the test side were kept separate and filtered through separate 0.2µm sterile analysis discs.

1.2.11 The analysis membranes were aseptically removed from the filter funnel, plated on Mueller-Hinton Agar and incubated for 48 hours at 32°C. Titre reduction (Tr) was calculated using the following formula:

$$T_R = \frac{\text{Number of organisms recovered on the control side (upstream)}}{\text{Number of organisms recovered on the test side (downstream)}}$$

2. Test for Submicron Microbiological Penetration, Brevundimonas (Pseudomonas) diminuta, Aerosol Test System

The split aerosol test procedure permits simultaneous direct monitoring of the input and recovery during an aerosol challenge test of a filter, thereby eliminating unnecessary variables and potential errors inherent in performing separate control and test challenges.

The procedure employs a two-channel timer controlling a three-way valve which directs air flow through either the test filter or an unfiltered “control” by-pass, using an alternating five second cycle per side. The filtered and unfiltered air streams are then passed through matched liquid impingers that recover aerosolised microorganisms and control flow rate by also acting as critical orifices. In contrast to the Andersen Viable Sampler, the maximum number of cells capable of being collected in a liquid impinger and qualified is not limited, so that actual incident challenges of greater than 10^9 microorganisms can be measured. When the challenge is completed, the volume of fluid and concentration of challenge organisms recovered in the liquid impingers are determined. The difference in total cells recovered by the impingers between the test and control sides of the system is a function of the test filter efficiency (i.e. filtered vs. unfiltered recoveries).

3. Validation of Aerosol Test System for Production of a Monodisperse Dry Bacterial Aerosol

The system is routinely validated for performance and for production of a monodisperse dry bacterial aerosol by the following general procedure. Initial validation and routine repeat validations of the system are essential to assure that the microbial particles are essentially free of water (not enclosed in droplets) and that the system is performing consistently and properly. Validation is performed using the Andersen Six-Stage Viable Sampler because these are capable of sorting viable particles (e.g. bacteria) on the basis of size. The sampler is fitted with a critical orifice (downstream) to control flow at 1.68 + 0.08 Nm^3/h. Install the sampler at the test filter position.

Tryptic soy agar (or equivalent) plates are placed on each stage of the Andersen Sampler. The plates are labeled with the sampler stage number. A 5 ml suspension of *Brevundimonas (Pseudomonas) diminuta* (1-2 x 10^3 cells/ml) is placed in the nebuliser.

The challenge protocol is performed as described below. At the end of the test, the agar plates are removed, covered and incubated at 32° C for 48 to 72 hours.

When properly designed and functioning, the test system will yield an approximate 90% recovery of the collected *Brevundimonas (Pseudomonas) diminuta* cells on stages 5 and 6, with the balance on stage 4.

Large numbers of colonies appearing on any of stages 1-3 is proof that the aerosol has not dried and that large droplets are present. Discrepancies between sampler recoveries indicate unequal flow rates or cycle time. If these conditions occur, corrective modifications must be made to the system.

4. Bacterial Challenge Procedure

Brevundimonas (Pseudomonas) diminuta (ATCC 19146) suspensions, prepared in sterile phosphate buffer from frozen cell stocks, were used to perform the test challenges (see Section 6.1)

4.1 Sterilise test stand, DeVilbiss No. 40 nebuliser, and all equipment used.

4.2 Dilute bacteria stock suspensions in sterile phosphate buffer to a concentration of 10^8 and 10^{10} per ml. Assemble test system. Liquid impingers are filled with 20 ml of sterile phosphate buffer (pH 7.0, 0.02M).

4.3 Preset two-way timer to change air flow to alternate sides every 5 seconds.

4.4 Add 5 ml of the lower concentration of bacteria suspension (10^8/ml) to nebuliser.

4.5 Initiate vacuum flow so that the vacuum gauges downstream of the impingers read above 15 inches Hg. Turn timer on.

4.6 Initiate sterile, desiccated air flow (minus 40°C dewpoint) so that an appropriate amount of make-up air 16.8 Nm^3/h is supplied to the system.

4.7 Initiate 633 mbar air pressure at DeVilbiss nebuliser. Adjust make-up air as required. Run test for 6 minutes.

4.8 Turn off air supply to nebuliser. Adjust make-up air.

4.9 Allow two minutes to elapse after turning off nebulizer before turning off vacuum and sterile desiccated make-up air and vacuum.

4.10 Turn off two-way timer.

4.11 Disconnect impingers.

4.12 Aseptically measure volume of fluid left in each impinger using sterile 25 ml pipettes.

4.13 Assay each sample of bacteria.

4.14 Repeat steps 4.4 through 4.13 using the higher concentration bacterial suspension (10^{10}/ml). Replace impingers with a new set.

5. Collection of Effluent Organisms

The challenge aerosol effluents are collected using matched, paired all-glass liquid impingement samplers (Ace Glass Inc., Vineland, New Jersey, 14 lpm). Impingers must be matched for flow rate and recovery. Distance from tip of capillary tube to flask bottom must be 4.0 + 0.5 mm. Impingement samplers were autoclaved prior to use (20 minutes, 121°C, slow exhaust) and aseptically filled with 20 ml of sterile 0.2M phosphate buffer, pH 7.0.

6. Microorganism Assay Methods

Brevundimonas (Pseudomonas) diminuta cells collected in challenge retention tests are assayed by removing aliquots of the impingement sampler fluid and passing the samples through sterile 0.2µm analysis membranes. Each membrane is aseptically placed onto Mueller-Hinton Agar plates, incubated at 32°C for 48-72 hours and observed for the presence of colonies.

6.1 Preparation of Stock Suspension from Frozen Cell Paste

6.1.1 Disinfect the tube containing the cell paste by dipping the tube in 95% ethyl alcohol and flaming just long enough to burn off of the alcohol. Use sterile tongs to hold the tube.

6.1.2 Aseptically remove the cap from the tube and drop the tube into a sterile Erlenmeyer flask containing a sterile magnetic stirring bar and 1 litre of a sterile solution of 0.9% NaCl which contains 2 ml of 1M $MgCl_2$ and 2 ml of 1M phosphate buffer at room temperature; e.g. transfer 50 ml aliquot of frozen cell paste into 1 litre sterile solution. Note: $MgCl_2$ and phosphate buffers must be in the solution prior to adding the frozen cell paste to prevent clumping during thawing.

6.1.3 Place the flask on a magnetic stirring unit, and mix until the entire contents of the tube are evenly suspended (30-40 minutes). Store up to three weeks in refrigerator at 3°C. Mix well prior to use.

6.1.4 Determine the concentration of viable cells according to Paragraph 6.4 (Expected concentration of cell suspension is 1-3 x 10^{10} cells/ml).

6.1.5 Identify the organism as *Brevundimonas (Pseudomonas) diminuta* per Paragraph 6.3.

6.2 Preparation of Challenge Suspension

6.2.1 Prepare volume of *Brevundimonas (Pseudomonas) diminuta* challenge suspension by diluting the appropriate amount of the stock suspension with sterile gel-phosphate and mix well.

Example: Stock Titre = 2 x 10^{10}/ml
Desired nebuliser input = 2 x 10^8
Volume of challenge suspension = 5 ml
To make challenge suspension add 0.5 ml of stock to 49.5 ml of sterile gel-phosphate

6.3 Organism Identification *(Brevundimonas (Pseudomonas) diminuta* ATCC No. 19146)

6.3.1 Colony Morphology

6.3.1.1 Colonies of *Brevundimonas (Pseudomonas) diminuta* are yellow-beige, slightly convex and shiny.

6.3.1.2 At 30°C colonies are microscopic to pinpoint at 24 hours, and 1 to 2 mm diameter at 36 to 48 hours.

6.3.2 Microscopic Examination

6.3.2.1 Prepare a gram stain

6.3.2.1.1 Examine the preparation with a compound light microscopic fitted with a calibrated binocular micrometer and an oil immersion objective lens with good resolving power (e.g., a planachromatic objective with an N. A. of 1.2 or greater). Observe several microscopic fields for organism size and arrangement of cells.

6.3.2.1.2 Stained preparation should reveal a gram negative, small, rod-shaped organism about 0.3 to 0.4µm by 0.8 to 1.0µm in size, occurring primarily as single cells.

6.3.2.2 (Optional) Prepare a flagella stain. *Brevundimonas (Pseudomonas) diminuta* is characterised by a single, polar flagellum.

6.3.3 Biochemical Characterisation

6.3.3.1 Perform the following biochemical tests to assure that the organism is positively identified as *Brevundimonas (Pseudomonas) diminuta* ATCC No.19146.

Test	Results
Spore formation	-
OF glucose medium open	-
OF glucose medium, sealed	-
Indole	-
Methyl red	-
Acetylmethylcarbinol	-
Gelatinase	-
Aerobe	+
Catalase	+
Cytochrome (Indophenol) oxidase	+
Growth on MacConkey Agar	+
Denitrification	+
DNAse (DNAse Test Agar)	-
Centrimide Tolerance	-

6.3.3.2 Alternatively, commercially available systems for bacteria identification may be used for identification of *Brevundimonas (Pseudomonas) diminuta.*

6.4 Viable Count

6.4.1 Aseptically remove an aliquot from the prepared suspension of *Brevundimonas (Pseudomonas) diminuta.*

6.4.2 Aseptically prepare decimal serial dilutions of the suspension.

6.4.3 Perform viable colony assay, in duplicate, using standard membrane filter assay procedures. Use 1 ml from each of the 10^{-7} to 10^{-9} dilutions of the cell stock suspension.

6.4.4 Transfer analysis membranes to petri plates containing Mueller-Hinton and incubate assay plates at 30°C ± 2°C for 48 hours.

6.4.5 Count colonies on plates showing between 20 and 200 colonies, and calculate the concentration (cells/ml) of the original suspension.

APPENDIX 2
PROCEDURE FOR AIR FLOW/DIFFERENTIAL MEASUREMENTS

1. Test Apparatus

Figure 6 is a schematic diagram of the assembly required to test a filter under vent conditions. The flowmeter should be calibrated against a traceable standard and give <2% uncertainty of reading. The preferable method is an orifice plate installed and operated in accordance with ISO5167-1. The maximum flow achieved by the test rig will be a function of the inlet and outlet connections of the test housing which should be as large as possible, preferably 63 mm (2 inches) nominal bore.

A clean air supply capable of delivering more than the required flow at the test pressure is necessary. Typically up to 500 Nm^3/h at 2 bar g pressure.

Figure 7 shows the assembly required to test a filter under pressure conditions.

In both cases, the pressure tapping must be within the general requirement of ISO5167-1.

Additionally, great care must be taken to ensure that the system is free of any leaks, since these will give spurious results.

2. Test Procedure

2.1 Vent Mode (Figure 6)

2.1.1 Install the test filter in the housing.

2.1.2 Shut the flow control valves, C and D.

2.1.3 Open the pressure controller (A) and the ball valve B.

2.1.4 Open the flow control valve C in stages (the smaller by-pass valve D is necessary for small flows and fine adjustment).

2.1.5 Record flow, temperature, upstream pressure and pressure differential (dP) at each flow rate.

2.1.6 Shut off the pressure at the control valve B and pressure controller A.

2.2 Pressure Mode (Figure 7)

2.2.1 Install the test filter in the housing.

2.2.2 Shut the flow control valves, C and D.

2.2.3 Open the ball valve B and adjust the pressure controller A until the desired test pressure is reached.

2.2.4 Open the flow control valve (C) in stages.

2.2.5 Record flow, temperature, upstream pressure and pressure differential (dP) at each flow rate.

2.2.6 Shut off the pressure at the ball valve B and pressure controller A.

Figure 6: *Test Set-up for Air Flow / Differential Pressure Measurements in Vent Mode*

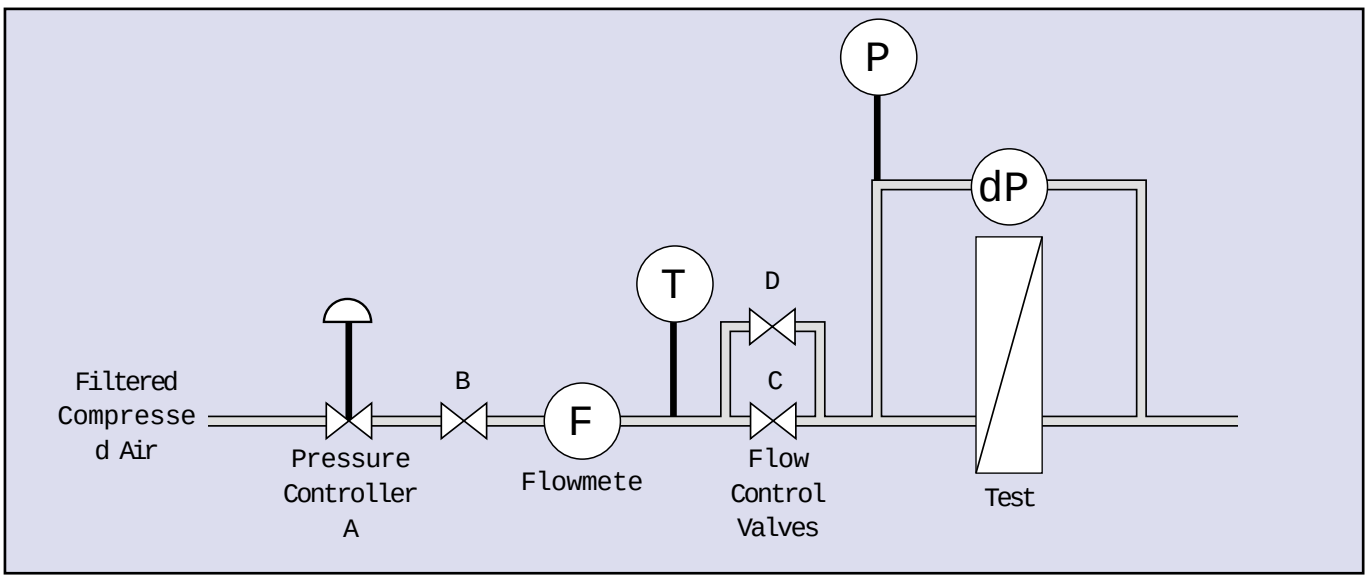
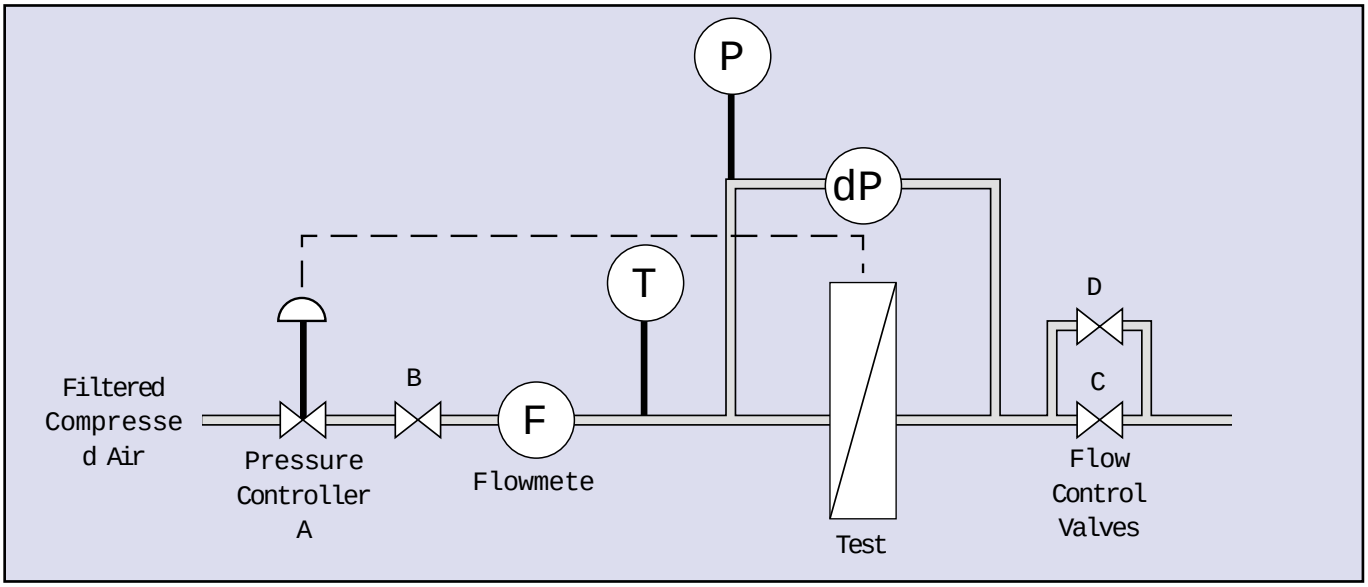


Figure 7: *Test Set-up for Air Flow / Differential Pressure Measurements in Pressure Mode*



APPENDIX 3
PROCEDURE FOR DETERMINATION OF EXTRACTABLES FROM FILTER ELEMENTS

1. Equipment Required

1.1 Automatic reciprocator capable of 20-40 full strokes per minute where 1 stroke is an upward and downward movement.

1.2 Tie rod and code 7 adaptor assembly - made of stainless steel and PTFE.

1.3 Glass graduated cylinders, 2000 ml ± 10 ml.

1.4 Glass round bottom flasks, 1000 ml.

1.5 Rotary evaporator.

1.6 Porcelain crucibles, 25 ml.

1.7 Small portable desiccator.

1.8 Vented circulating oven - Calibrated with an accuracy of ±5%.

1.9 Vacuum source with vacuum measurement device (vacuum pump, aspirator, house vacuum or suitable source of vacuum with vacuum gage).

1.10 Analytical balance - calibrated.

1.11 A furnace maintaining 500°C or higher.

1.12 No. 8 one-hole rubber stopper fitted with glass tube for vacuum connection.

2. Reagents and Materials

2.1 D.I. Water, 0.45µm filtered or better and of high resistivity (greater than 17 M -cm), with known residue of less than 3 ppm.

2.2 95% Ethyl Alcohol, USP/NF

2.3 Teflon* wrap, Parafilm and Aluminum foil

2.4 Laboratory Soap

2.5 Chromerge** Glassware Cleaner

* Teflon is a trademark of E.I. duPont de Nemours, Inc.

** Chromerge is a trademark of Monostat Corporation.

3. Preparation of Apparatus and Materials

3.1 The graduated cylinders and round bottom flasks are cleaned with soap and water, followed by filtered deionised water rinse. They are then cleaned with Chromerge glass cleaner (a 95% sulphuric acid, 5% chromium trioxide mixture), followed by several filtered deionised water rinses.

3.2 Tie rods, adaptors, seal nuts and weights (sealed PTFE bottles, filled with stainless steel beads) are cleaned with soap and water, and rinsed thoroughly with filtered deionised water.

3.3 **Teflon** caps and stainless extension rods are also cleaned as in Step 3.2.

3.4 The glassware is allowed to dry and is covered with aluminum foil or an appropriate cover.

3.5 Test filters are prepared for extraction procedure by integrity testing, flushing, autoclaving using Pall recommended procedures, and allowing to cool to ambient temperature.

4. Extraction Procedure

4.1 Alcohol Extraction

4.1.1 For each series of extractions, a control (blank), in the appropriate solvent (deionised water or 95% ethanol) is also to be performed. The control should consist of the same assembly as the sample, except for the filter cartridge sample. The control consists of the extraction cylinder filled with the same extraction fluid of the same manufacturer and lot number with the same type of tie rod assembly.

4.1.2 Clean gloves must be worn while handling all filters to avoid the possibility of contamination.

4.1.3 Fill the graduated cylinders with 1500 ml of the test fluid (95% ethanol). Record the exact volumes (±10 ml) for both the sample cylinder and control.

4.1.4 Use a precleaned tie rod/adaptor/seal nut/cover assembly to attach the filter to the reciprocating stand.

4.1.5 Immerse the cartridge into the alcohol slowly, allowing the trapped air to escape.

4.1.6 Adjust the apparatus so that the filter is submerged on the downstroke and emerges on the upstroke. The stroke should be equal such that the filter submerges the same distance that it emerges, typical one to two inches.

4.1.7 Reciprocate the filter for 4 hours.

4.1.8 At the end of the extraction period, lift the filter out of the alcohol and allow it to drain into the cylinder. Carefully remove the filter and pour the volume from the filter core into the cylinder. Record the final volume (±10 ml).

4.2 Water Extraction

4.2.1 Follow steps 4.1.1 through 4.1.3 above, using the deionised water in place of 95% ethanol.

4.2.2 Immerse filter bomb fin down into the extracting fluid so that the water comes to the weld line between the code adaptor and the filter element.

4.2.3 Pull vacuum on the core of the filter for 2 minutes by fitting the o-ring adaptor with a clean no. 8 one-hole stopper having a glass tube in it connected to a vacuum line.

4.2.4 After 2 minutes, break the vacuum and remove the stopper.

4.2.5 Perform step 4.1.4 through 4.1.8 above.

5. Procedure for Determination of Non-volatile Residue

5.1 Evaporate the extracting solution, in aliquots, using a clean 1000 ml glass round bottom flask.

5.2 Adjust and maintain the temperature of the water bath to 80°C. Evaporate 1000 ml of sample in aliquots. Evaporate the last aliquot to less than 25 ml.

5.3 Clean porcelain crucibles by heating in furnace at 500°C or higher for approximately 30 minutes. Allow them to cool to room temperature in a desiccator and weigh to the nearest 0.0001 g. Repeat until constant weight is obtained (± 0.0002 g). Store in desiccator.

5.4 Quantitatively transfer the concentrated extract to the tared crucible contained in the desiccator. If residue remains in the round bottom flask, add a few drops of fresh deionised water, swirl and add to crucible. If more than a few drops are needed, note the volume used.

5.5 Carefully place the desiccator containing the crucibles in a circulating oven maintained at 1° below the boiling point of the solvent. Evaporate to dryness.

5.6 Remove the desiccator from the oven after evaporation of the extracting solution, cover and allow to cool to room temperature.

5.7 Weigh the crucibles to the nearest 0.0001 g and record.

5.8 Calculate the non-volatile residue (NVR) for the volume evaporated as follows:

$$NVR_V\ (mg) = C_R\ (mg) - C_C\ (mg)$$

where NVR_V = NVR for volume evaporated, in mg

C_R = constant weight of crucible and

C_C = constant weight of crucible

5.9 Calculate the total NVR for both the control and each sample.

$$NVR_T\ (mg) = NVR_V\ (mg) \times \frac{V_I}{V_E}$$

where NVR_T (mg) = Total NVR, in mg

V_I = initial solvent volume used for extraction

V_E = volume of solvent taken from final volume for evaporation

5.10 Calculate the Net NVR for each sample as follows:

$$\text{Net NVR (mg)} = NVR_S - NVR_C$$

where NVR_S = total NVR of sample, in mg

NVR_C = total NVR of control, in mg

SOUTH MOUNTAIN LABORATORIES, INC.
380 LACKAWANNA PLACE SOUTH ORANGE, NJ 07079

DATE: November 1, 1995 SM# 9503596

SPONSOR: Janet Mathus
Pall Corporation
25 Harbor Park Drive
Port Washington, NY 11050

PRODUCT: Pall Emflon PFA Filter
P.O. #98016

RE: CLASS VI PLASTIC - 121C

REQUIRED: Systemic Injection Test
Intracutaneous Test
Implantation Test

METHOD: U.S.P. XXIII

EXTRACTS:
1. Sodium Chloride Injection
2. 1 in 20 Solution of Alcohol in Sodium Chloride Injection
3. Polyethylene Glycol-400
4. Sesame Oil

RESULTS:

Systemic Injection Test MEETS the requirements.

Intracutaneous Test MEETS the requirements.

Implantation Test MEETS the requirements.

The data for each test is attached.

Analyst: Geri Doerflein 11/3/95

Reviewed by: Barbara Paterson 11/3/95

pa

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