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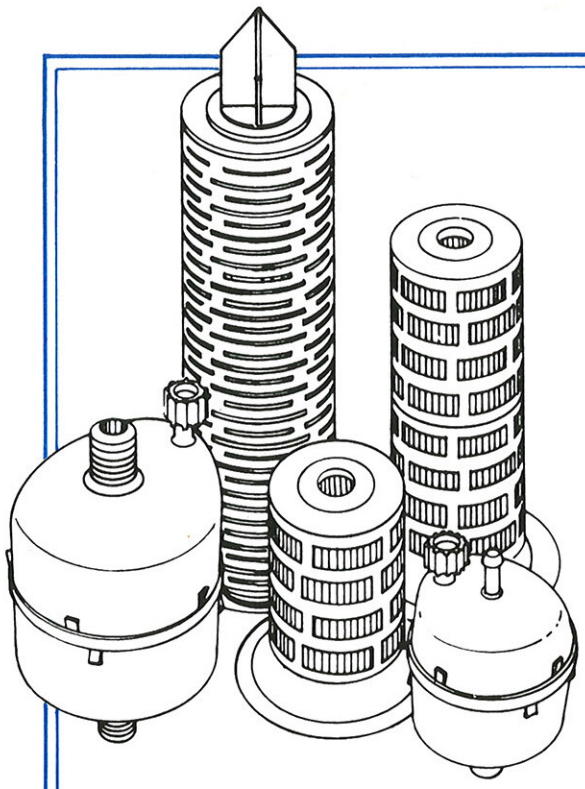

VALIDATION GUIDE for Pall 0.2 µm Emflon® Membrane Cartridges

PALL

Introduction

This guide contains validation data applicable to the Pall Corporation 0.2μm microbially-rated Emflon® elements.

The test program included Extractable Tests, Biological Safety Tests, and Forward Flow Integrity Tests with Microbial Retention Tests.

The letter P in the part numbering code indicates that these elements are intended for pharmaceutical service, and that they are manufactured in Clean Room environments, and are subject to stringent quality control, including stringent in-process control, and testing of the elements as follows:

1. Forward Flow Test, on a 100% basis; and
2. Oxidizables (USP XX) and pH Tests,
3. Effluent Cleanliness Test,
4. Limulus Test, and
5. Microbial Challenge Tests, on a statistical sampling basis.

Materials of construction and performance parameters of the Emflon filters are described in detail in Pall Bulletin EM650A which accompanies this guide.

Pall Corporation will be happy to supply any additional information you may require. Further information may be obtained from the Scientific & Laboratory Services Department, Pall Corporation, Glen Cove, NY 11542.

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SECTION I

VALIDATION OF PALL EMFLON® PRODUCTION FILTERS AS 0.2μm BACTERIALLY RATED FILTERS

Introduction

The correlation between a non-destructive integrity test and assurance of bacterial retention is extremely important for filters used to sterilize fluids.

The industry-accepted non-destructive tests used to verify filter integrity are the *forward flow test* and the *bubble point test*. Both are performed by applying a preset air pressure to a wetted filter, but the forward flow test quantitatively measures the sum of *diffusive flow* and *flow through any pores larger than a specified size,* while the bubble point test is qualitative, and determines only the bulk air flow point of a wetted filter. Pall Corporation uses the forward flow test for non-destructive integrity testing on a 100% production basis.

Parameter Determination

Based on numerous bacteria challenge tests during development of the Pall Emflon filter, it was determined that a forward flow test pressure of 11 psi was suitable for integrity testing Pallsol®* wetted Emflon 0.2μm filters, and that the maximum allowable forward flow value should be set at 20 cc of air per minute for a 8.8 square foot filter cartridge. This test has been applied, on a 100% basis, to production Emflon filter cartridges.

In order to validate the use of 11 psi air pressure and an allowable flow of 20 cc/minute, a number of elements were subsequently selected from routine production.

Validation Bacterial Challenge Procedure

Emflon filter cartridges of 8.8 square-feet surface area were tested as follows, using the apparatus and materials described in Appendix I:

1. Installed in housing.
2. Wetted by flowing Pallsol through the filter.
3. Forward flow integrity tested at 11 psi and test value recorded.
4. Filter in housing was flushed with water to remove the Pallsol, wrapped for autoclaving, autoclave sterilized at 123°C for 60 minutes, slow exhaust, and allowed to cool to room temperature.
5. Filter was installed on the test system and aseptically connected to analysis manifold (see Figure I-1).
6. Valves A, B and C on the analysis manifold were closed and Valve D (Control) was opened.
7. The flow was initiated and the system was run for 15 minutes as a sterility control check.
8. Valves A, B and C were then opened and Valve D was closed. The water flow rate was adjusted to the test flow rate of 1 liter per minute and bacterial input was initiated. Analysis membrane D was removed, plated on Mueller Hinton Agar, and incubated at 32°C for 72 hours.

   Note: *P. diminuta* input was by metering pump at a constant rate for the length of the test with the challenge being 1-3.5 x 10^{12} viable cells. The water flow rate was maintained at 1 liter per minute throughout the test by use of an automatic flow control regulator.
9. At the end of the challenge period, bacterial input was terminated and water flow was allowed to continue for 10 minutes. The test was then stopped and analysis membranes A, B and C were removed aseptically, plated on Mueller Hinton Agar, and incubated for 72 hours at 32°C. Titre Reduction (T_R) was calculated using the formula:

$$T_R = \frac{\text{Number of organisms incident on the filter}}{\text{Total Number of organisms on the three test discs}}$$

   If the effluent is sterile, T_R is greater than 1 x 10^{12}, and retention is 100%.
10. The test filter was again forward flow tested and the value recorded. The higher of the two forward flow values was used in tabulating the data of Table I-1.

    Any test having a contaminated control analysis membrane was considered invalid.

Results

The data from the validation study are listed in order of increasing forward flow values in Table I-1. The data shows that all Emflon filters retained 100% of the *Pseudomonas diminuta* challenge.

Conclusion

The forward flow integrity test maximum value of 20 cc/min per8.8 ft² surface area Emflon filter tested at 11 psig, Pallsol wet, incorporates a substantial safety factor. Emflon filters which have forward flow values equal to or lower than 20 cc/min will provide absolute removal of incident *Pseudomonas diminuta.*

*Pallsol, a solution of tertiary butyl alcohol in water in a ratio of 25 parts by volume of the alcohol in 75 parts by volume of water, is a generally non-flammable wetting liquid for integrity testing of Emflon elements and is miscible with water in all proportions. Note, however, that Pallsol has a flash point in open air of 28°C.

The surface tension of Pallsol is 26.7 dynes/CM. Solution density is 0.966 g/cc and refractive index is 1.3518 to 1.3540 (all at 25°C).

Emflon elements also can be forward flow tested with adequate sensitivity using a 60/40 (v/v) isopropyl alcohol–water mixture. The volumes should be measured separately and then mixed. This fluid is generally more readily available than PALLSOL, but is flammable. The surface tension is very close to that of PALLSOL.

Adequate ventilation must be used when working with alcohols. All containers must be tightly closed to avoid loss of vapor as well as consequent possible significant changes in concentration.

TABLE I

Comparison of Forward Flow with *P. diminuta* Retention for AB1FRP (0.2μm rated) Emflon® Filters

Filter Cartridge Serial Number	Forward Flow at 11 psig (PallSol® Wet) cc/min	Sterile Effluent
82780Z21	7.4	Yes
102180AG	9.4	Yes
110480AE	9.7	Yes
110480AK	10.0	Yes
102180AI	10.0	Yes
110480AB	10.0	Yes
92580I	11.0	Yes
N2090177	16	Yes
92580AG	20	Yes
102180V	24	Yes
N2090094	28	Yes
110480O	30	Yes
110480D	30	Yes
N2090003	31	Yes
N7780464	33	Yes
N7780462	37	Yes
N2090024	40	Yes
92580B	50	Yes
112180U	54	Yes
N2080837	59	Yes
N2070793	65	Yes
112180Z16	71	Yes
92580AF	80	Yes
102480C	98	Yes
92580AZ	100	Yes
N7780470	119	Yes
112180L	132	Yes
102480L	155	Yes
92580K	228	Yes
110480F	233	Yes

Test Challenge: $1.0 - 4.1 \times 10^{12}$ Total *P. diminuta*
($1.4 - 5.7 \times 10^{8}$ per cm^2)

Fluid: Sterile Water
Flow Rate = 1 liter/min.
Total Time = 60 minutes

APPENDIX I
BACTERIAL CHALLENGE PROCEDURE

1. CULTURE AND MEDIA PREPARATION

1.1. Preparation of *Pseudomonas diminuta* (ATCC No. 19146) Stock Culture.

1.1.1 Reconstitute lyophilized preparations of *P. diminuta*, ATCC No. 19146 from the American Type Culture Collection per ATCC directions. Check the purity of the reconstituted culture via streak plates. Examine for uniform colony morphology, and identify single cell isolates as *P. diminuta* per Section 2.
Prepare stock cultures from single cell isolates. Inoculate Trypticase Soy Agar (TSA) slants and incubate at 30 ± 2°C for 24 hours.

1.1.2 Long Term Storage of Cultures
Overlay slants with sterile mineral oil and store at 4°C. Check for viability and purity weekly.
Lyophilize or store in liquid nitrogen a culture prepared from 1.1.1.

1.2 Preparation of Frozen Cell Paste of *P. diminuta*

1.2.1 Inoculate 10 ml of sterile Growth Medium A with the stock culture (1.1.1) and incubate at 30 ± 2°C for 24 hours.

1.2.2 Transfer 10 ml of the bacteria suspension from 1.2.1 into 500 ml of sterile Growth Medium A and incubate at 30 ± 2°C for 24 hours.

1.2.3 Prepare 10 liters of a seed culture by transferring 200 ml of the bacterial suspension from 1.2.2 into 10 liters of sterile Growth Medium A. Incubate at 30 ± 2°C for 24 hours.

1.2.4 Inoculate the 10 liters of the seed culture into 500 liters of Growth Medium A. Grow aerobically at 30 ± 2°C. Monitor growth spectrophotometrically at 500 nm, and plot growth curve.

1.2.5 When the culture reaches the stationary phase, harvest the cells by continuous flow centrifugation.

1.2.6 Resuspend cells in two to three volumes of cold sterile Harvesting Buffer.

1.2.7 Centrifuge suspension and resuspend cells in an equal volume of Harvesting Buffer. Determine the cell concentration. (Expected concentration of viable cells is $2\text{-}6 \times 10^{11}$/ml.)

1.2.8 Transfer aliquots, e.g. 50 ml, of cell paste into sterile plastic centrifuge tubes, and freeze using dry ice-acetone bath or liquid nitrogen. Store frozen cell paste at −60°C or lower.

1.3 Preparation of Stock Suspension from Frozen Cell Paste

1.3.1 Disinfect the tube containing the cell paste by dipping tube in 80% ethyl alcohol and flaming just long enough to burn off most of the alcohol. Use sterile tongs to hold tube.

1.3.2 Aseptically remove the cap from the tube and drop the tube into a sterile Erlenmeyer flask containing a sterile magnetic stirring bar and twenty volumes of a sterile solution of 0.9% NaCl which contains 0.002 *M* $MgCl_2$ at room temperature, e.g. transfer 50 ml aliquot of frozen cell paste into 1 liter sterile solution.
Note: $MgCl_2$ must be in the solution prior to adding the frozen cell paste to prevent clumping during thawing.

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

1.3.4 Determine the concentration of viable cells according to Section 3. (Expected concentration of cell suspension is $1\text{-}3 \times 10^{10}$ cells/ml.)

1.3.5 Identify the organism as *Pseudomonas diminuta* per Section 2.

1.4 Preparation of Challenge Suspension

1.4.1 Prepare one liter of *P. diminuta* challenge suspension by diluting the appropriate amount of the stock suspension with sterile 0.9% saline and mix well.
Example: Stock titre = 2×10^{10}/ml
Desired Total Challenge = 2×10^{12}
Volume of stock required = 100 ml
To make challenge suspension add 100 ml of stock to 900 ml of sterile saline and mix well.

1.4.2 Split challenge volume into two 500 ml aliquots and store in refrigerator until needed. This volume is sufficient for one test.

1.4.3 Immediately prior to starting test, ultrasonically disperse the cells in one of the 500 ml aliquots (from 1.4.2) by placing the container in a 100-watt ultrasonic cleaning bath and sonicating for 10 minutes. This treatment breaks up cell aggregates, but does not damage the cells.

1.4.4 Add a sterile stirring bar to the dispersed suspension, place suspension on magnetic stirring plate, and insert metering pump inlet line. Stir at moderate rate during test.

1.4.5 When volume remaining is about 75 to 100 ml, ultrasonically disperse second 500 ml aliquot of challenge suspension (as in 1.4.3) and add to suspension on stirring unit.
Note: Cells will reaggregate slowly. Sequential addition of freshly sonicated suspension prevents this from occurring during the test period.

1.5 Media Preparation

1.5.1 Trypticase Soy Agar (TSA)
Prepare per manufacturer's directions.

1.5.2 Trypticase Soy Broth (TSB)
Prepare per manufacturer's directions.

1.5.3 Growth Medium A.

	per liter
Trypticase Peptone (or Casitone)	7.5 g
Yeast Extract	2.5 g
NaCl	0.5 g
$MgSO_4$	0.002*M* final

Bring to 1 liter with H_2O, pH 6.8-7.2, autoclave at 121°C for 15 minutes.

1.5.4 Harvesting Buffer

KH_2PO_4	790 mg
K_2HPO_4	1.0 gram

1.5.4 Harvesting Buffer *(continued)*

Glycerol	100 ml
Water	900 ml

Adjust to pH 7.2 with 0.1 *N* KOH, autoclave at 121°C for 15 minutes.

1.5.5 Mueller Hinton Agar

Prepare per manufacturer's directions.

2. ORGANISM IDENTIFICATION

Identification of *Pseudomonas diminuta* (ATCC No. 19146)

2.1 Colony Morphology

2.1.1 Colonies of *Pseudomonas diminuta* are yellow-beige, slightly convex, shiny and entire.

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

2.2 Microscopic Examination

2.2.1 Prepare a gram stain

2.2.1.1 Examine the preparation with a compound light microscope fitted with a calibrated ocular 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.

2.2.1.2 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.

2.2.2 (Optional) Prepare a flagella stain. *Pseudomonas diminuta* is characterized by a single, polar flagellum.

2.3 Biochemical Characterization

2.3.1 Perform the following biochemical tests to assure that the organism is positively identified as *Pseudomonas diminuta*, ATCC #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	–

2.3.2 Alternatively, commercially available systems for bacteria identification may be used for identification of *Pseudomonas diminuta*.

3. VIABLE COUNT

Viable Count of *P. diminuta* Stock and Challenge Suspensions

3.1 Aseptically remove an aliquot from the prepared suspension of *Pseudomonas diminuta*.

3.2 Aseptically prepare decimal serial dilutions of the suspension.

3.3 Perform viable colony assay, in duplicate, using the standard membrane filter assay procedures as follows:

Use 1 ml from each of the 10^{-7} to 10^{-10} dilutions of the frozen cell stock suspension, and from each of the 10^{-6} to 10^{-9} dilutions of the challenge suspension. Place 20 ml of sterile saline into the funnel of the filter holder prior to adding the 1.0 ml aliquots of the decimal dilutions. Filter and wash the walls of the funnel with 20 ml of sterile saline.

3.4 Transfer analysis membranes to petri plates containing TSA and incubate assay plates at 30 ± 2°C for 72 hours.

3.5 Count colonies on plates showing between 12 and 100 colonies, and calculate the concentration (cells/ml) of the original suspension.

4. EQUIPMENT

Note: All system piping, unless otherwise noted, is ½ NPT type 304 stainless steel (SS).

4.1 System Upstream of Test Filter

1 – Ball Valve, ½ NPT, Type 304 SS
1 – Pressure Gauge, 0-150 psi, SS, Marshalltown 89917
1 – Pressure Regulator, 125 psi, bronze or SS
2 – Sanitary Connectors, Triclover 23 BMP, 1" Thermometer caps, Type 304 SS
3 – Clamps, Triclover 13 MHHA, 1½", Type 304 SS
2 – Sanitary Housing, Pall Corp., SANT1G723
4 – Ball Valves (Vent and Drain), ¼ NPT, SS
1 – Flow Rate Controller (Automatic), Kates Type MFA, 0.2-3 GPM, 316 SS, ½ NPT
1 – Metering Pump, Fluid Metering Inc., RPG-6-1-CSC (¼" ceramic piston), and Low Flow Teflon Tube Fittings Kit, Teflon Tubing – ⅛" OD X 1/16 I.D.
1 – Quick Connect Body, Swagelok SS-QC8-B8PF-VT with viton O-ring, ½" NPT, SS

4.2 Test Filter Assembly

1 – Quick Connect Stem, Swagelok SS-QC8-S8PT, ½ NPT, SS
1 – Sanitary Connector, Triclover 23 BMP, 1" Thermometer Cap, Type 304 SS
2 – Clamps, Triclover 13 MHHA, 1½", Type 304 SS
1 – Sanitary Housing, Pall Corp., SANT 1G723
1 – Indicating Pressure Switch, 0-160 psi, ¼" NPT, U.S. Gauge #19029
1 – Vent Valve, needle type, ¼ NPT, SS
1 set – Quick Connect Body and Stem, Swagelok, SS-QC-4-S4PF and SS-QC4B4PM-VT, SS

4.3 Analysis Manifold

1 – Sanitary Connector, Triclover 23 BMP, 1" Thermometer Cap, Type 304 SS
1 – Clamp, Triclover 13 MHHA, 1½", Type 304 SS
1 – Hose Connector, ½" I.D., Hose barb to ½" NPT, SS
1 – Hosing ½" I.D. Tygon, reinforced, 18" long
1 – Pressure Gauge, 0-30 psi, Marshalltown 89917 (severe service gauge), ¼" NPT
4 – Ball Valves, Hoke Rotoball Barstock (#7223F8V), ½" FNPT – ½" FNPT, with Viton O-rings
4 – Hose Connectors, ⅜" ID Hose barb to ½" NPT, SS
4 – Filter Disc Holders, 142 mm diameter, Type 304 SS
4 – Hosing, ⅜" I.D., reinforced Tygon, 12" long.

4.4 Analysis Membrane Filters

4 – Membrane Filter Discs, 0.2μm, 142 mm diameter.

5. EQUIPMENT PREPARATION

5.1 Assemble the equipment listed within each subsection above, e.g. 4.1 (Use Figure I-1 as guide).

5.1.1 Wet the O-rings of the test filter cartridge with water prior to installing the filter in its housing.

5.1.2 Install the 142 mm analysis membrane filter discs (0.2μm) in the housings.

5.2 Pre-Challenge Integrity Test of Test Filter

5.2.1 Install the filter in its housing and flush with Pallsol at a flow rate of 2 liters per minute for 15 minutes. Open the vent on the test filter housing upon initiating flush to completely fill test filter housing, then close the vent.

5.2.2 Perform the Forward Flow integrity test using a test pressure of 11 psig. Record integrity test value (cc/min) obtained.

5.2.3 Flush filter with water at 2 LPM for 10 min. to rinse out Pallsol.

6. EQUIPMENT STERILIZATION AND SET-UP

6.1 Analysis membrane filters in housings.

6.1.1 Connect a short piece (8-10") of autoclavable tubing to vents (vent valves open), and a 12" length of reinforced or pressure resistant autoclavable tubing to the inlet port.

6.1.2 Wrap all open ports and ends of tubing with autoclave paper.

6.1.3 Autoclave at 123°C for 30 minutes, slow exhaust. Allow to cool to room temperature, then close vent valves.

6.2 Analysis manifold with pressure gauge.

6.2.1 Open all valves on manifold.

6.2.2 Wrap all open ports and connecting tubing with autoclave paper.

6.2.3 Autoclave at 123°C for 30 minutes. Allow to cool at room temperature.

6.3 Test filter in housing.

6.3.1 Wrap inlet, outlet and vent (open) with autoclave paper.

6.3.2 Autoclave the test filter in its housing for 60 minutes at 123°C, slow exhaust.

6.4 Aseptic Assembly of Sterilized Equipment (Refer to Figure I-1)

6.4.1 Connect the test filter inlet to the test stand.

6.4.2 Aseptically connect the test filter outlet to the analysis manifold.

6.4.3 Aseptically connect each of the analysis membrane filters to the analysis manifold.

7. CHALLENGE PROTOCOL

7.1 Negative Control (performed immediately prior to Bacteria Challenge).

7.1.1 Connect a piece (~6" long) of tubing to the vent valve on the test filter housing to facilitate venting.

7.1.2 With valves, A, B, C closed and Valve D (control) open, flow water through the test filter and control analysis filter at a flow rate of 2 liters per minute for 15 minutes.

7.1.3 Open valves, A, B, and C and close valve D. Vent the air from each test analysis filter holder.

7.1.4 Aseptically remove the control analysis filter holder and transfer to laminar flow hood.

Note: This should be done before starting bacterial test challenge. Then start challenge per Section 7.2 and continue with 7.1.5 and 7.1.6.

7.1.5 Apply vacuum to downstream side of control analysis filter holder to remove residual liquid.

7.1.6 Aseptically transfer the control analysis filter disc to a Mueller Hinton Agar Plate and incubate at 32°C for 72 hours.

7.2 Bacteria Challenge

7.2.1 Adjust the system flow rate to 1 LPM for each Emflon filter.

7.2.2 Connect the tubing from the positive displacement metering pump, and the bacteria suspension, to the water line upstream of the test filter.

7.2.3 Start the positive displacement (metering) pump to inject the bacteria suspension (prepared per Section 1) into the water feed. The pump rate is adjusted to provide the total bacteria challenge over the test time of approximately 1 hour, giving a total challenge of 1-2 x 10^{12} viable organisms. System flow is continued for 10 additional minutes (1-2 x 10^3/CTA^2).

7.2.4 Shut off water flow and then close valves A, B, and C.

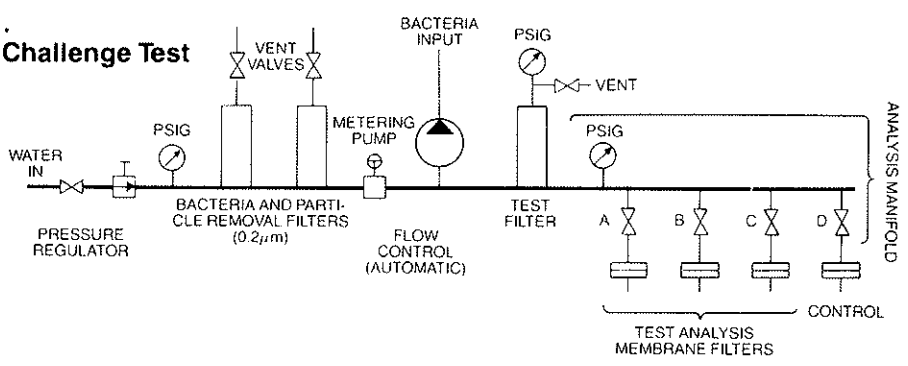
7.2.5 Aseptically remove each test analysis filter holder and transfer to laminar flow hood.

7.2.6 Apply vacuum to the downstream side of each test analysis filter holder to remove residual liquid.

7.2.7 Aseptically transfer each test analysis filter disc onto a labelled Mueller Hinton Agar plate and incubate at 32°C for 72 hours. If colonies appear, count and perform identification per Section 2.

7.2.8 Post-challenge integrity test: Repeat the Forward Flow integrity test on the test filter using the test pressure of 11 psig. Record value obtained.

FIGURE I-1.
Test Set-Up For Bacteria Challenge Test



SECTION II

EXTRACTABLES REPORT AND TEST PROCEDURES ON EMFLON® PRODUCTION FILTERS

Introduction

The purpose of this test series was to determine the amount of material which can be extracted from the 0.2 μm microbial-rated Emflon membrane filter cartridges by water and by commonly used solvents. General Purpose Elements and Special Purpose Elements were tested as completely assembled ten-inch cartridges without O-rings, adaptors or bomb fins.

Summary of Method

Representative filter cartridges were autoclaved unwrapped for one hour at 123°C, using slow exhaust, prior to extraction. A metal basket was used to hold the elements when in the autoclave. Visible droplets of water remaining on the elements after autoclaving were allowed to evaporate at room temperature. Extraction was then performed in the commonly used solvents listed in Table II-1, at the temperatures indicated.

Extraction was performed by immersing the filter cartridge in the solvent and gently reciprocating the cartridge up and down in 1500 ml of the solvent. The bottom of the cartridge was plugged; this ensures that flow of the extracting solvent (eluant) takes place through the filter medium as a result of the pressure head of solvent that is created each time the element is partially lifted out of the solvent during the extraction. After the extraction, 1000 ml of the eluate was evaporated to dryness and the non-volatile extractables were determined gravimetrically. The detailed procedure is described in Appendix II at the end of this Section.

Results

The quantities listed in Table II are in milligrams per filter cartridge. Cartridges were autoclaved for 1 hour at 18 psig prior to testing.

Conclusions

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

The levels of extractables reported are typical of Emflon production elements; some variations from element to element may occur.

Actual service will impose different conditions, such as different exposure times, temperature, solvent purity, etc., hence, evaluation under process conditions is recommended.

TABLE II
Extractables levels of Emflon FRV and FRS Filter Elements:

Non-volatile extractables, determined on 7.54 sq. ft. cartridges by 4 hours exposure in agitated solvent.

	Temp °C	Emflon FRV mg.	Emflon FRS mg.
Acetone	r.t.	92	5
Acetonitrile	r.t.	—	< 1
Acetone/H_2O 70/30 (v/v)	—	—	4
Ethanol, SDA-3A 190 proof	r.t.	62	18
Ethyl Acetate	r.t.	—	14
Hydrochloric Acid 10%	r.t.	54	—
Isopropyl Alcohol	r.t.	62	26
Methylene Chloride	r.t.	500	85
Water①	r.t.	3	—
Water①	80°	3	—

①Element prewet by dip in 2 liters of a 60/40 (v/v) IPA-H_2O solution, then flushed with two successive lots of 1600 ml of H_2O prior to the extraction test.

APPENDIX II

PROCEDURE FOR DETERMINATION OF EXTRACTABLES FROM FILTER ELEMENTS

Preparation of Apparatus and Materials

It is essential that care be taken to prevent the filter cartridge, the eluate and, subsequently, the non-volatile residue from being contaminated with any material which does not originate in the filter itself. Therefore, all apparatus used in this test must be scrupulously clean, and the solvents used must be sufficiently pure so that any non-volatile impurities in the solvent do not contribute significantly to the non-volatile residue obtained upon evaporation of the eluate. Solvents containing more than 2 mg/L non-volatiles (as determined by steps A III.1 or B III.1 of this procedure) should be purified prior to use, preferably by distillation.

This test exists in two modifications: Procedure A for solvents not sensitive to air, and Procedure B for solvents sensitive to air. Filter cartridges used in these procedures were ten inches in length, completely assembled, but without o-rings, adaptors and bomb fins.

PROCEDURE A—FOR SOLVENTS NOT SENSITIVE TO AIR

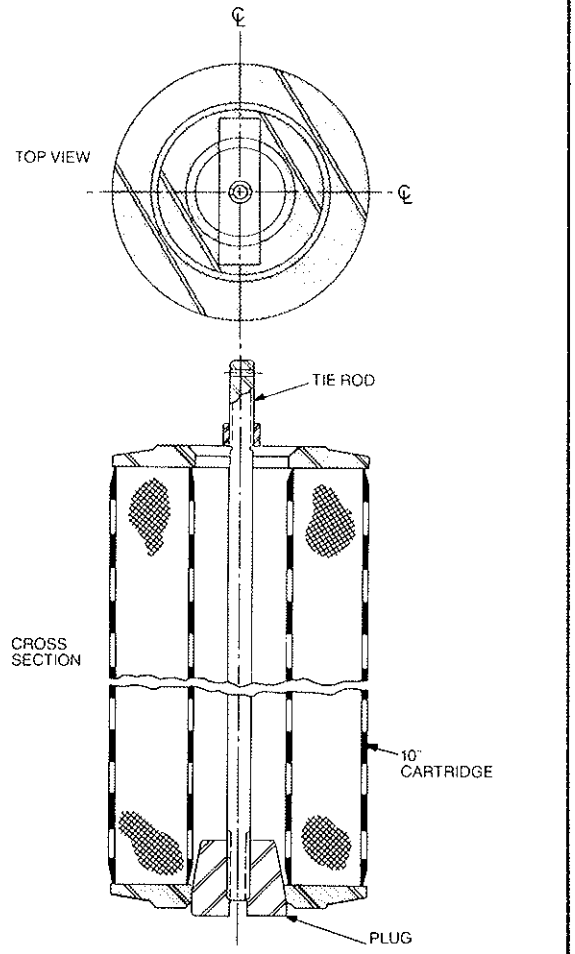
I. Equipment Required

1. 2000 ml Pyrex graduated cylinder, cleaned with chromic acid
2. 1500 ml solvent
3. Aluminum foil
4. 12 rpm motor with eccentric cam, having a 7.5 cm stroke during full cycle, and vertical shaft with hanger
5. Tie rod and plug assembly (see Figure II-1)
6. Rotary evaporator
7. Vacuum pump capable of maintaining pressures of ≤1 mm Hg (for high boiling solvents)
8. 25 ml platinum crucible
9. Circulating air oven
10. Vacuum oven (for high boiling solvents)
11. 3 L Resin kettle (or other flask) with heating mantle
12. Reflux Condenser

II. Extraction Procedure

1. Fit cartridge with tie rod and plug assembly (see Figure II-1).
2. Fill graduated cylinder to 1500 ml mark with solvent. For extraction at elevated temperatures, substitute a 3L resin kettle equipped with a heating mantle and reflux condenser.
3. Immerse cartridge into the measured solvent slowly, allowing all entrained air to escape.
4. Attach shaft of motor to the tie rod assembly (see Figure II-2).
5. Adjust apparatus so that cartridge is submerged at least 1 cm at downstroke, and emerges at least 2 cm at upstroke.
6. Cover graduated cylinder with aluminum foil, slotted to accommodate hanger from cam. For extraction at elevated temperatures, position the hanger through the reflux condenser.
7. Reciprocate for 4 hours at room temperature or other temperature desired.

FIGURE II-1
Tie Rod and Plug Assembly

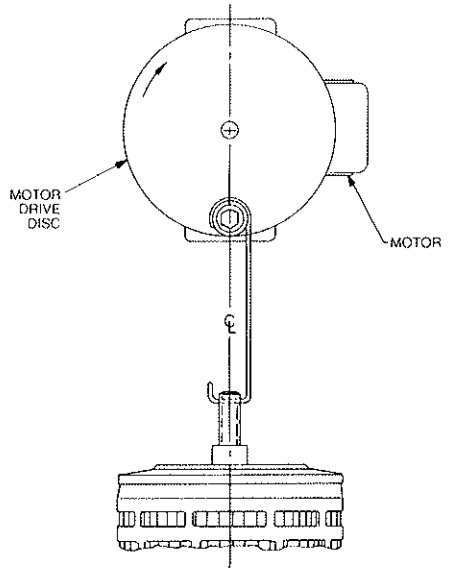


8. At the end of 4 hours remove the filter cartridge from the liquid, allow the solvent to drain into the cylinder, remove the cartridge, and cover the graduated cylinder.
9. If further testing of the cartridge is required, the cartridge should be retained. If no immediate testing is required, rinse the cartridge by successively drawing 2000 ml Fotocol 190 through it, and allow the cartridge to air dry overnight. When dry, seal the cartridge in a plastic bag.

III. Procedure for Determination of Non-volatile Residue

1. Determine solvent blank by allowing 1500 ml pure solvent to remain in a clean 2000 ml graduated cylinder for 4 hours at room temperature and determine the non-volatile residue in the same manner as the following procedure for the filter eluate (i.e. steps 2-8 below).
2. Transfer approximately 250 ml of the eluate into a clean container to be used as a retention sample (e.g. in visual examination for haze after dilution with deionized water), allowing 1000

FIGURE II-2
Attachment of Tie Rod Assembly to Motor Drive Disc



ml to remain in the graduated cylinder. Note that approximately 250 ml of the eluate is retained by the filter cartridge.

3. Concentrate the 1000 ml sample to less than 25 ml by transferring it into a round-bottom flask and using a rotary evaporator at aspirator pressure.

   CAUTION: Aspirator must be located in a vented hood, as the solvent vapors will be delivered in its effluent.

   Bath temperature during evaporation should be approximately 55°C for water and 45°C for other solvents. For high boiling (>150°C) or relatively non-volatile solvents, a vacuum pump should be used. The bath temperature may be raised, if necessary, but should be kept as low as possible. On occasion the bath temperature can be raised above 55°C to enable evaporation at aspirator pressure. Record the pressure and the bath temperature during evaporation of the eluate.
4. Clean a platinum crucible (volume *ca.* 25 ml) by flaming to red heat over a burner for 10 minutes. When cool, tare the crucible, weighing to the nearest 0.0001 g.
5. Quantitatively transfer the concentrate (from Step 3) to the tared platinum crucible.
6. Place crucible, loosely covered with Pyrex evaporating dish, into a circulating air oven maintained at 1°C above the boiling point of the solvent to effect mass transfer and evaporate to dryness. For solvents with b.p. >150°C, or relatively non-volatile solvents, place the crucible, loosely covered with a Pyrex evaporating dish, into a vacuum oven maintained at the same temperature as the evaporation bath (cf. Step 3).
7. Weigh the crucible and its contents to the nearest 0.0001 g. Repeat the drying and weighing (steps 6 and 7) at two-hour intervals until no further change in weight is noted. Save the residue for further tests, if necessary.
8. Calculate non-volatile residue (NVR) as follows:
   NVR = [weight dried (Step 7) − weight crucible (Step 4)] $\times \frac{1500}{1000}$
9. Calculate net non-volatile residue per cartridge as follows:
   Net NVR = NVR total (Step 8) − blank (Step 1)

PROCEDURE B – FOR AIR-SENSITIVE SOLVENTS

I. Equipment Required

1. 2000 ml Pyrex graduated cylinder, cleaned with chromic acid
2. 1500 ml solvent
3. Aluminum foil
4. 12 rpm motor with eccentric cam, having a 7.5 cm stroke during full cycle, and vertical shaft with hanger
5. Tie rod and plug assembly (see Figure II-1)
6. Dry, oil-free nitrogen supply with needle valve control and inlet tube
7. Rotary evaporator
8. Vacuum pump (optional)
9. Vacuum oven

II. Extraction Procedure

1. Fit cartridge with tie rod and plug assembly (see Figure II-1).
2. Fill graduated cylinder to 1500 ml mark with solvent.
3. Immerse cartridge into the measured solvent slowly, allowing all entrained air to escape.
4. Attach shaft of motor to the rod assembly (see Figure II-2)
5. Adjust apparatus so that cartridge is submerged at least 1 cm at downstroke, and emerges at least 2 cm at upstroke.
6. Affix nitrogen inlet tube to inside of graduated cylinder at the top in such a fashion that it will not interfere with the reciprocation. Maintain a slow flow of nitrogen for the duration of the extraction.
7. Cover graduated cylinder with aluminum foil, slotted to accommodate hanger from cam.
8. Reciprocate for 4 hours at room temperature. Extraction at elevated temperatures was not performed with air-sensitive solvents.
9. At the end of 4 hours remove the filter cartridge from the liquid, allow the solvent to drain into the cylinder, remove the cartridge, and cover the graduated cylinder.
10. If further testing of the cartridge is required, the cartridge should be retained. If no immediate testing is required, rinse the cartridge by successively drawing 2000 ml acetone and 2000 ml Fotocol 190 through it, and allow the cartridge to air dry overnight. When dry, seal the cartridge in a plastic bag.

III. Procedure for Determination of Non-volatile Residue

1. Determine a solvent blank by allowing 1500 ml pure solvent to sit in a clean 2000 ml graduated cylinder for 4 hours at room temature under a slow flow of nitrogen and deter-

mining the non-volatile residue in the same manner as the following procedure for the filter eluate (i.e. steps 2-7 below).

2. Transfer approximately 250 ml of the eluate to a clean container to be used as a retention sample (e.g. in visual examination for haze after dilution with deionized water), allowing 1000 ml to remain in the graduated cylinder. Note that approximately 250 ml of the eluate is retained by the filter cartridge.
3. Clean, using chromic acid, a 1000 ml round-bottom flask with a ground-glass joint to fit a rotary evaporator, and dry by flaming. Tare the flask by weighing to the nearest 0.0001 g, preferably, although the nearest 0.001 g is adequate.
4. Evaporate the eluate (1000 ml) to near dryness in the tared flask using the rotary evaporator. Aspirator or vacuum pump pressure should be used as necessary. Bath temperature should be kept at 45°C, but may be raised, if necessary, keeping it as low as possible. Record the pressure and the bath temperature during evaporation of the eluate. At the end of the evaporation period there should be no drops or other evidence of solvent in the flask.
5. Dry the flask (with residue) in a vacuum oven set at 45°C, or the same temperature as the bath in Step 4.
6. Weigh the flask with the residue to the nearest milligram or tenth of a milligram (i.e. the same accuracy as in Step 3). Repeat the drying and weighing (Steps 5 and 6) until no change in weight is observed. Save the residue for further tests, if necessary.
7. Calculate non-volatile residue (NVR) as follows:
   NVR = [weight dried (Step 6) – weight flask (Step 3)] $\times \frac{1500}{1000}$
8. Calculate net non-volatile residue per cartridge as follows:
   Net NVR = NVR total (Step 7) – blank (Step 1).

SECTION III

BIOLOGICAL SAFETY REPORTS AND TEST PROCEDURES ON EMFLON® PRODUCTION FILTERS

Introduction

The purpose of these tests was to evaluate the biological suitability of the materials of construction of the Emflon membrane filters. This was done by performing the Biological Tests for Plastics, as described in the *United States Pharmacopeia*.

Summary

The testing procedures described in the USP include injection of extracts of plastic materials, as well as implantation of the material itself into animal tissue. Four extracting media are listed which simulate parenteral solutions and body fluids. These include: Sodium Chloride Injection, 1 in 20 Solution of Alcohol in Sodium Chloride Injection, Polyethylene Glycol 400, and Vegetable Oil (sesame or cottonseed oil). Extracts are 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 membrane filters will be autoclaved during use, and since the most stringent condition not resulting in physical changes in the plastic is recommended, they were extracted at 121°C.

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 extracts were injected intravenously. Vegetable Oil extract and Polyethylene Glycol 400 extract were injected intraperitoneally.

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 was also performed, in order to subject the materials of construction to the most stringent conditions included in the USP. Each of the components of the Emflon membrane filter cartridges was implanted separately.

Results

The Emflon membrane filter cartridges were found to meet the requirements of the USP for Class VI (121°C) Plastics. The tests were conducted by South Mountain Laboratories, 380 Lackawanna Place, South Orange, New Jersey 07079, and the test reports are provided in Appendix III.

SM SOUTH MOUNTAIN
LABORATORIES · INC.

380 LACKAWANNA PLACE • SOUTH ORANGE, NEW JERSEY 07079 • (CODE 201) 762-0045

DATE: May 8, 1981

REPORT TO: Dr, Hyman Katz
Pall Corporation
Glen Cove, New York

RE: Corrugated Filter Medium & Mesh Support
Lot AB1FRP-PTFE & AB1FRP-PP
SM No. 813107

TESTS REQUIRED: Safety
Class VI

METHOD OF ASSAY: USP XX

RESULTS:

Implant:

Method of Sterilization:	Steam
Rabbits Used:	2
Tissue Examination:	72 Hours

The sample appeared normal and was entirely free from hemorrhage, film or encapsulation. It therefore meets the USP XX requirements for the test. Photos are enclosed.

The sample was found to meet all requirements of the USP XX for Class VI safety. The protocols are enclosed.

C. N. Mangieri

CNM:mf
encl.

C. N. Mangieri
Director

SM SOUTH MOUNTAIN
LABORATORIES · INC.
380 LACKAWANNA PLACE • SOUTH ORANGE, NEW JERSEY 07079 • (CODE 201) 762-0045

DATE: May 8, 1981

REPORT TO: Pall Corporation
Glen Cove, New York

RE: Corrugated Filter Medium & Mesh Support
Lot AB1FRP-PTFE & AB1FRP-PP
SM No. 813107

CLASS PLASTIC VI EXTRACTION TEMP 121 °C 72 HRS.

TESTS REQUIRED: x Extraction; x Systemic Injection, Mice; x Intracutaneous Injection, Rabbit; x Implantation, Rabbit: Pyrogen

METHOD OF ASSAY: USP XX

RESULTS: SYSTEMIC INJECTION; MICE

Physiological Saline-Blank	50 ml/kg. I.V. 5 out of 5 mice	survived 72 hours.
Physiological Saline-Sample	50 ml/kg. I.V. 5 out of 5 mice	survived 72 hours.
	Sample passes Test Requirements.	
Physiological Saline-Alcohol, Blank	50 ml/kg. I.V. 5 out of 5 mice	survived 72 hours.
Physiological Saline-Alcohol, Sample	50 ml/kg. I.V. 5 out of 5 mice	survived 72 hours.
	Sample passes Test Requirements.	
Polyethylene Glycol-400, Blank	10 g/kg. I.P. 5 out of 5 mice	survived 72 hours.
Polyethylene Glycol-400, Sample	10 g/kg. I.P. 5 out of 5 mice	survived 72 hours.
	Sample passes Test Requirements.	
Sesame Oil — Blank	50 ml/kg. I.P. 5 out of 5 mice	survived 72 hours.
Sesame Oil — Sample	50 ml/kg. I.P. 5 out of 5 mice	survived 72 hours.
	Sample passes Test Requirements	

Conclusion: The test sample meets the Systemic Injection Test Requirements.

C. N. Mangieri
Director

SM SOUTH MOUNTAIN
LABORATORIES · INC.
380 LACKAWANNA PLACE • SOUTH ORANGE, NEW JERSEY 07079 • (CODE 201) 762-0045

INTRACUTANEOUS INJECTION

DATE: May 8, 1981

REPORT TO: Pall Corporation
Glen Cove, New York

RE: Corrugated Filter Medium & Mesh Support
Lot AB1FRP-PTFE & AB1FRP-PP
SM No. 813107

EXTRACTS OF SAMPLE

	Physiol. Saline 750			Physiol. Saline Alc. 752-1			Polyethylene Glycol-400 754-1			Sesame Oil 756		
Hours	24	48	72	24	48	72	24	48	72	24	48	72
Erythema	0	0	0	0	0	0	0	0	0	0	0	0
Eschar	0	0	0	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0	0	0	0
	Blank			Blank			Blank			Blank		
Hours	24	48	72	24	48	72	24	48	72	24	48	72
Erythema	0	0	0	0	0	0	0	0	0	0	0	0
Eschar	0	0	0	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0	0	0	0

EVALUATIONS OF SKIN REACTIONS

Erythema and Eschar:	No erythema	0
	Very slight (barely perceptable) erythema	1
	Well defined erythema	2
	Moderate to severe erythema	3
	Severe erythema (beet redness) to slight eschar; injuries in depth	4
Edema:	No edema	0
	Very slight edema (barely perceptable)	1
	Slight edema (edges of area well defined by definite raising	2
	Moderate edema (raised approx. 1mm)	3
	Severe edema (raised more than 1mm and extending beyond area of exposure)	4

CONCLUSION: The test material meets ~~does not meet~~ the USP requirements for the intracutaneous test.

C. N. Mangieri
Director

SM SOUTH MOUNTAIN
LABORATORIES · INC.
380 LACKAWANNA PLACE • SOUTH ORANGE, NEW JERSEY 07079 • (CODE 201) 762-0045

INTRACUTANEOUS INJECTION

DATE: May 8, 1981

REPORT TO: Pall Corporation
Glen Cove, New York

RE: Corrugated Filter Medium & Mesh Support
Lot AB1FRP-PTFE & AB1FRP-PP
SM No. 813107

EXTRACTS OF SAMPLE

	Physiol. Saline			Physiol. Saline Alc.			Polyethylene Glycol-400			Sesame Oil		
	751			753-1			755			757		
Hours	24	48	72	24	48	72	24	48	72	24	48	72
Erythema	0	0	0	0	0	0	0	0	0	0	0	0
Eschar	0	0	0	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0	0	0	0
	Blank			Blank			Blank			Blank		
Hours	24	48	72	24	48	72	24	48	72	24	48	72
Erythema	0	0	0	0	0	0	0	0	0	0	0	0
Eschar	0	0	0	0	0	0	0	0	0	0	0	0
Edema	0	0	0	0	0	0	0	0	0	0	0	0

EVALUATIONS OF SKIN REACTIONS

Erythema and Eschar:	No erythema	0
	Very slight (barely perceptable) erythema	1
	Well defined erythema	2
	Moderate to severe erythema	3
	Severe erythema (beet redness) to slight eschar; injuries in depth	4
Edema:	No edema	0
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	Slight edema (edges of area well defined by definite raising	2
	Moderate edema (raised approx. 1mm)	3
	Severe edema (raised more than 1mm and extending beyond area of exposure)	4

CONCLUSION: The test material meets ~~does not meet~~ the USP requirements for the intracutaneous test.

C. N. Mangieri
Director

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