Validation Guide for
Pall Ultipor® VF™ DV50 Ultipleat™ AB Style Virus Removal Filter Cartridges

PALL

Introduction

This guide contains validation data applicable to Ultipor® VF™ Grade DV50 Virus Removal Filters in Ultipleat™ AB Style cartridge configurations.

The test program included steam sterilizations, Extractables Tests, and Biological Reactivity Tests, in addition to correlation of Forward Flow Integrity Test with Viral Retention Tests.

The letter "P" in the part numbering code indicates that these filters are tested for use in pharmaceutical service, that they are manufactured in controlled environments, and are subjected to stringent quality control including in-process controls and testing of the filter elements as follows:

(1) Forward Flow Test, on a 100% basis; and

(2) Oxidizables and pH Tests,

(3) Effluent Cleanliness Test,

(4) Limulus Amebocyte Lysate Test, and

(5) Viral Challenge Tests, on a sampling basis.

Materials of construction and performance parameters of the Ultipor VF filters are described in detail in Pall Publication DV50, which is intended to be used in conjunction with this guide.

Further information which you may require can be obtained from the Pall Scientific & Laboratory Services Department or Pall Ultrafine Filtration Co., East Hills, NY 11542. Call 1-800-645-6532 or (in NY State) 516-484-5400.

TABLE OF CONTENTS

	Page
Section I	
• Validation of Pall Ultipor VF Grade DV50 Virus Removal Filter	4
• Viral Challenge Validation Procedure	7
Section II	
• Extractables Report and Test Procedures on Ultipor VF Virus Removal Filter Cartridges	9
• Procedure for Determination of Extractables from Ultipor VF Grade DV50 Ultipleat AB Style Filter Elements	10
Section III	
• Biological Reactivity Reports and Test Procedures on Ultipor VF Grade DV50 Virus Removal Filter Cartridges	12
• Appendix: Copies of Laboratory Test Reports	13
Section IV	
• Repeated Steam Sterilization Cycle Report and Test Procedures for Ultipor VF Grade DV50 Virus Removal Filter Cartridges	21

SECTION I

VALIDATION OF PALL ULTIPOR® VF™ GRADE DV50 VIRUS REMOVAL FILTERS

Introduction

The correlation between a non-destructive integrity test and assurance of microbial retention is extremely important for filters used in critical fluid processes. Such processes often occur at the terminal production stages, but may also be introduced at key intermediate stages. Examples include filtration sterilization of parenterals, biological liquids and fluids for fermentation.

Pall Corporation uses the industry accepted Forward Flow Test method on a 100% production basis for the non-destructive integrity testing of sterilizing grade filter elements. For integral filters, these Forward Flow values are measured air volume flow rates (cubic centimeters per minute) due to diffusion of air through the liquid film wetting the pores of the filter membrane. Forward Flow measurement may be performed downstream of the wetted filter membrane under constant test pressure, or on the upstream side of the wetted filter membrane by measuring the air flow required to maintain constant test pressure.

The Pressure Hold Test is a modified form of upstream Forward Flow testing in which the filter housing is pressurized to a predetermined setting, then isolated from the pressure source, and the diffusion of air across the wetted membrane is quantitatively measured as a decay in pressure over a specified period of time. Upstream integrity tests are particularly useful in critical fluid processes since they can be performed without compromising the sterility of the downstream system.

Correlating filter integrity test values with microbial challenge testing is an empirical process resulting in validation data applicable to the test parameters and fluid attributes utilized.

Parameter Determination

Based on viral challenge and forward flow integrity tests during the development of the Pall AB style Ultipor VF Grade DV50 filter cartridges, it was determined that a Forward Flow integrity test pressure of 85 psi was suitable for integrity testing Ultipor VF Grade DV50 filters, wet with dilute (30%) isopropyl alcohol, and the maximum allowable Forward Flow value could be set at 12.5 cc of air per minute for one 10-inch filter cartridge.

In order to validate the use of 85 psi air pressure and a maximum allowable flow of 12.5 cc/minute, filter elements were sampled from production lots and tested as described below using the non-enveloped spherical bacteriophage PR772, which was sized at 53 nm by scanning electron microscopy.

Summary of Viral Challenge Validation Procedure

Pall Ultipor VF Grade DV50 filter cartridges, part number AB1UDV507PH4 filter cartridges (effective filter area ~17.5 ft^2), were tested using the following procedure:

1. Install the filter in the housing.
2. Perform a pre-autoclave, pre-challenge integrity test of the filter as follows: flush with 1.5 liters of 30:70 (v/v) isopropyl alcohol at a flow rate of 250 ml/min. Forward flow integrity test at a test pressure of 85 psi. Record the integrity test value in cc/min.
3. Flush the filter with deionized water at a flow rate of 1 liter per minute for 20 minutes using 30 psi backpressure.
4. Sterilize the wet filter, in the housing, by autoclaving at 15 psi, 121°C, for 60 minutes, on a slow exhaust cycle.
5. Flush the filter with deionized water at a flow rate of 1 lpm for 20 minutes using 30 psi back-pressure. Perform a post-autoclave aseptic forward flow integrity test *in-situ* at 85 psi.
6. Perform the viral challenge as follows:
   a. Fill the sterile pressure vessel with the challenge suspension.
   b. Connect the outlet port of the pressure vessel to the inlet port of the filter housing and the inlet port of the pressure vessel to a regulated air source.
   c. Adjust the air pressure to the pressure vessel, as necessary, to maintain an approximate flow rate of 1 lpm.
   d. Collect 1 liter of effluent.
   e. Stop the challenge flow by clamping the downstream tubing and releasing the pressure from the pressure vessel.
   f. Remove the filter from the housing and sanitize by submerging in 1% sodium hypochlorite overnight.
7. Assay the input and effluent samples for virus content using the agar overlay method. Assay ten 1 ml aliquots of the undiluted effluent.

8. Incubate the plates at 37 ± 2°C overnight and calculate the virus removal efficiency of the filter as follows:

$$\text{Titer Reduction } (T_R) = \frac{\text{Concentration of challenge virus in the influent (PFU/ml)}}{\text{Concentration of challenge virus in the effluent (PFU/ml)}}$$

When no challenge virus is detected downstream of the filter (i.e., the effluent aliquots tested are sterile), 1 is substituted for the recovery (i.e., virus concentration in the effluent) and the T_R is expressed as greater than (>) the calculated value.

9. Following sanitization of the filter, after the viral challenge, drain the sanitizing agent and flush the filter with D.I. water at 250 ml/min for 20 minutes.
10. Perform a post-challenge integrity test of the filter in 30% isopropyl alcohol at 85 psi.

Results

The data from the validation study are listed in Table 1. AB1UDV50 cartridges with Forward Flow values from 6.5 cc/min to 12.8 cc/min when wet with 30:70 (v/v) isopropyl alcohol: water and tested at 85 psi test pressure gave titer reductions for bacteriophage PR772 (sized at 53 nm) of greater than or equal to 10^6, under the test conditions, with virus concentrations of greater than or equal to 10^6 pfu/ml. AB1UDV50 cartridges with Forward Flow values from 13.2 to 18.3 gave titer reductions greater than 10^5.

Conclusion

The Forward Flow integrity test performed at 85 psi in 30:70 (v/v) isopropyl alcohol: water establishes that Ultipleat AB style Ultipor VF grade DV50 filter cartridges with diffusional flows of up to 12.5 cc/min are retentive for 53 nm bacteriophage PR 772 with a titer reduction of > 10^6. Filters with Forward Flow values between 12.5 cc/min and 13.7 cc/min represent a safety margin above the limit value, while filters with Forward Flow values as high as 18.3 demonstrate an additional safety interval where titer reductions of greater than 10^5 can still be expected.

The data showing a reduced titer reduction at higher Forward Flow is indicative of a size exclusion removal mechanism and suggests that viruses larger than approximately 53 nm will also be retained with similar or greater efficiency by a UDV50 filter under these test conditions.

TABLE 1

Correlation of Forward Flow Values of Ultipor® VF™ Grade DV50 AB1UDV50 Filter Elements with Retention of Bacteriophage PR 772 Sized at 53 nm

Filter Element Lot/Serial No.	Forward Flow (cc/min), at 85 psi, 30/70 IPA/Water-Wet	Titer Reduction (T_R) ≥10^6	Titer Reduction (T_R) where <10^6
EF0930104	6.5	Yes	
EF0930103	6.5	Yes	
EF0930105	7.3	Yes	
EF0680098	7.5	Yes	
EF1110132	7.5	Yes	
EF0930102	7.5	Yes	
EF0930101	7.8	Yes	
EF1110237	7.9	Yes	
EF0680100	7.9	Yes	
EF0680137	8.2	Yes	
EF0680105	8.3	Yes	
EF0680035	8.7	Yes	
EF0930145	8.7	Yes	
EF1110090	8.9	Yes	
EF0930214	8.9	Yes	
EF0930124	9.2	Yes	
EF0680209	9.3	Yes	
EF0680044	9.5	Yes	
EF1110048	9.5	Yes	
EF0930134	9.8	Yes	
EF1110139	9.8	Yes	
EF1110244	10.0	Yes	
EF0680154	10.6	Yes	
EF1110240	10.6	Yes	
EF0930128	10.6	Yes	
EF1110078	10.7	Yes	
EF0930136	10.7	Yes	
EF0930139	10.7	Yes	
EF1110297	10.9	Yes	
EF1110083	10.9	Yes	
EF0930140	10.9	Yes	
EF1110134	11.0	Yes	
EF1110130	11.3	Yes	
EF1110206	11.3	Yes	
EF1110329	11.5	Yes	
EF1110313	11.5	Yes	
EF1110109	11.8	Yes	
EF1110315	11.9	Yes	
EF1110142	11.9	Yes	
EF1110186	12.5	Yes	
EF1110247	12.6	Yes	
EF1110317	12.8	Yes	
EF1110104	13.2	No	9.4×10^5
EF1110198	13.2	Yes	
EF1110105	13.5	Yes	
EF1110137	13.7	Yes	
EF1110001	14.0	No	6.0×10^4
EF1110257	14.0	Yes	
EF1110129	14.6	No	6.6×10^5
EF1110177	15.1	Yes	
EF0930106	15.5	No	2.2×10^5
EF1110179	16.5	No	8.2×10^5
EF1110135	16.9	No	2.5×10^5
EF1110289	17.1	No	1.3×10^5
EF1110148	17.6	Yes	
EF1110072	18.0	No	6.8×10^5
EF1110251	18.3	No	4.4×10^5
EF1110189	18.4	No	3.3×10^4
EF0680165	21.8	Yes	

VIRAL CHALLENGE VALIDATION PROCEDURE

1. MICROBIAL STOCKS AND MEDIA PREPARATION

1.1 Bacteriophage and Bacterial Host

Bacteriophage PR772 and its host *Escherichia coli* K12 strain J53-1 were obtained from the Reference Center for Bacterial Viruses, Quebec, Canada.

1.2 Preparation of Bacterial Culture for Use as Phage Host

1.2.1 Host suspensions may be prepared by one of the following methods:

1.2.1.1 Broth may be inoculated using frozen stocks contained in cryogenic tubes. For preparation of the frozen bacterial stocks, following growth of the bacterial culture in Tryptic Soy Broth (TSB) to early logarithmic phase, the culture was aliquoted into 1 ml aliquots containing 10% glycerol and frozen. For use, the frozen stock was allowed to thaw and was then used to inoculate 50 ml of TSB.

1.2.2 Incubate the culture at 37 ± 2°C as required (usually 3-7 hours). Take an OD_{550} reading and record the result before use.

Note: Actively growing bacterial cultures are required to provide a bacterial host lawn for phage assays.

1.3 Preparation of Viral Stocks

1.3.1 Determine the appropriate dilution of stock bacteriophage required to give semi-confluent lysis on the bacterial lawn. (For example, on the surface of a 150 mm Tryptic Soy Agar (TSA) plate, use a dilution of bacteriophage stock that will yield 10^5 plaque forming units per ml (PFU/ml)).

1.3.2 Prepare an actively growing bacterial host culture as described in Section 1.2.

1.3.3 To a sterile tube containing 9 ml of soft agar add the specific dilution of the bacteriophage as prepared in 1.3.1 and appropriate volume of the bacterial host (usually 2 ml). Pour the mixture on to the surface of a 150 mm TSA plate. Allow the plate to solidify at room temperature. Incubate overnight at 37 ± 2°C.

1.3.4 Following incubation the plates should demonstrate semi-confluent to confluent lysis. Harvest the plates using 15 ml TSB per plate. Pool the wash from all the plates into 250 ml centrifuge bottles.

1.3.5 Centrifuge at approximately 3000 rpm for 20 minutes. Decant the supernatant into sterile centrifuge tubes. Centrifuge again using the same conditions. Decant the supernatant into a sterile container.

1.3.6 Filter the supernatant through a sterile 0.2 μm rated membrane filter. Aliquot the bacteriophage stock into 50 ml centrifuge tubes, label and store at 4°C for use in bacteriophage challenges of membranes and filter elements.

1.3.7 Determine the stock bacteriophage titer using the agar overlay method described in Sec 2.0.

1.4 Preparation of Viral Challenge Suspension

1.4.1 Remove phage stock of known titer from refrigerator and mix thoroughly via a Vortex mixer.

1.4.2 Prepare the challenge suspension to the desired challenge level in appropriate volume of carrier fluid that has been prefiltered.

1.5 Media Preparation

1.5.1 Tryptic Soy Agar (TSA): Prepare as per manufacturer's specifications

1.5.2 Tryptic Soy Broth (TSB): Prepare as per manufacturer's specifications

1.5.3 Dilution blanks: Prepare TSB as per manufacturer's specifications, autoclave, and aseptically dispense 4.5 ml into sterile disposable tubes.

1.5.4 Soft agar for use as agar overlay:

1.5.4.1 Prepare soft agar (0.6% nutrient agar) and autoclave as per manufacturer's specifications.

1.5.4.2 Aseptically dispense 4.5 ml of soft agar into sterile glass test tubes and keep molten in 48 - 50°C water bath until use.

1.6 Carrier Fluid (MEM-10) for Viral Challenge

Dulbecco's Modified Eagle Medium (MEM) supplemented with 10% newborn calf serum:

1.6.1 Supplement MEM with an appropriate volume (to provide a 10% concentration) of newborn calf serum which has been thawed.

1.6.2 Filter the entire contents through an 0.2 μm-rated Bio-Inert® filter (Pall P/N SLK7002NRLP) which has been forward flow integrity-tested and sterilized (autoclaved 121°C, 60 minutes, slow exhaust). Add the appropriate concentration of the challenge virus to the carrier fluid.

2. BACTERIOPHAGE ASSAYS

2.1 Assay Procedure

2.1.1 Make serial ten-fold dilutions in TSB of the sample(s) to be assayed.

2.1.2 Dispense 0.5 ml of appropriate bacterial host into a sterile test tube containing 4.5 ml of molten soft agar.

2.1.3 Add 1 ml of the undiluted or diluted phage suspension to the soft agar-bacterial host

mixture. Mix gently and pour onto the surface of a 100 mm TSA plate. Swirl plate gently to ensure that the entire surface of the plate is covered.

2.1.4 All phage assays are done in duplicate.

2.1.5 Following solidification of the plates, incubate overnight at 37 ± 2°C.

2.2 Calculation of Titer Reduction

The virus removal efficiency of the Ultipor VF Grade DV50 filter cartridge, evaluated in terms of the Titer Reduction (T_R), is calculated as follows:

$$\text{Titer Reduction } (T_R) = \frac{\text{Concentration of challenge virus in the influent (PFU/ml)}}{\text{Concentration of challenge virus in the effluent (PFU/ml)}}$$

When no challenge virus is detected downstream of the filter (i.e., the effluent aliquots tested are sterile), 1 is substituted for the recovery (i.e., virus concentration in the effluent) and the T_R is expressed as greater than (>) the calculated value.

3.0 TEST EQUIPMENT

Equipment for Viral Challenge

1 - Pressure Vessel, Mensco (CA), 2 gal., P/N 72-02-V-M type 304 SS

1 - Sanitary Filter Housing, Pall Ultrafine Filtration Co., East Hills, NY, VSANT1G723

1 - Sterile side arm vacuum flask, 1 liter

1 - Fairchild Model # 30252, pressure regulator, 0-100 psi

1 - 0-60 psi gauge 2.5", 0.25 NPTLM Wika # 111.10

2 - Clamps, 1.5" tri-clamp 13MHHA type 304 SS

1 - Vent valve, needle type, 1/4" NPT ss

1 - Quick connect ss-QC8-S-8PM male NPT

1 - Quick connect ss-QC8-B-8PM female NPT

2 - Sanitary connector, tri-clamp 23BMP, 1" thermometer cap, type 304 SS
Braided tubing (Sanitech), 3/8" I.D.

4. EQUIPMENT PREPARATION, STERILIZATION AND SET-UP

4.1 Pre-autoclave, Pre-challenge Forward Flow Integrity Test of Filter

4.1.1 Install test filter into housing and flush with 1.5 l of 30:70 isopropyl alcohol at a flow rate of 250 ml/min. Record the upstream pressure during the flush. Open the vent on the test filter housing upon initiating the flush to ensure that the test filter housing is completely full; close vent.

4.1.2 Perform the forward flow integrity test at a test pressure of 85 psi. Record the integrity test value in cc/min.

4.1.3 Flush the filter with deionized water at a flow rate of 1 lpm for 20 minutes using 30 psi back-pressure. Backpressure is accomplished by attaching a valve downstream of the filter housing with a gauge situated between the downstream of the housing and the valve. Record the upstream pressure during the flush. Proceed directly to sterilization of the filter and test equipment.

4.2 Sterilization of Test Equipment and Filter

4.2.1 Attach two 12" lengths of autoclavable braided tubing to both the inlet and outlet sides of the test filter housing.

4.2.2 Connect an 8-10" length of tubing to the bleed port vent (vent valve open).

4.2.3 Wrap the bleed tube and upstream and downstream sides of the test housing with autoclave paper.

4.2.4 Place pressure vessel, the test hardware and all tubing to be used for challenge into the autoclave. The test filter must be autoclaved wet in the filter housing. If a considerable amount of time has lapsed since the integrity test has been performed it may be necessary to re-wet the filter prior to positioning it in the filter housing. Autoclave all test equipment including the filter at 15 psi, 121°C, for 60 minutes, on a slow exhaust cycle. Allow filter and housing to cool to ambient temperature prior to performing the post-autoclave aseptic forward flow integrity test and proceeding with the viral challenge.

4.3 Post-autoclave, Pre-challenge Forward Flow Integrity Test of Filter

4.3.1 While maintaining aseptic conditions downstream of the sterilized filter, flush with deionized water at a flow rate of 1 lpm for 20 minutes using 30 psi backpressure. Perform a water-wet Forward Flow test *in-situ* at 85 psi. Proceed with the viral challenge.

5. CHALLENGE PROTOCOL

5.1 Viral Challenge of Filters

5.1.1 Fill the sterile pressure vessel with the challenge suspension.

5.1.2 Take an input sample of the challenge suspension from the pressure vessel to determine the input titer.

5.1.3 Attach the outlet port of the pressure vessel to the inlet port of the filter housing. Connect the inlet of the pressure vessel to a regulated air source.

5.1.4 Attach side arm of a sterile vacuum flask to the downstream tube of the test housing, leaving the top of the flask loosely covered with aluminum foil.

5.1.5 Clamp off the downstream tube and allow challenge suspension to flow through the bleed valve of test housing to properly bleed the system.

5.1.6 Close the bleed valve and remove clamp from the downstream side of the test housing. Adjust the air pressure to the pressure vessel, as necessary, to maintain an approximate flow rate of 1 lpm.

5.1.7 Collect 1 liter of effluent in the sterile vacuum flask.

5.1.8 Stop the challenge flow by clamping the downstream tubing and releasing pressure from the pressure vessel. Disconnect the test housing and connect a second test housing. Repeat steps 5.1.3 through 5.1.8 for the other filters to be challenged.

5.1.9 Remove the filters from the housings and sanitize as described in Sec. 5.3

5.2 Viral Assay

5.2.1 Proceed with assay of the input and effluent samples using the agar overlay procedure described in Sec. 2.

5.2.2 To increase the sensitivity of the assay, in addition to dilutions of the effluent, assay ten 1 ml aliquots of the undiluted effluent.

5.3 Sanitization of Filters Post Use

5.3.1 Submerge the filters in 1% sodium hypochlorite overnight.

5.4 Post-Challenge Integrity Test of Filters

5.4.1 Drain the sanitizing agent and rinse the filter.

5.4.2 Place the filter in the housing and flush the filter with deionized water at a flow rate of 250 ml/min for 20 minutes.

5.4.3 Drain the housing and perform the Forward Flow Integrity Test in water at 85 psi. Record the value obtained in cc/min.

5.4.4 Flush the filter with 1.5 l of 30:70 isopropyl alcohol at 45 psi forward pressure. Drain the housing and forward flow integrity test at 85 psi. Record the value in cc/min.

SECTION II

EXTRACTABLES REPORT AND TEST PROCEDURES ON ULTIPOR® VF™ VIRUS REMOVAL FILTER CARTRIDGES

Introduction

The purpose of these tests is to quantify and characterize the materials which may be extracted from the Ultipor VF Grade DV50 filter cartridge into aqueous products when the filter is used for virus reduction in accordance with the procedures validated by Pall Corporation. Ten inch (254 mm) Ultipleat AB style filters (17.5 ft^2 filter media surface area), constructed of a polyvinylidene difluoride filter membrane, polyester drainage material, and polypropylene molded endcaps and molded components, from routine production were tested for non-volatile, water-soluble extractable matter. This matter was then analyzed by infrared and ultraviolet spectrophotometry.

Summary of Method

Ultipor VF Grade DV50 filter cartridges AB1UDV507PH4 from inventory were tested for extractable matter in the state the filters would typically be in at the onset of filtration. Whenever the Ultipor VF Grade DV50 filter cartridge is used in the processing of pharmaceutical or biological products the filter would normally be integrity tested and sterilized before filtration. These procedures were carried out according to the procedures validated by Pall.

1. Integrity testing in 30/70 (V/V%) IPA-water mixture;
2. Flushing with 0.2μ filtered deionized water;
3. Steam sterilizing at 121°C for one hour;
4. Flushing with 0.2μ filtered deionized water, and (optionally) performing a post-autoclave aseptic integrity test, water-wet.

After these procedures have been carried out, the filter is in the state it would be in at the onset of the actual filtration step.

The test filters were then extracted by reciprocating for 24 hours in 1500 ml deionized water at ambient temperature according to the procedure described below. At the end of the 24-hour period the ultraviolet absorption spectrum of the eluates were measured over the range 200-360 nm. 1000 ml of each eluate was evaporated to dryness and the amount of non-volatile residue was determined. The residue was characterized by infrared spectroscopy.

Results

After the recommended procedures for integrity testing and sterilization, the UDV50 filters tested showed ≤1.5 mg of non-volatile materials when extracted with water at ambient temperature.

The ultraviolet absorption spectrum of this eluate was measured using a Hewlett-Packard Model 8452A Diode Array Spectrophotometer. The eluate showed essentially no UV absorbance over the range of 200 to 360 nm measured.

The infrared spectrum of the non-volatile residue was measured for sample in the form of a KBr pellet using a Nicolet Model 510P Fourier Transform Infrared Spectrophotometer. The spectrum obtained is shown in Fig. 1. The infrared absorptions at 1720 cm^{-1}, 1630 cm^{-1}, 1384 cm^{-1} and 1102 cm^{-1} indicate that the material consists mainly of acrylate residues from the chemically bonded polymer coating which makes the filter membrane surface hydrophilic.

Conclusions

The levels of aqueous extractables found for Ultipor VF Grade DV50 filter cartridges are extremely low. Actual service may impose different conditions, such as different fluids, exposure times, and temperature. Evaluation under process conditions is suggested.

PROCEDURE FOR DETERMINATION OF EXTRACTABLES FROM ULTIPOR VF GRADE DV50 ULTIPLEAT AB STYLE FILTER ELEMENTS

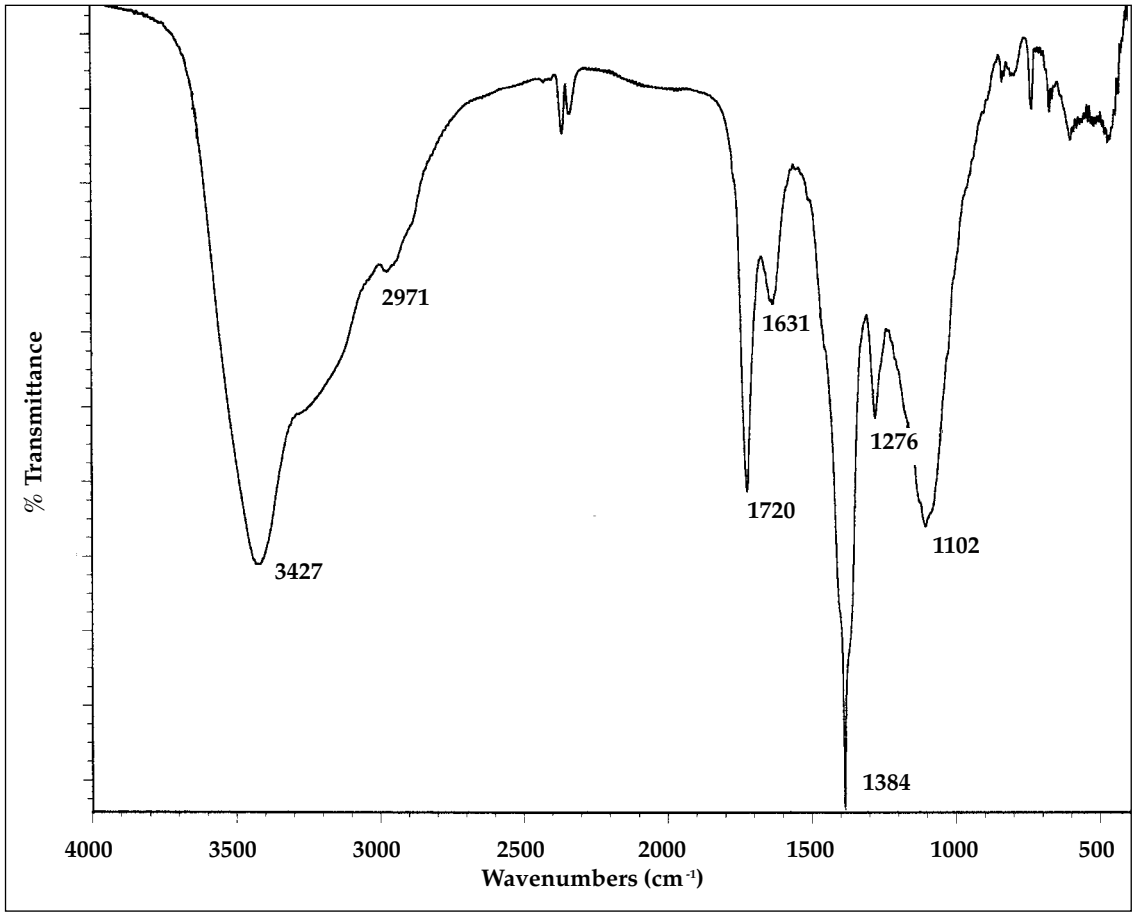
I. Equipment Required

1. Automatic reciprocator capable of 20-40 full strokes per minute where 1 stroke is an upward and downward movement, or 12 RPM motor.
2. Teflon* Pall Code 7 or Code 8 Adapter Assembly with stainless steel extension rod.
3. Glass Graduated Cylinders, 2000 ml ± 10 ml
4. Glass Round Bottom Flasks, 1000 ml
5. Rotary Evaporator
6. Porcelain Crucibles, 25 ml
7. Small Portable Desiccator
8. Vented Circulating Oven - calibrated with an accuracy of ±5, capable of maintaining 100°C.
9. Vacuum Source with Vacuum Measurement Device (vacuum pump, aspirator, house vacuum, or suitable source of vacuum with vacuum gage)

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

FIGURE 1

Infrared Spectrum of Non-Volatile Residue as KBr Pellet



10. Analytical Balance - calibrated and capable of measuring at least 130g with reproducibility of ±0.1 mg and linearity of ±0.2 mg
11. Furnace capable of maintaining 500°C or higher.

II. Reagents and Materials

1. Sterile Water for Injection (WFI)
2. Aluminum foil
3. Laboratory Soap
4. Chromerge Glassware Cleaner or similar

III. Preparation of Apparatus and Materials

1. The graduated cylinders and round bottom flasks are cleaned with soap and water, followed by filtered deionized water rinse. They are then cleaned with Chromerge glass cleaner (a 95% sulfuric acid, 5% chromium trioxide mixture) or a suitable cleaner, followed by several filtered deionized water rinses.
2. Tie rods, adapters, teflon caps and stainless extension rods are cleaned with soap and water, and rinsed thoroughly with filtered deionized water.
3. The glassware is allowed to dry and is covered with aluminum foil.
4. Test filters are prepared for extraction procedure by integrity testing, flushing, autoclaving and post-autoclave integrity testing using Pall recommended procedures.

IV. Extraction Procedure

1. For each series of extractions, a control (blank) is also to be performed. The control consists of the extraction cylinder filled with the same Water for Injection (WFI) fluids.
2. Clean gloves (powder free) must be worn while handling all filters to avoid the possibility of contamination.
3. Fill the graduated cylinders with 1500 ml of the WFI. Record the exact volumes (±10 ml) for both the sample cylinder and control.
4. Use a precleaned tie rod/adapter/extension rod assembly to attach the filter to the reciprocating motor or stand.
5. Immerse the cartridge into the WFI slowly, allowing the trapped air to escape.
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.
7. Cover the top of the cylinder with an appropriate cover (i.e. aluminum foil or Teflon).
8. Reciprocate the filter for 24 hours.
9. At the end of the extraction period, lift the filter out of the water 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).

V. Procedure for Determination of Non-Volatile Residue

1. Evaporate the water, in aliquots, using a clean 1000 ml glass round bottom flask.
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.
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.
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 deionized water, swirl and add to crucible. If more than a few drops are needed, note the volume used.
5. Carefully place the desiccator containing the crucibles in a circulating oven maintained at 95°C. Evaporate the water to dryness.
6. Remove the desiccator from the oven after evaporation of the water, cover and allow to cool to room temperature.
7. Weigh the crucibles to the nearest 0.0001 g and record.
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 my
C_R = constant weight of crucible and residue
C_C = constant weight of crucible

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

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

where NVR (mg)= Total NVR, in mg
V_I = initial solvent volume used for extraction
V_E = volume of solvent taken from final volume for evaporation

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

SECTION III

BIOLOGICAL REACTIVITY REPORTS AND TEST PROCEDURES ON ULTIPOR® VF™ GRADE DV50 VIRUS REMOVAL FILTER CARTRIDGES

Introduction

The purpose of these tests was to evaluate the biological suitability of the materials of construction of the Pall Ultipor® VF™ Grade DV50 virus removal filter cartridges. This was done by performing the Biological Reactivity Tests, In-Vivo, for Plastics, as described in the *United States Pharmacopeia (USP)* Chapter <88>.

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 Ultipor VF Grade DV50 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 filter cartridges was implanted separately.

Results

The Ultipor® VF™ Grade DV50 virus removal 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 07979, and copies of the tests reports are provided herein.

SOUTH MOUNTAIN LABORATORIES, INC.

380 LACKAWANNA PLACE • SOUTH ORANGE, NEW JERSEY 07079 • (201) 762-0045 • FAX: (201) 762-4685

DATE: September 29, 1994

SM #9403252

REPORT TO: Janet Mathus

Pall Corporation
30 Sea Cliff Ave.
Glen Cove, NY 11542

RE: Pall Ultipor VF Filters
P.O. #98016

TESTS REQUIRED: Class VI

FPO

METHOD OF ASSAY: U.S.P. XXII

RESULTS: The sample was found to meet the requirements of the U.S.P. XXII for Class VI plastics. The data is attached.

Analyst: _____ 10/7/94

Reviewed by: Julia Gbur 10/7/94

pa

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LABORATORIES · INC.
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DATE: September 24, 1994 SM #9403252

REPORT TO: Janet Mathus

Pall Corporation
30 Sea Cliff Ave.
Glen Cove, NY 11542

RE: Pall Ultipor VF Filters
P.O. #98016

CLASS PLASTIC CLASS VI EXTRACTION TEMP 121°C 1 HRS.

TESTS REQUIRED: Extraction; (Systemic Injection, Mice;) Intracutaneous

Injection, Rabbit; Implantation, Rabbit: Pyrogen

METHOD OF ASSAY: U.S.P. XXII

FPO

RESULTS: SYSTEMIC INJECTION; MICE

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

Conclusion: The test sample meets ~~does not meet~~ the Systemic Injection Test Requirements.

ANALYST: [signature] 10/7/94

REVIEWED BY: Julia Glass 10/7/94

SM SOUTH MOUNTAIN LABORATORIES · INC.
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INTRACUTANEOUS INJECTION

DATE: September 29, 1994 SM #9403252

REPORT TO: Janet Mathus

Pall Corporation
30 Sea Cliff Ave.
Glen Cove, NY 11542

RE: Pall Ultipor VF Filters
P.O. #98016

EXTRACTS OF SAMPLE

METHOD OF ASSAY: U.S.P. XXII CLASS VI

	Physiol. Saline			Physiol. Saline Alc.			Polyethylene Glycol-400			Sesame Oil		
Rabbit #:	I652			I656			I658			I660		
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	1	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	1	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.

ANALYST: [signature] 10/7/94

REVIEWED BY: Julia Glass 10/7/94

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INTRACUTANEOUS INJECTION

DATE: September 29, 1994 SM #9403252

REPORT TO: Janet Mathus

Pall Corporation
30 Sea Cliff Ave.
Glen Cove, NY 11542

RE: Pall Ultipor VF Filters
P.O. #98016

EXTRACTS OF SAMPLE

METHOD OF ASSAY: U.S.P. XXII CLASS VI

	Physiol. Saline			Physiol. Saline Alc.			Polyethylene Glycol-400			Sesame Oil		
Rabbit #:	40E			I655			56			I659		
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	1	0	0	0	0	0	1	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	1	0	0	0	0	0	1	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.

ANALYST: _____ 10/7/94

REVIEWED BY: Julia Gbur 10/7/94

SOUTH MOUNTAIN LABORATORIES, INC.

380 LACKAWANNA PLACE • SOUTH ORANGE, NEW JERSEY 07079 • (201) 762-0045 • FAX: (201) 762-4685

DATE:	September 29, 1994	SM #9403252
REPORT TO:	Janet Mathus	Pall Corporation 30 Sea Cliff Ave. Glen Cove, NY 11542
RE:	Pall Ultipor VF Filters P.O. #98016	
TESTS REQUIRED:	Animal Implant	
METHOD OF ASSAY:	U.S.P. XXII	

FPO

RESULTS:

Method of Sterilization:	Steam
No. of rabbits used:	2
Tissue examination:	72 hours

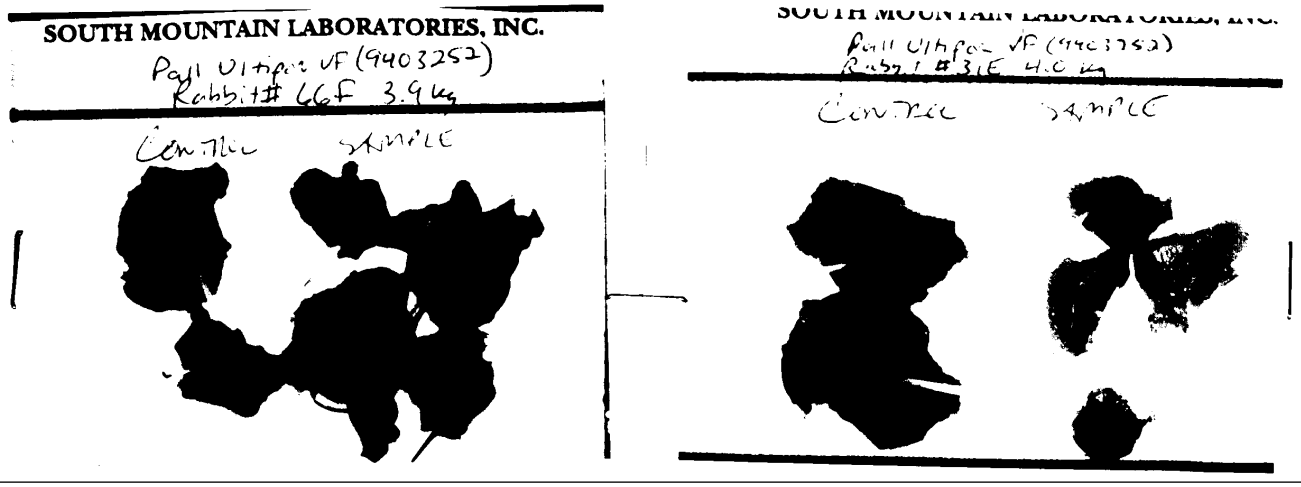
The sample appeared normal and was entirely free from hemorrhage, film or encapsulation.

The sample, therefore, meets the requirements of the test.

Photographs are enclosed.

Analyst: _____ 10/7/94

Reviewed by: Julia Gbur 10/7/94



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380 LACKAWANNA PLACE • SOUTH ORANGE, NEW JERSEY 07079 • (201) 762-0045 • FAX: (201) 762-4685

DATE: October 14, 1994

SM #9403252

REPORT TO: Janet Mathus

Pall Corporation
30 Sea Cliff Ave.
Glen Cove, NY 11542

RE: Ultipor VF Filters
Polypropylene end caps
P.O. #98016

TESTS REQUIRED: Animal Implant

METHOD OF ASSAY: U.S.P. XXII

FPO

RESULTS:

Method of Sterilization:	Steam
No. of rabbits used:	2
Tissue examination:	72 hours

The sample appeared normal and was entirely free from hemorrhage, film or encapsulation.

The sample, therefore, meets the requirements of the test.

Photographs are enclosed.

Analyst: [signature] 10/14/94

Reviewed by: [signature] 10/14/94

SOUTH MOUNTAIN LABORATORIES, INC

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380 LACKAWANNA PLACE • SOUTH ORANGE, NEW JERSEY 07079 • (201) 762-0045 • FAX: (201) 762-4685

DATE: October 14, 1994

SM #9403252

REPORT TO: Janet Mathus

Pall Corporation
30 Sea Cliff Ave.
Glen Cove, NY 11542

RE: Ultipor VF Filters
Polyester drainage material
P.O. #98016

TESTS REQUIRED: Animal Implant

FPO

METHOD OF ASSAY: U.S.P. XXII

RESULTS:

Method of Sterilization:	Steam
No. of rabbits used:	2
Tissue examination:	72 hours

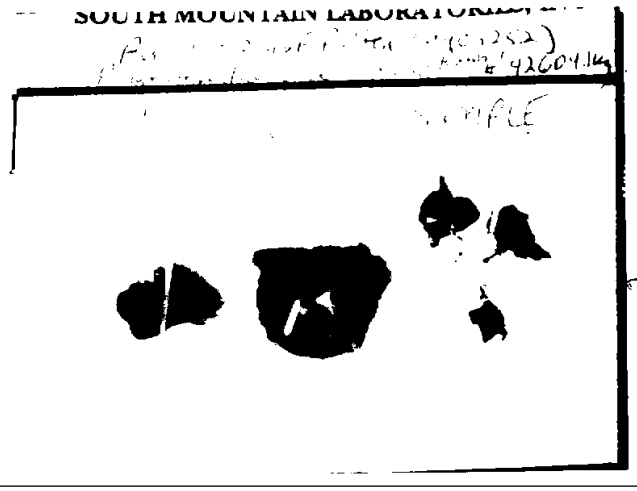
The sample appeared normal and was entirely free from hemorrhage, film or encapsulation.

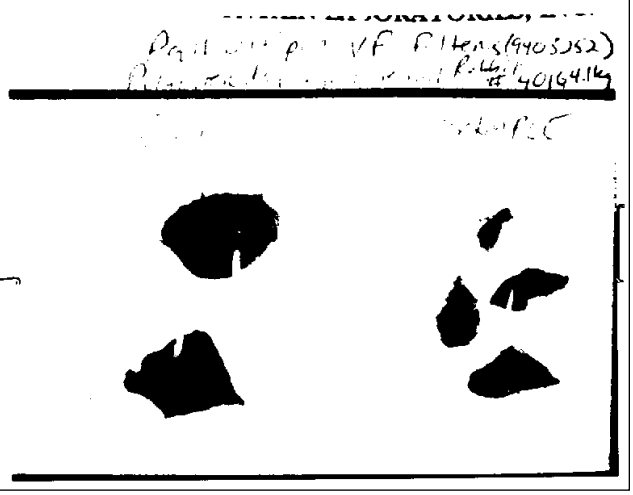
The sample, therefore, meets the requirements of the test.

Photographs are enclosed.

Analyst: [signature] 10/14/94

Reviewed by: [signature] 10/14/94





SOUTH MOUNTAIN LABORATORIES, INC.

380 LACKAWANNA PLACE • SOUTH ORANGE, NEW JERSEY 07079 • (201) 762-0045 • FAX: (201) 762-4685

DATE:	October 14, 1994	SM #9403252
REPORT TO:	Janet Mathus	Pall Corporation 30 Sea Cliff Ave. Glen Cove, NY 11542
RE:	Ultipor VF Filters PVDF hydrophilic filter medium (corrugated membrane) P.O. #98016	
TESTS REQUIRED:	Animal Implant	
METHOD OF ASSAY:	U.S.P. XXII	

FPO

RESULTS:

Method of Sterilization:	Steam
No. of rabbits used:	2
Tissue examination:	72 hours

The sample appeared normal and was entirely free from hemorrhage, film or encapsulation.

The sample, therefore, meets the requirements of the test.

Photographs are enclosed.

Analyst: [signature] 10/14/94

Reviewed by: [signature] 10/14/94

SECTION IV

REPEATED STEAM STERILIZATION CYCLE REPORT AND TEST PROCEDURES FOR ULTIPOR® VF™ GRADE DV50 VIRUS REMOVAL FILTER CARTRIDGES

Introduction

The purpose of these tests is to demonstrate that the Ultipor VF Grade DV50 Ultipleat AB style filter cartridges reliably retains integrity after repeated cycles of steam sterilization, performed according to the procedures recommended by Pall Corporation. The integrity of 10-inch (254 mm) filter cartridges from routine production was verified at the beginning of the test and then verified again after each sterilization cycle.

Summary of Method

Three Ultipor VF Grade DV50 filter cartridges, p/n AB1UDV507PH4 from production inventory were tested for integrity by performing a forward flow test. The filters were wetted thoroughly with 30/70 (v/v%) IPA-water mixture and a test pressure of 85 psi was used. All filters tested met the forward flow limit recommended in Section I of this Guide.

The filters were then flushed with water to expel any alcohol and were then sterilized by steam autoclaving for 1 hour at 125°C. After autoclaving (and flushing again to ensure complete wetting with water), a water-wet forward flow test at 85 psi was performed as a first indication of retaining integrity after autoclaving. The filters were then dried overnight and the integrity was verified by means of the virus-correlated IPA-water forward flow test at 85 psi.

This cycle of:

1. flushing with IPA-water,
2. performing the virus correlated integrity test,
3. flushing residual alcohol with water,
4. autoclaving at 121°C for one hour,
5. flushing again with water and integrity testing, and subsequently
6. drying

was carried out for a total of three cycles. At the end of the third cycle the integrity of the dry filters was again verified by means of the virus correlated IPA-water forward flow test.

Results

All filters tested were fully integral at the onset of the test. The filters remained integral and fully functional as indicated by the virus-correlated IPA-water forward flow test after each of three cycles of steam sterilization and drying.

Actual forward flow data in IPA-water at 85 psi are:

Forward Flow (cc/min) at 85 psi

S/N	start	cycle 1	cycle 2	cycle 3
EF0680206	7.0	5.3	6.1	6.3
EF0680222	7.3	5.8	6.2	6.7
EF0680109	8.0	7.0	7.8	7.5

Conclusions

At the beginning of this test and at all times over a total of three cycles of steam sterilization and drying, the Forward Flow of these filters remained below the limit of 12.5 cc/min, which was validated (Section I) to correlate with retention of the virus PR772 according to the test method described. Since the service life of the filter will be affected by process conditions (such as exposure to oxidizers, cleaning agents or solvents), the actual service life will vary with the specific conditions of use.

PROCEDURE FOR VERIFYING INTEGRITY OF FILTERS AFTER REPEATED STEAM STERILIZATION

Equipment required

1- Pressure Vessel, Mensco (CA), 2 gal., P/N 72-02 V-M type 304 SS

Reinforced rubber tubing, capable of operating at 85 psi

Autoclavable tubing

1- Sanitary Housing, Pall Corporation, NY, VSANTIG723

1- Fairchild Model #30252, pressure regulator, 0-100 psi

Sanitech braided tubing, 3/8" I.D.

1 - 0-60 psi gauge 2.5", 0.25 NPTLM Wika #111.10

2 - Clamps, 1.5" tri-clamp 13MHHA type 304SS

1 - Vent valve, needle type, 1/4" NPT ss

1 - Quick connect ss-QC8-S-8PM male NPT

1 - Quick connect ss-QC8-B-8PM female NPT

2- Sanitary connector, tri-clamp 24BMP, 1" thermometer cap, type 304 SS

1- Flowmeter or pneumatic trough capable of measuring an air flow of 12.5 cc/min

Test procedure

1. Install the test filter into a housing. Using a pressure vessel, flush the filter with 1.5 L of 30/70 (v/v %) isopropyl alcohol/water at a flow rate of 250 ml/min. Open the vent on the test filter housing upon initiating the flush to bleed out all air; then close the vent as soon as the housing is full.
2. Perform the forward flow integrity test at a test pressure of 85 psi. Measure and record the forward flow in cc/min.
3. Flush the filter with deionized water at a flow rate of 1 lpm for 20 minutes using 30 psi back pressure. Back pressure is accomplished by attaching a valve downstream of the filter housing with a pressure gauge between the downstream of the filter and the valve.
4. Isolate the filter housing from the water lines and connect an 8-10" length of autoclavable tubing to the bleed port vent (vent valve open).
5. Wrap the bleed tube and upstream and downstream side of the test housing with autoclave paper.
6. Autoclave the filter in the housing at 15 psi, 121°C, for 60 min., on a slow exhaust cycle. This should be done immediately after flushing; do not allow the filter to dry out before autoclaving. After autoclaving allow the filter and housing to cool to ambient temperature prior to performing the post-autoclave integrity test.
7. Flush the filter with deionized water at a flow rate of 1 lpm for 20 minutes using 30 psi backpressure as in step 3.
8. Remove the filter from the housing and dry in a circulating air oven at 150°F for 14 hours and allow to cool to ambient temperature before performing an integrity test again.
9. Flush the filter with 30% (v/v) IPA and perform a forward flow integrity test as in steps 1 and 2.
10. Repeat steps 3 - 9 for a total of 3 cycles.

Pall Ultrafine Filtration Company
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http://www.pall.com

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