BioPharmaceuticals

Validation Guide

VG-DV20

Pall ULTIPOR® VF Grade DV20
AB Style Virus Removal Filter Cartridges

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Validation Guide

Pall ULTIPOR® VF Grade DV20 AB Style Virus Removal Filter Cartridges

Introduction

This guide contains validation data applicable to ULTIPOR® VF grade DV20 virus removal filters in AB style cartridge configurations.

The test program included Steam Sterilization, Extractables Tests, Protein Transmission Studies, and Biological Reactivity Tests, in addition to correlation of Forward Flow Integrity Test with Viral Retention Tests.

These filters are manufactured and certified for use in pharmaceutical service, and this is indicated by the letter "P" in the part numbering code. The filters are manufactured in a controlled environment 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, on a sampling basis:
(2) Total Organic Carbon (TOC), Conductivity and pH Tests,
(3) Effluent Cleanliness Test,
(4) Limulus Amebocyte Lysate Test, and
(5) Viral Challenge Tests.

The materials of construction and performance parameters of the ULTIPOR VF grade DV20 filters are described in detail in Pall Element Data Sheet E-67, Ultipor VF Grade DV20 Virus Filter Cartridges, which is intended to be used in conjunction with this Guide.

Further information may be obtained from Pall BioPharmaceuticals, East Hills, NY 11548 (USA); Phone: 1-800-717-PALL (7255) or 516-484-5400, or through any Pall subsidiary or distributor.

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Introduction

Manufacturers of biologicals and biotherapeutics are mandated by regulatory agencies to incorporate adequate virus contamination-control strategies to ensure the virological safety of the final product. Multiple methodologies are recommended to ensure overlapping and complementary levels of protection (ICH Harmonized Tripartite Guideline, 1997). Of the available viral clearance (inactivation and removal) strategies, filtration is often a method of choice, being considered a robust technique not highly susceptible to minor changes in process conditions. Additionally, filtration is one of the least invasive of processes; it does not require inclusion of stabilizers or other additives that have a potential for toxicity; it does not alter the antigenicity of the target protein or induce the formation of neoantigens, and, in general, it is very amenable to inclusion into the manufacturing process.

From a process standpoint, application of filtration for virus removal from biologicals requires documentation of the filter's performance through a physical test, performed during manufacturing, which can be correlated to virus retention performance. Physical integrity tests currently in use include either destructive tests, such as challenge with particulates or colloids (e.g., colloidal gold) or nondestructive tests such as air diffusion (Forward Flow) tests. Nondestructive, Forward Flow-based integrity tests are the method of choice as they are amenable to use both pre- and post- filtration.

Pall Corporation uses the industry accepted Forward Flow Test method on a 100% production basis for the nondestructive integrity testing of sterilizing grade and virus removal filter elements. For integral filters, these Forward Flow values are measured air volume flow rates (ml/min) due to diffusion of air through a suitable 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 with predetermined or measured upstream volume is pressurized to the predetermined forward flow test pressure, then isolated from the pressure source, and the diffusion (forward flow) of air across the wetted membrane is quantitatively measured as a decay in upstream pressure over a specified period of time.

Correlating filter integrity test values with viral challenge testing is an empirical process resulting in validation data applicable to the test parameters and fluid attributes utilized. This document provides data from the viral retention (for bacteriophages PR772 and PP7) versus forward flow validation studies. Typical retention data for mammalian viruses, such as parvoviruses and other viruses are available from Pall Corporation upon request.

Parameter Determination

Based on the viral challenge and forward flow integrity tests which were conducted during the development of the Pall AB style ULTIPOR VF Grade DV20 filter cartridges, it was determined that a Forward Flow integrity test air pressure of 85 psi (5.86 bar) is suitable for integrity testing the ULTIPOR VF Grade DV20 filters when the filters are wet with dilute (30%) isopropyl alcohol (IPA). Under these test conditions, the maximum allowable Forward Flow value for one 10-inch filter cartridge was determined to be 7.0 ml of air per minute.

In order to validate the use of 85 psi (5.86 bar) air pressure and the maximum allowable flow of 7.0 ml/minute (for 30% IPA) filter elements were sampled from multiple production lots and tested as described below. Bacteriophage PP7, which is sized at 25 nm, is often used as a surrogate for mammalian viruses such as parvoviruses and poliovirus, which are in the similar size range (Aranha-Creado and Brandwein, 1999). Bacteriophage PP7 is a non-enveloped icosahedral phage with cubic symmetry. The method of assembly of PP7 within the bacterial host and its cubic symmetry preclude aggregation. Bacteriophage PR772 is an icosahedral non-enveloped bacteriophage sized at 53 nm. The non-aggregated status of the challenge bacteriophages (PP7 and PR772) was confirmed by transmission electron microscopy.

Summary of Viral Challenge Validation Procedure

Pall ULTIPOR VF Grade DV20 filter cartridges, part number AB1DV207PH4 filter cartridges [effective filter area, EFA, ~10.8 ft^2 (~1.0 m^2)], were tested as follows:

1. Filters were installed in the housing.

2. A pre-autoclave, pre-challenge integrity test of the assembly was performed after flushing with 3 liters of 0.1 µm-filtered 30:70 (v/v) isopropyl alcohol/water. A forward flow integrity test was performed using a PALLTRONIC® TruFlow automated integrity tester unit (model TF01) at an air test pressure of 85 psi (5.86 bar). A 10-minute stabilization period was followed by a 10-minute test period. The filter was then flushed with deionized water for 20 minutes.

   Note: For some filters, Forward Flow testing was also conducted using 20:80 (v/v) ethanol/water as the wetting fluid to develop alternative integrity test fluid values which are available on request from Pall BioPharmaceuticals.

3. Following wetting, the filter was sterilized in the housing by autoclaving at 15 psi, 121°C, for 60 minutes, on a slow exhaust cycle.

4. After autoclaving, the filter was flushed with 0.1µm-filtered deionized water for 20 minutes. A post-autoclave aseptic forward flow installation integrity test was performed water wet *in-situ* at 85 psi (5.86 bar).

5. The viral challenge was performed by:
   a. Filling a sterile pressure vessel with a pre-filtered carrier fluid (BSA/PBS [10 mg/ml]) inoculated with bacteriophages PR772 and PP7 at a concentration of approximately 10^6 plaque forming units (PFU)/ml.
   b. Connecting 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. Adjusting the air pressure to the pressure vessel to maintain 30 psi (2 bar) differential pressure across the filter during challenge.
   d. Allowing 1 liter of effluent to pass through the filter, then collecting an aliquot for viral assays.
   e. Stopping the challenge flow by clamping the downstream tubing and releasing the pressure from the pressure vessel.
   f. Removing the filter from the housing and sanitizing by submerging in 1% sodium hypochlorite overnight.

6. Input and effluent samples were assayed for virus content using an agar overlay plaque assay method. Serial dilutions of the input samples were made to confirm the initial concentration of PP7 and PR772. Ten 1 ml aliquots of the undiluted effluent were assayed to determine any low level PR772 transmission, while ten-fold serial dilutions of the effluent were made to assay for PP7 transmission.

7. All plaque assay plates were counted after incubating at 37 ± 2°C overnight. The virus removal efficiency of the filter was calculated as follows:

$$\text{Log Titer Reduction } (T_R) = \text{Log}_{10} \frac{\text{Concentration of challenge virus in input (PFU/ml)}}{\text{Concentration of challenge virus in effluent (PFU/ml)}}$$

   When challenge virus was not detected downstream of the filter (i.e., the effluent aliquots tested are sterile), 1 was substituted for the recovery (i.e., virus concentration in the effluent) and the LTR was expressed as greater than (>) the calculated value.

8. The sanitizing agent was drained from the filter and the filter was installed in a housing and flushed with 3 liters of 0.1 µm-filtered 30:70 (v/v) isopropyl alcohol at 45 psi (3.1 bar).

9. A post-challenge integrity test of the filter was performed in 30:70 (v/v) isopropyl alcohol at 85 psi (5.86 bar) air test pressure, using the PALLTRONIC TruFlow integrity tester unit. A 10-minute stabilization period was followed by a 10-minute test period.

A detailed description of this procedure is included in Appendix A of this report.

Results

The data from the viral retention versus forward flow validation study are listed in Table 1. ULTIPOR VF grade DV20 AB1DV20 10″ (254 mm) cartridges with Forward Flow values from 4.0 ml/min to 9.8 ml/min – when wet with 30:70 (v/v) isopropyl alcohol:water and tested at 85 psi (5.86 bar) air pressure – gave titer reductions for bacteriophages PR772 (53 nm) and PP7 (sized at 25 nm) of greater than or equal to 6 logs and 3 logs, respectively, under the test conditions. The test conditions included challenge with bacteriophage viruses at concentrations of greater than or equal to 10^6 pfu/ml in an isotonic protein solution (phosphate buffered saline + 10 mg/ml bovine serum albumin) with an applied differential operating pressure of 30 psi (2 bar) differential, and effluent was sampled and analyzed post-filtration after a 1 L challenge throughput volume.

Conclusions

The AB1DV20 cartridges have been validated to provide titer reductions of ≥ 6 logs for bacteriophages PR772 (53 nm) and ≥ 3 logs for PP7 (sized at 25 nm) under the specified test conditions. The Forward Flow integrity test performed at 85 psi (5.86 bar) in 30:70 (v/v) isopropyl alcohol:water establishes that AB style ULTIPOR VF grade DV20 filter cartridges with diffusional flows of up to 7.0 ml/min are retentive for bacteriophages PR772 and PP7 (25 nm) with titer reduction of ≥ 6, and ≥ 3, respectively. Filters with forward flow values between 7.1 and 9.8 ml/min are also retentive of viruses to the product specification for ULTIPOR VF grade DV20 filters, indicating a safety margin above the limit value.

Table 1.
Correlation of Forward Flow Values[1] of ULTIPOR VF Grade DV20 AB1DV20 Filter Elements with Retention of Bacteriophage PP7 (sized at 25 nm) and PR772 (sized at 53 nm)

Filter Serial No.	Forward Flow[1] [ml/min at 85 psi (5.86 bar), 30:70 IPA:Water]	Log Titer Reduction for Phage PR 772 (53 nm)	Log Titer Reduction for Phage PP7 (25 nm)
EK0410100	4.0	> 6	4.9
EK0260020	4.1	> 6	5.6
EK0410117	4.1	> 6	4.8
EK0300042	4.4	> 6	4.5
EK0410027	4.4	> 6	4.7
EK0300022	4.6	> 6	5.2
EK0410126	4.6	> 6	5.0
EK0300068	4.9	> 6	5.2
EK0300013	5.0	> 6	4.0
EK0300017	5.1	> 6	4.9
EK0300066	5.4	> 6	5.5
EK0260032	5.4	> 6	5.3
EK0410091	5.4	> 6	4.3
EK0410034	5.4	> 6	3.5
EK0260006	5.5	> 6	4.8
EK0300046	5.6	> 6	5.7
EK0260039	5.6	> 6	5.1
EK0300009	5.7	> 6	5.0
EK0260030	5.7	> 6	4.6
EK0260025	5.8	> 6	4.7
EK0260040	5.8	> 6	4.9
EK0260028	6.0	> 6	4.8
EK0300052	6.1	> 6	5.7
EK0260085	6.3	> 6	5.5
EK0260077	6.4	> 6	5.2
EK0300063	6.7	> 6	4.5
EK0410014	7.1	> 6	4.2
EK0300035	7.9	> 6	4.9
EK0410122	9.8	> 6	5.3
EK0260003[2]	9.9	< 4.0	3.9
EK0410084	11.8	> 6	3.1
EK0410006	11.8	> 6	4.5
EK0300038	13.0	> 6	4.8
EK0410093[2]	14.3	> 6	2.9
EK0410085	17.7	> 6	3.5

[1] Forward flow (FF) data reported for the higher of the 2 FF values (pre- v/s post-challenge FF values)
[2] Filter cartridges with less than the stated log reduction claim, i.e., < 6 logs for bacteriophage PR772 and/or < 3 logs for bacteriophage PP7

Introduction

The purpose of these tests was to quantify and characterize the materials that may be extracted from the ULTIPOR VF Grade DV20 filter cartridge into aqueous products when the filter is used for virus removal in accordance with the procedures validated by Pall Corporation. ULTIPOR VF grade DV20 cartridges are constructed from a hydrophilic acrylate-modified polyvinylidene fluoride (PVDF) filter membrane, polyester nonwoven support and drainage layers, and polypropylene molded components. In these tests, two 10-inch (254 mm) AB-style filters (EFA: ~10.8 ft^2, ~1.0 m^2) from each of 3 routine production lots were tested for nonvolatile, water-soluble extractables. These extractables were then analyzed by infrared and ultraviolet spectrophotometry.

Summary of Method

The ULTIPOR VF grade DV20 filter cartridges AB1UDV207PH4 were obtained from production inventory and were tested for extractable matter in a state the filters would typically be in at the onset of filtration. Whenever the ULTIPOR VF grade DV20 filter cartridge is used in the processing of pharmaceutical or biological products, the filter would normally be integrity tested and sterilized before filtration is begun. In these tests all of these steps were carried out according to the following procedures validated by Pall.

1. Integrity testing after wetting with 30/70 (v/v) IPA/water mixture,
2. Flushing with 0.2 µm filtered deionized water,
3. Steam sterilizing at ≥ 121°C for one hour,
4. Flushing with 0.2 µm filtered deionized water, and (optionally) performing a post-autoclave aseptic installation 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.

Each test filter was then extracted by submerging the filters (open end upwards) in 1500 ml deionized water at ambient temperature and gently moving the filters up and down in the liquid at a rate of 20 cycles per minute for a period of 24 hours. In each cycle the top of the filter cartridge is brought above the liquid level, causing flow of the extracting liquid through the filter. 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.

A detailed description of these procedures is provided in Appendix B.

Results

After the recommended procedures for integrity testing and sterilization, the AB1DV20 filters tested showed a maximum of 1.2 mg of nonvolatile materials when extracted with water at ambient temperature.

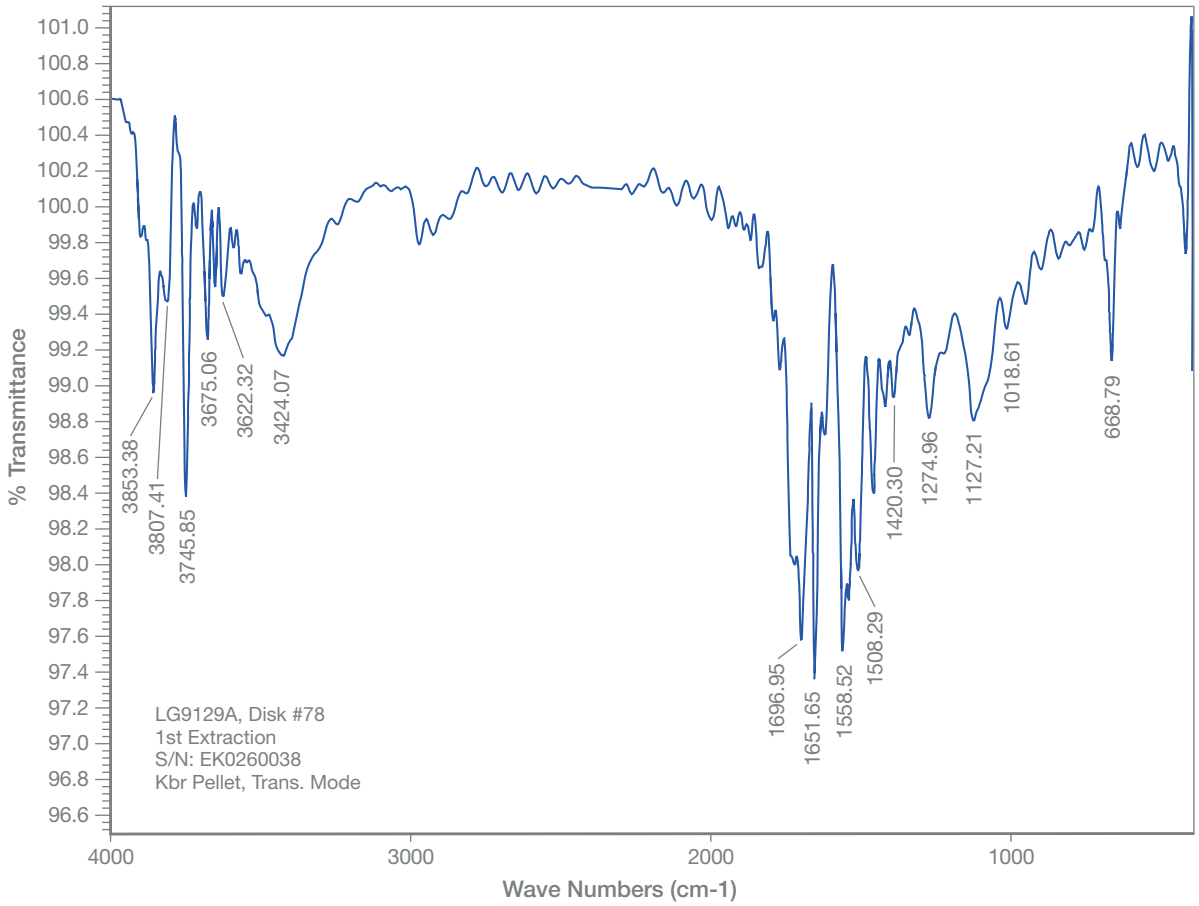
The ultraviolet absorption spectrum of this eluate was measured using a Hewlett-Packard Model 8452A Diode Array Spectrophotometer. The eluate showed a maximum absorbance of about 0.065A at 200 nm and a small secondary maximum (0.02A) at about 240 nm. This is due to the presence of small amounts of water-soluble terephthalate and isophthalate oligomers originating from the polyester drainage materials of the filter.

The infrared spectrum of the nonvolatile 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. II-1. The infrared absorptions from approx. 1700 - 1730 cm^{-1}, 1651 cm^{-1}, 1558 cm^{-1}, at 1270 cm^{-1} and at 1127 cm^{-1} indicate that the material consists mainly of a mixture of the terephthalate and isophthalate oligomers from the polyester support and drainage layers and acrylate residues from the chemically bonded polymer coating, which makes the filter membrane surface hydrophilic.

Conclusions

The level of aqueous extractables found from ULTIPOR VF Grade DV20 filter cartridges is extremely low. Actual service may impose different conditions, such as different fluids, exposure times, and temperature. Evaluation under process conditions is suggested. For assistance in reporting product- and process-related extractables, contact Pall Corporation.

Fig. II-1
Infrared Spectrum of Nonvolatile Residue as KBr Pellet



Section III Biological Reactivity Test Procedures and Results for ULTIPOR VF Grade DV20 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 DV20 Virus Reduction 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 DV20 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 tests were conducted by Gibraltar Laboratories, 122 Fairfield Road, Fairfield, New Jersey 07004-2405 and copies of the test reports are provided in Appendix C.

Conclusions

The ULTIPOR VF Grade DV20 Virus Removal filter cartridges were found to meet the requirements of the USP for Class VI-121°C Plastics.

Section IV Repeated Steam Sterilization Cycle Report for ULTIPOR VF Grade DV20 AB Style Virus Removal Filter Cartridges

Introduction

The purpose of these tests is to demonstrate that the ULTIPOR VF Grade DV20 AB-style filter cartridge reliably retains integrity after repeated cycles of either wet or dry steam sterilization, performed according to the procedures recommended by Pall Corporation. In the case of wet steaming, two filters from each of three production lots were tested. The integrity of each 10-inch (254 mm) filter was verified at the beginning of the test and then verified again after each of 6 wet sterilization cycles. In the case of dry steaming, one filter from each of three production lots was tested. The integrity of each 10-inch (254 mm) filter was verified at the beginning of the test and then verified again after each of 3 dry sterilization cycles.

Summary of Method

A. Wet steaming

The ULTIPOR VF Grade DV20 filter cartridges, P/N AB1DV207PH4, were obtained from production inventory and were tested for integrity by performing a forward flow test. In these tests the filters were wetted thoroughly by flushing with 30/70 (v/v) IPA/water mixture, after which forward flow was measured at a test pressure of 85 psi (5.86 bar). All filters tested met the forward flow limit recommended in Section I of this Guide. The forward flow tests were performed using the Palltronic™ TruFlow TF01 electronic integrity test kit.

The filters were then flushed with water to expel any alcohol and were then subjected to *in-situ* steaming for 1 hour at 125°C. After steaming the wet filters were allowed to cool to ambient temperature. The filters were then dried overnight and their integrity was verified by means of the virus-correlated IPA-water forward flow test at 85 psi (5.86 bar).

This cycle, which consisted of the following steps, was carried out for a total of six cycles:

1. Flushing with 30/70 (v/v) IPA-Water
2. Performing the virus correlated integrity test at 85 psi (5.86 bar)
3. Flushing residual alcohol from the filter with water
4. *In-situ* steaming at 125°C for one hour
5. Allowing filter to cool to ambient temperature
6. Drying the filter at 65°C for 14 hours

At the end of the sixth cycle the integrity of the dry filters was again verified by means of the virus correlated forward flow test in 30/70 (v/v) IPA/water.

A detailed description of these procedures is provided in Appendix D.

B. Dry steaming

The ULTIPOR VF Grade DV20 filter cartridges, P/N AB1DV207PH4, were obtained from production inventory. The filters were installed dry in an appropriate housing and were subjected to *in-situ* steaming for 1 hour at 125°C. After steaming the filters were allowed to cool to ambient temperature. The filters were then flushed with water and the system integrity was tested by means of a water-wet forward flow test at 85 psi (5.86 bar). This test is not virus-correlated and may be used as an installation test. Filter integrity was then tested after steaming by flushing the filters with 30% IPA/water and performing the virus-correlated forward flow test at 85 psi (5.86 bar). After this the filters were flushed with water to remove the alcohol and dried in an oven at 65°C for 14 hours.

This cycle, which consisted of the following steps, was carried out for a total of three cycles:

1. *In-situ* steaming dry at 125°C for one hour, allowing filter to cool to ambient temperature
3. Flushing the filter with water for 20 minutes
4. Performing an installation forward flow test at 85 psi (5.86 bar)
5. Flushing the filter with 30% IPA/water and performing the virus-correlated forward flow test at 85 psi (5.86 bar)
6. Flushing the filter with water and drying the filter at 65°C for 14 hours

A detailed description of these procedures is provided in Appendix D.

Results

A. Wet steaming

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 six cycles of steam sterilization and drying.

Actual forward flow data in 30/70 (v/v) IPA/water at 85 psi (5.86 bar) are shown in Table 2 below.

Additional proof of functionality after steam sterilization is given by the results of viral challenge presented in Section I of this Guide, which describes removal of viruses measured after subjecting each filter to a 1-hour autoclave cycle at 125°C.

Table 2. Forward Flow After Wet Steaming (ml/min) at 85 psi (5.86 bar)

Cartridge S/N	Start of Test	End of Cycle 1	End of Cycle 2	End of Cycle 3	End of Cycle 4	End of Cycle 5	End of Cycle 6
EK0260128	6.1	4.8	4.8	4.0	4.8	5.0	6.3
EK0260135	5.7	4.4	4.0	4.5	5.3	4.7	4.9
EK0300006	5.9	5.1	4.7	4.8	5.6	3.9	4.9
EK0300045	5.6	4.9	4.2	5.0	3.8	6.3	5.8
EK0410048	4.7	4.1	3.5	3.2	3.2	3.2	3.5
EK0410082	6.7	5.1	2.8	2.5	2.5	2.6	2.9

B. Dry steaming

All filters tested remained integral and fully functional as indicated by the virus-correlated forward flow test in 30:70 (v:v) IPA:Water after each of the three cycles of dry steam sterilization.

Actual forward flow data in water and in 30/70 (v/v) IPA/water at 85 psi (5.86 bar) air test pressure are shown in Table 3 below.

Table 3. Forward Flow After Dry Steaming (ml/min) at 85 psi (5.86 bar)

Cartridge S/N	End of Cycle 1 (Water)	End of Cycle 1 (IPA)	End of Cycle 2 (Water)	End of Cycle 2 (IPA)	End of Cycle 3 (Water)	End of Cycle 3 (IPA)
EK0260029	5.1	3.7	4.8	2.0	4.9	6.2
EK0300039	5.6	4.5	4.3	2.6	5.6	3.0
EK0410052	4.0	3.7	2.1	3.6	3.3	3.7

Conclusions

At the beginning of this test and at all times after multiple cycles of either wet or dry cycles of steam sterilization, the Forward Flow of these filters remained at or below the limit of 7.0 ml/min which was validated (Section I) to correlate with retention of the viruses PR772 and PP7 according to the test method described. Since the service life of the filter will be affected by sterilization conditions (such as temperature or additional exposure to oxidizers, cleaning agents or solvents), the actual service life will vary with the specific conditions of use.

Introduction

Biologicals and biopharmaceutical products, being proteinaceous in nature, are often not amenable to sterilization by heat or other invasive methods; with these products filtration is often the method of choice. In the case of filtration for virus removal, concomitant with the need for high viral retention is the requirement for optimal recovery of the target protein since the effective size of the target protein may sometimes approach that of small viruses. High product recovery is a prime consideration especially in the case of several recombinant-derived products which have clinical efficacy in the microgram quantities range and are, consequently, high premium products. This study was undertaken to demonstrate the ability of the ULTIPOR VF grade DV20 to provide high protein transmission while at the same time providing high efficiency of removal of viruses as small as 20 nm.

Summary of Method

A solution containing 2.5 mg/ml (0.25%) of a highly purified (clinical grade) murine monoclonal antibody in phosphate-buffered saline solution was filtered under a constant pressure of 30 psid (2.0 bard), through 47 mm membrane discs (EFA: 0.015 ft^2; 13.935 cm^2) of ULTIPOR VF grade DV20 virus removal membrane taken from 3 different lots, at typical flow rate of about 0.35 ml/min. Extrapolated to the area of a 10-inch DV20 filter cartridge (EFA: ~10.8 ft^2, ~1.0 m^2) this is equivalent to a flow rate of about 15 L/hour. The effluent through the disc was collected in 2-ml fractions for the first 10 ml and in 10-ml fractions thereafter for a total of approximate 100 ml (equivalent to a volume of about 67 L through a 10-inch cartridge). Each fraction was analyzed spectrophotometrically (A_{280}) for percentage of protein transmitted in each fraction and the total amount of protein recovered during the filtration was calculated.

A detailed description of this procedure is provided in Appendix D.

Results

In each case >95% of the influent concentration was found after approximate 12 ml effluent was collected, 99% transmission was found when approximate 34 ml effluent was collected and 100% transmission was found before 55 ml effluent was collected and the total amount of IgG recovered was >97%.

The data are summarized below:

Lot Number	Volume at 95% Transmission	Volume at 99% Transmission	Volume at 100% Transmission	Total IgG Recovery
KW-2-97	12 ml	33 ml	53 ml	98%
KW-3-02	11 ml	33 ml	44 ml	97%
KW-3-04	12 ml	34 ml	55 ml	98%

Conclusions

ULTIPOR VF grade DV20 filter membranes and cartridges are capable of allowing high transmission of proteins as large as IgG and a high level of protein recovery can be expected under normal conditions of use.

Ackermann, H-W and M.S. DuBow. 1987. "Viruses of Prokaryotes." CRC Press Inc., Boca Raton, Fl.

Aranha-Creado, H. and Brandwein, H. (1999), "Application of bacteriophages as surrogates for mammalian viruses: a case for use in filter validation based on precedents and current practices in medical and environmental virology," PDA J. Pharm. Sci. Technol. 53: 75-82.

ICH harmonized tripartite guideline, "Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin," pp. 1-27, IFPMA, Geneva, Switzerland (1997).

United States Pharmacopeia XXIII, The United States Pharmacopeial Convention, Rockville, Maryland, 1994.

Appendix A: Procedure for Viral Challenge Validation

1.0 Microbial Stocks and Media Preparation

1.1 Media Preparation

1.1.1 Tryptic Soy Agar plates (TSA), (Remel, Lenexa, KS)

1.1.2 Tryptic Soy Broth (TSB), (Difco, Detroit, MI) Prepare as per manufacturer's specifications. Autoclave at 121°C 15 psi (1 bar) for 20 minutes using a slow exhaust cycle.

1.1.3 Dilution blanks: Use TSB from section 1.1.2 and aseptically dispense 4.5 ml into sterile disposable tubes.

1.1.4 Soft agar for use as agar overlay:

1.1.4.1 Prepare soft agar (0.6% nutrient agar) (Difco, Detroit MI). Prepare as per manufacturer's specifications. Autoclave at 121°C, 15 psi (1 bar) for 20 minutes using a slow exhaust cycle.

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

1.2 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. Bacteriophage PP7 (ATCC 15692-B2) and its host *Pseudomonas aeruginosa* (ATCC 15692) were obtained from the American Type Culture Collection (ATCC), Rockville, MD.

1.3 Preparation of Bacterial Culture for Use as Phage Host

1.3.1 Host suspensions are prepared by the following methods:

1.3.2 Storage Stock:

1.3.2.1 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 (-70°C).

1.3.2.2 Allow a frozen stock tube to thaw and use it to inoculate 50 ml of TSB. Incubate the culture at 37 ± 2°C as required (usually 3-7 hours). Take an OD_{550} reading (a minimum absorbance of 0.2 is required) and record the result before use.

1.3.2.3 Streak the host onto several TSA plates. Incubate the plates at 37 ± 2°C for 24 hours. Wrap the plates in parafilm and refrigerate (4°C) for use in preparation of overnight cultures.

1.3.3 Preparation of overnight bacterial host culture for use as inoculum.

1.3.3.1 Place 10 ml of TSB in a sterile glass tube along with one isolated colony obtained from the plates mentioned in section 1.3.2.3.

1.3.3.2 Incubate the culture at 37 ± 2°C for 15 hours. Take an OD_{550} reading and record it.

1.3.4 Preparation of Bacterial Culture for Use in Agar Overlay Assay:

1.3.4.1 Use 2 ml of the overnight culture (section 1.3.3) to inoculate 50 ml of TSB. Incubate the culture at 37 ± 2°C as required (usually 3-5 hours). Take an OD_{550} reading (a minimum absorbance of 0.2 is required) and record before use. **NOTE:** Actively growing bacterial cultures are required to provide a bacterial host lawn for phage assays.

1.4 Preparation of Viral Stocks

1.4.1 Determine the appropriate dilution of stock bacteriophage required to produce 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).

1.4.2 Prepare an actively growing bacterial host culture as described in 1.3.4. To a sterile tube containing 9 ml of soft agar add the specific dilution of the bacteriophage as prepared in 1.4.1 and appropriate volume of the homologous 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.

1.4.4 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.

1.4.5 Centrifuge at 3000 rpm for 20 minutes. Decant the supernatant into sterile centrifuge tubes. Repeat centrifugation. Decant the supernatant into a sterile container.

1.4.6 Aseptically vacuum filter the supernatant through a sterile 0.45 µm-rated membrane filter followed by filtration through a sterile 0.2 µm-rated filter. Aliquot the bacteriophage stock into 2 ml cryogenic vials, label and store at 4°C for use in bacteriophage challenges of membranes and filter elements.

1.4.7 Determine the stock bacteriophage titer using the agar overlay method described in section 2.0.

1.5 Carrier Fluid (1% Albumin Bovine [BSA]) for Viral Challenge

Albumin Bovine (ICN Biomedicals,Aurora OH) 10g/L is prepared in Phosphate Buffered Saline (PBS) pH 7.4 (Sigma Diagnostics, St. Louis MO).

1.5.1 Prepare PBS as per manufacturer's specification utilizing deionized water. Once dissolved, add BSA to provide a 1% concentration.

1.5.2 Filter the entire contents through an ULTIPOR VF Grade DV50 filter cartridge (Pall P/N AB1UDV507PH4) which has been forward flow integrity-tested and sterilized (autoclaved 121°C, 60 minutes, slow exhaust). Add the appropriate concentration of the challenge viruses to the carrier fluid.

1.6 Preparation of Viral Challenge Suspension

1.6.1 Remove phage stocks of known titer from refrigerator and vortex.

1.6.2 Prepare the challenge suspension to the desired challenge level in appropriate volume of carrier fluid that has been pre-filtered as described in section 1.5.

2.0 Bacteriophage Assays

2.1 Assay Procedure

2.1.1 Make ten-fold serial dilutions in TSB of the input and effluent 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. *E.coli* K-12 strain J53-1 and *P. aeruginosa* (ATCC 15692) are the hosts used for bacteriophages PR772 and PP7, respectively.

2.1.3 Add 1.0 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 minimally 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 DV20 filter cartridge, evaluated in terms of the Log Titer Reduction (LTR), is calculated as follows:

$$LTR = Log_{10} \frac{\text{Concentration of challenge virus in the input (PFU/ml)}}{\text{Concentration of challenge virus in the effluent (PFU/ml)}}$$

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 titer reduction is expressed as greater than (>) the calculated value.

3.0 Test Equipment

Equipment for Viral Challenge

1 – Pressure Vessel, 30 liters, P/N 89810-002 Type 304 Alloy Products, Waukesha, WI
1 – Sanitary Filter Housing, Pall Ultrafine Filtration Co., (Pall BioPharmaceuticals), East Hills, NY, Part No. VSANT1G723
3 – Glass cylinders (minimum 4 liter capacity)
2 – Sterile disposable 100 ml cylinders
1 – Fairchild Model # 30252, pressure regulator, 0-100 psi
2 – 0-60 psi gauge 2.5″, 0.25 NPTLM Wika # 111.10
1 – Metering valve P/N SS-IVM4-A R.S Crum & Co, Albertson, NY
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.

4.0 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 3 liters of 0.1 µm-filtered 30:70 (v/v) isopropyl alcohol utilizing 50 psi (3.4 bar) upstream pressure and 20 psi (1.38 bar) backpressure. Upstream pressure is set on the main gauge attached to the air source. Backpressure is accomplished by attaching a valve downstream of the filter housing with a gauge situated between the outlet port of the housing and the valve. Open the vent on the test filter housing upon initiating the flush to ensure that the test filter housing is completely full; close vent. Once pressure is stabilized record flow rate (ml/min).

4.1.2 Perform the forward flow integrity test using PALLTRONIC TruFlow unit at a test pressure of 85 psi (5.86 bar). Allow for a 10-minute stabilization period followed by a 10-minute test sequence. Record the integrity test value in ml/min. **NOTE:** For some filters, Forward Flow testing was also conducted using 20:80 (v/v) ethanol/water as the wetting fluid to develop alternative integrity test fluid values which are available on request from Pall BioPharmaceuticals.

4.1.3. Flush the filter with deionized water for 20 minutes using 50 psi (3.4 bar) upstream-pressure and 20 psi (1.38 bar) backpressure. Place a gauge between the water source and the upstream of the filter housing to monitor upstream pressure. Backpressure is

accomplished by attaching a valve downstream of the filter housing with a gauge situated between the outlet port of the housing and the valve. Open the vent on the test filter housing upon initiating the flush to ensure that the test filter housing is completely full; close vent. Once pressure is stabilized, record flow rate (ml/min).

4.1.4 Proceed to sterilization of the filter and test equipment.

4.2 Sterilization of Test Equipment and Filter

4.2.1 Cut two 12″ lengths of autoclavable braided tubing for the inlet and outlet sides of the test filter housing. Attach a male quick connect to one end of the first tubing and place a sanitary connector on the other end. Attach a female quick connect to the second tubing, along with a sanitary connector. Wrap both ends of each tubing with autoclave paper.

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

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 the challenge into the autoclave. The test filter must be autoclaved wet in the filter housing. Autoclave all test equipment including the filter at 15 psi (1 bar), 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 Test of Filter

4.3.1 While maintaining aseptic conditions downstream of the sterilized filter, flush with deionized water. The water used for the flush is pre-filtered through an ULTIPOR Nylon 6,6 filter cartridge (Pall P/N AB1NT7PH4) which has been forward flow integrity tested and sterilized (autoclave 121°C, 60 minute, slow exhaust cycle). Attach the sterile pre-filter to the deionized water source. Place a gauge between the pre-filter and the test filter to monitor upstream pressure. Backpressure is accomplished by attaching a valve downstream of the filter housing with a gauge situated between the outlet port of the housing and the valve. Perform a flush for 20 minutes using 50 psi (3.4 bar) upstream pressure and 20 psi (1.38 bar) backpressure. Once pressure is stabilized record flow rate (ml/min). Perform a water-wet Forward Flow test *in-situ* at 85 psi (5.86 bar) as described in section 4.1.2. Proceed with the viral challenge.

5.0 Challenge Protocol

5.1 Viral Challenge of Filters

5.1.1 Fill the sterile pressure vessel with the pre-filtered carrier fluid. Inoculate the carrier fluid with bacteriophages PR772 and PP7 to yield a concentration of approximately 10^6 pfu/ml.

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 first tubing mentioned in section 4.2.1 to the inlet port (upstream side) of the filter housing. Attach the second tubing mentioned in section 4.2.1 to the outlet port (downstream side) of the filter housing.

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

5.1.5 Place the downstream tube of the test housing in a 4 liter cylinder, leaving the top of the cylinder loosely covered with aluminum foil.

5.1.6 Clamp off the downstream tube. Adjust air pressure to 5 psi and allow challenge suspension to flow through the bleed valve of test housing to properly bleed the system.

5.1.7 Close the bleed valve and remove clamp from the downstream side of the test housing. Adjust the air pressure to 30 psi (2 bar).

5.1.8 After 1-liter of the challenge fluid is filtered collect a 50 ml aliquot in a sterile 100 ml cylinder. (**NOTE:** If a larger volume is to be collected, record the time after each liter of effluent is collected. During collection switch out cylinders as needed. Take a final 50 ml aliquot in a sterile 100 ml cylinder). Initial and final effluent samples are saved for viral assay.

5.1.9 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.

5.1.10 Remove the filters from the housings and sanitize as described in section 5.2.

5.2 Sanitization of Filters Post Use

5.2.1 Submerge the filters in 1% sodium hypochlorite overnight.

5.3 Viral Assay

5.3.1 Proceed with assay of the input and effluent samples using the agar overlay procedure described in section 2.0.

5.3.2 *E. coli* K-12 is the bacterial host used to assay for bacteriophage PR772. To determine PR772 input concentration make tenfold serial dilutions in TSB. To evaluate PR772 retention, assay ten 1 ml aliquots of the undiluted effluent. Proceed with assay using the agar overlay method as described in sections 2.1.3-2.1.5.

5.3.3 To evaluate the input and effluent samples for bacteriophage PP7, make tenfold serial dilutions in TSB and follow the procedure described in section 2.1.3-2.1.5 using the *P. aeruginosa* host.

5.4 Post-Challenge Integrity Test of Filters

5.4.1 Drain the sanitizing agent and install the filter in the housing.

5.4.2 Flush the filter with 3 liters of 0.1 µm-filtered 30:70 (v/v) isopropyl alcohol at 45 psi (3.1 bar). Record flow rate (ml/min).

5.4.3 Perform a forward flow integrity test as described in section 4.1.2.

Appendix B: Procedure for Determination of Extractables

I. Equipment

1. Automatic reciprocator capable of 20-40 full strokes per minute where 1 stroke is an upward and downward movement.
2. PTFE Adapter Assembly with stainless steel extension rod
3. Glass Graduated Cylinders, 2000 ± 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 gauge)
10. Analytical Balance - calibrated and capable of measuring at least 130 g 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. Glassware Cleaners

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, PTFE 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 Nonvolatile 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 cooling to room temperature.

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

8. Calculate the nonvolatile 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.

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

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,

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REPORT No. G-20058
07/30/99
Page 1 of 2

LABORATORY REPORT

FINAL REPORT

Sponsor:(0850)
Pall Corporation
25 Harbor Park Drive
Port Washington,NY,11050
Attn: Janet Mathus
Purchase Order #:402026

GBL Ref.: 1394- 196- 7176
GBL Sample No.:09467/1-5.30
Lot #1:DV20
Lot #2: None
Lot #3: None
Date Received: 07/07/99
Date Tested: 07/09/99
Date Completed: 07/22/99

USP 23 CLASS VI on Ultipor^{R} VF^{TM} DV20 Filter
Interim Report Activity:

Description:
One white plastic filter cartridge with a spike at one end and two orange O-rings at the other. The following components to be tested as a composite for Intracutaneous Irritation and Systemic Toxicity and individually for Implantation: /1 = PVDF hydrophilic filter medium (corrugated membrane); /2 = polyester drainage material; /3 = polypropylene core or adapter; /4 = polypropylene outer body; /5 = polypropylene endcaps.

1 Purpose
To determine the reaction of normal animal tissue and living animals to the presence of extracts and/or portions of the test material.

2 Test System
2.1 New Zealand albino rabbits, either sex, 2.08 to 3.18 kg.
Two per extract and two per implant.
2.2 Swiss Webster albino mice, male, 19.3 to 29.9 grams.
Five test and control (intravenous and intraperitoneal injection).

3. Method: Test Material Preparation and Extraction
A composite of the following materials was extracted in 20 mL of each of the below solvents. Each component was tested separately for implantation.
/1 24 cm^2 - PVDF Hydrophillic Filter Medium
/2 24 cm^2 - Polyester Drainage Material
/3 0.8 gram - Polypropylene Core or Adaptor
/4 0.8 gram - Polypropylene Outer Body
/5 0.8 gram - Polypropylene Endcaps

3.1 Solvents
(X) USP Sodium Chloride for Injection (Saline)
(X) 5% Ethanol in Sodium Chloride (ETOH/Saline)
(X) Cottonseed Oil (CSO)
(X) Polyethylene glycol 400 (PEG)

Conclusion: The material conforms to the requirements of this test.

Respectfully Submitted,
GIBRALTAR LABORATORIES, INC.

Date
Written: 07/30/99
Analyst: 41

Approved By: Daniel L. Prince, Ph.D.

AUDIT #P7130199 GBL

Protocol #: None

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LABORATORY REPORT

3.2 Extraction Conditions
(X) 121C for one hour

3.3 Dosing Procedures
(X) IC Injection - three day observation period
(X) Systemic Injection (IV and IP) - three day observation period
(X) Intramuscular Implantation - seven day observation period

4. Results: See Tables 1 to 7.

Table 1: Intracutaneous Irritation

Extract	Average Test Score	Average Control Score	Difference
Saline	(0.00)	(0.00)	(0.00)
ETOH/Saline	(0.00)	(0.00)	(0.00)
CSO	(0.92)	(1.50)	(0.58)
PEG 400	(0.00)	(0.00)	(0.00)

Table 2: Systemic Toxicity

	(Test Group)		(Control Group)		
Extract	Death	Morbidity	Death	Morbidity	Difference
Saline	0/5	0/5	0/5	0/5	0/5
ETOH/Saline	0/5	0/5	0/5	0/5	0/5
CSO	0/5	0/5	0/5	0/5	0/5
PEG 400	0/5	0/5	0/5	0/5	0/5

Table 3: Intramuscular Implantation - PVDF Hydrophilic Filter Medium

Sample Size	Average Test Score	Average Control Score	Difference
1 x 10 mm	(0.00)	(0.50)	(0.50)

Table 4: Intramuscular Implantation - Polyester Drainage Material

Sample Size	Average Test Score	Average Control Score	Difference
1 x 10 mm	(0.125)	(0.500)	(0.375)

Table 5: Intramuscular Implantation - Polypropylene Core or Adaptor

Sample Size	Average Test Score	Average Control Score	Difference
1 x 10 mm	(0.00)	(0.50)	(0.50)

Table 6: Intramuscular Implantation - Polypropylene Outer Body

Sample Size	Average Test Score	Average Control Score	Difference
1 x 10 mm	(0.00)	(0.00)	(0.00)

Table 7: Intramuscular Implantation - Polypropylene Endcaps

Sample Size	Average Test Score	Average Control Score	Difference
1 x 10 mm	(0.25)	(0.00)	(0.25)

Appendix D: Procedure for Repeated Steam Sterilization Cycle Testing

A. Wet steaming

1. Immerse filter vertically with the open end up slowly into a solution of 30% (v/v) isopropyl alcohol (IPA) in water, allowing air to be expelled from the filter as it becomes wetted with the liquid. Hold the filter in the liquid for at least 15 minutes after the core of the filter has been completely filled with liquid. This may be done in a clean 2000 ml glass graduated cylinder using a minimum of 1500 ml liquid.

2. Using vacuum, draw at least 2 liters of 30% (v/v) IPA in water through the filter and allow the filter to drain for 10 minutes.

3. Install the filter in an appropriate clean, stainless steel housing and measure Forward Flow at 85 psi (5.86 bar) using a Palltronic TruFlow integrity test instrument. Set the inlet pressure of the TruFlow to 90 psi (6.2 bar) and both the stabilization time and test time to 10 minutes.

4. Flush the filter with DI water for 20 minutes using a differential pressure of 30 psid (2 bard) across the filter.

5. Attach the housing with the filter to a steam line fitted with a pressure regulator capable of regulating steam pressure over the range of 0–25 psi (0-1.72 bar). Apply live steam upstream of the filter. With the downstream of the filter housing open to atmosphere, vent the housing upstream to expel all air from the system by increasing the steam pressure gradually, maintaining no more than 5 psid (0.34 bard) across the filter, until the inlet temperature is 125°C (pressure is 19 psi; 1.31 bar). Maintain this temperature for 1 hour.

6. Shut off steam, properly venting housing to avoid reverse pressurization of the filter. Open housing and remove filter. Allow filter to cool until slightly warm to the touch.

7. Dry filter in an air oven at 65°C for 14 hours.

8. Repeat steps 1–7 for a total of 6 cycles of steaming. After the filter has been dried at the end of the 6th cycle wet the filter with 30% IPA in water and measure Forward Flow at 85 psi (5.86 bar) according to steps 1 and 2.

B. Dry steaming

1. Install the filter in an appropriate clean, stainless steel housing. Attach the housing with the filter to a steam line fitted with a pressure regulator capable of regulating steam pressure over the range of 0–25 psi (0–1.72 bar). Apply live steam upstream of the filter. With the downstream of the filter housing open to atmosphere, vent the housing upstream to expel all air from the system and increase the steam pressure gradually, maintaining no more than 5 psid across the filter, until the inlet temperature is 125°C (pressure is 19 psig). Maintain this temperature for 1 hour.

2. Shut off steam, properly venting housing to avoid reverse pressurization of the filter. Open housing and remove filter. Allow filter to cool until slightly warm to the touch.

3. Flush the filter with DI water for 20 minutes using a differential pressure of 30 psid (2 bard) across the filter.

4. Measure Forward Flow at 85 psi (5.86 bar) using a Palltronic TruFlow integrity test instrument. Set the inlet pressure of the Palltronic TruFlow to 90 psi (6.2 bar) and both the stabilization time and test time to 10 minutes.

5. Using a pressure vessel, flush 3 liters of 30% IPA/water (v/v) through the filter.

6. Measure Forward Flow at 85 psi (5.86 bar) using a Palltronic TruFlow integrity test instrument. Set the inlet pressure of the Palltronic TruFlow to 90 psi (6.2 bar) and both the stabilization time and test time to 10 minutes.

7. Flush the filter with DI water for 20 minutes using a differential pressure of 30 psid (2 bard) across the filter. Dry filter in an air oven at 65°C for 14 hours.

8. Repeat steps 1–7 for a total of 3 cycles of steaming.

Appendix E: Procedure for Determination of Protein Transmission

1.0 Test Apparatus

47 mm disc filter holders
Calibrated pressure gauge
Tubing manifold
Nalgene filtration equipment
Ultraviolet spectrophotometer capable of measuring absorbance at 280 nm
0.5 cm cuvette
Graduated cylinders
Micropipettes (Rainin P-200, P-1000, P-5000 or equivalent);
Test equipment assembly:
- Test stand
- Pressure regulator/tubing assembly
- Pressure vessel
- Metal clamp (to attach pressure vessel to pressure regulator/tubing assembly)

2.0 Materials

2.1 Reagents

IgG (murine monoclonal antibody; clinical grade)
PBS, Sigma, Cat # 1000-3, or equivalent

2.2 Materials

2 ml centrifuge tubes
15 ml polypropylene tubes
Tygon tubing
White tubing ties
Small O-ring for filter holder vents
Large O-rings for filter holder outlets
Plastic support for filter holders

3.0 Prefiltration of IgG solution

Prefilter the IgG solution using grade DVD membrane (Pall P/N FTKDVD). **Note:** The prefiltration disc must be prewetted in deionized water. Save 2 ml of the pre-filtered solution. The remainder will be used as influent in the IgG filtration test.

4.0 Measurement of IgG in effluent samples

4.1 Preparation of IgG standards:

4.1.1 To prepare standards, first dilute the IgG solution as received to a concentration of 3.0 mg/ml.

4.1.2 Dilute this solution serially in 2 X increments, by mixing 1 ml IgG solution in 1 ml PBS. Create 1.5, 0.75, 0.37, 0.18, and 0.09 mg/ml solutions.

4.1.3 Read absorbance of the following at 280 nm:

PBS (assay blank)

Standards in increasing order of IgG concentration.

Use a cuvette with 0.5 cm cell path. If low concentrations are needed (A_{280} < 0.37), then 1 cm cuvette may be used to read both standards and samples with low absorbance. Use the equation $C = k_0 + (k_1*A) + (k_2*A*A)$

4.1.4 Remove standards from the standard curve, if they have percent error values greater than 10%. Prepare a new standard if the standard is needed.

4.1.5 Save all data obtained from standards before continuing.

4.2 Reading samples on spectrophotometer

4.2.1 Measure the absorbance of effluent samples. If samples need to be diluted, record the dilution information.

5.0 Determination of IgG concentration

5.1 Determine the IgG concentration in each sample using the standard curves.

5.2 Calculate the fraction transmitted in each fraction as

% = (mg/ml in fraction) / (mg/ml in influent)

5.3 Calculate amount of IgG in each fraction as

mg IgG = (mg/ml in fraction) x (fraction volume in ml)

6.0 Calculate the total IgG recovered as

Total recovered = $\sum$ mg IgG (step 5.2)

7.0 Calculate the fraction of total IgG recovered as

$$\frac{\text{Total recovered (step 6.0)}}{\text{(total effluent volume) x (mg/ml in influent)}}$$

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