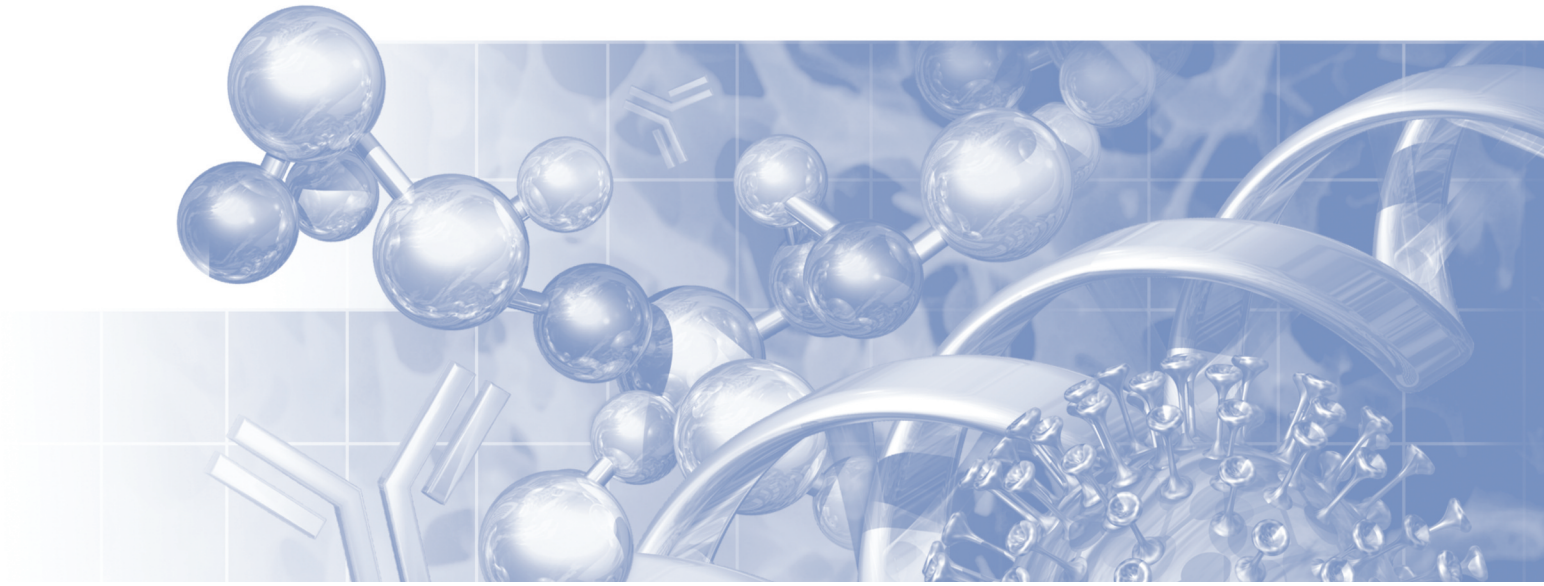
PALL Life Sciences

Validation Guide

USTR 2839[1]

Pegasus™ SV4 Virus Removal Filter Cartridges



*Filtration. Separation. Solution.*SM

Contents

1. Overview

1.1 Introduction

This document contains validation data applicable to Pegasus grade SV4 virus removal filters in AB-style cartridge configurations.

Pegasus grade SV4 virus removal filter cartridges are direct flow filters that combine robust, high viral clearance (> 4 log reduction of small viruses > 20 nm) with high throughput capacity and stable flow rates, in both dilute and complex concentrated biological fluids.

Pegasus grade SV4 sanitary AB-style filter cartridges are designed for highly efficient clearance of small and large viruses, whether they are enveloped or not, from biological solutions. The validation data presented in this document have demonstrated robust > 4 log titer reduction for ~ 25 nm PP7 bacteriophage (model for parvovirus), as recommended in PDA Technical Report 41-08, Virus Filtration.

Pegasus grade SV4 AB-style filter cartridges incorporate a unique, patented laid-over pleating construction for increased effective filtration area that maximizes process productivity and reduces process costs by reducing the required amount of filter elements per installation, which also minimizes the hold-up volume of the virus filtration system.

Pegasus grade SV4 AB-style filter cartridges are made from a hydrophilic modified polyvinylidene fluoride (PVDF) filter membrane, polyester support and drainage layers, and polypropylene molded components. Pegasus grade SV4 AB-style filter cartridges are intended for installation in suitable stainless steel or disposable filter housing assemblies.

Pegasus grade SV4 AB-style filter cartridges are manufactured in accordance with an ISO 9001 certified Quality Management System.

Pegasus grade SV4 AB-style filter cartridges may be used in conformance with current Good Manufacturing Practices (cGMP) in Manufacturing, Processing, Packing or Holding of Drugs per Title 21 of the U.S. Code of Federal Regulations (21 CFR Part 210) and cGMP for Finished Pharmaceuticals (21 CFR Part 211).

The tests that were performed to qualify the performance of Pegasus grade SV4 filters under a range of test conditions were as follows:

- Viral (bacteriophage) retention tests correlated with Forward Flow integrity testing
- Endurance to in-situ steam sterilization and autoclaving
- Determination of water flow characteristic
- Extractables post autoclaving using water after a pre-flush of 10 liters
- Biological reactivity tests on filter components – compliance with the requirements for biological reactivity, in vivo, under United States Pharmacopoeia (USP) <88> (for Class VI-121 °C plastics) and in vitro, under USP <87>

This guide may be combined with other documentation for Pegasus grade SV4 filters, namely

- Datasheet USD2847: Pegasus™ Grade SV4 Virus Removal Filter Cartridges
- Certificate of Test for Pegasus™ Grade USV4 Virus Removal Filters (included in each filter cartridge packaging)
- Application Note USD2846: Filterability Testing and Virus Challenge of Pegasus™ SV4 Virus Removal Membrane Filter Discs
- Application Note USD2778: Filterability Testing and Virus Challenge of Pall® Minidisc Capsules with Pegasus™ SV4 Virus Removal Filter Membrane

This comprehensive package substantiates the product specification and quality control standards applied to Pegasus grade SV4 filters.

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

(1) 100 % fabrication integrity tested – correlated to PP7 bacteriophage removal

(2) Viral reduction tested with PP7 bacteriophage lot release test

(3) 100 % fabrication water flow test

(4) pH Tests

(5) Effluent Cleanliness Test

(6) Endotoxin Test

Note: The units of pressure quoted in this document are “bar” and “pounds force per square inch (psi)”. The following figures can be used to convert these units of pressure to Pascal (Pa):

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

1.2 Summary of Conclusions

Viral (Bacteriophage) Retention Tests Correlated with Forward Flow Integrity Testing

Pegasus grade SV4 filters were tested using viral (bacteriophage) challenge tests with bacteriophage PP7 (~ 25 nm). The results demonstrated a > 4 log titer reduction for this test organism.

The Forward Flow integrity test was shown to be a suitable non-destructive integrity test for Pegasus grade SV4 filters and test parameters have been set as follows for 254 mm (10 inch) filters (part number AB1USV47PH4).

Table 1

Forward Flow Integrity Test Parameters for 254 mm (10 inch) Pegasus Grade SV4 AB-Style Filter Cartridges, Part Number AB1USV47PH4

Test pressure	60 psi (4.150 bar)
Wetting liquid	Water
Temperature	20 °C ± 5 °C (68 °F ± 9 °F)
Test gas	Air
Maximum allowable Forward Flow limit*	30 mL/min

** During the test period the temperature of the filter assembly should not vary more than + 1 °C (2 °F).*

Endurance to In-Situ Steam Sterilization and Autoclaving

Pegasus grade SV4 filters have been demonstrated to be capable of withstanding multiple in-situ steam sterilization and autoclave cycles under various standard steaming conditions. Tests performed demonstrate that Pegasus grade SV4 filters are robust and capable of withstanding differential pressures up to 300 mbar (4.35 psi) in the forward direction during steaming. Test conditions involved exposure to 130 °C (266 °F) temperature during in-situ steaming and autoclave testing, to cover possible temperature variation in actual use when targeting 125 °C (257 °F) temperature exposure.

The data presented in this report support the following product claims for in-situ steaming and autoclaving 254 mm (10 inch) Pegasus grade SV4 filters in sanitary AB-style cartridges:

Filter Part Number	Steam Conditions	Steam Life Claim
AB1USV47PH4	In-situ steam cycles at 125 °C (257 °F)	2 one-hour cycles
AB1USV47PH4	Autoclave cycles at 125 °C (257 °F)	2 one-hour cycles

Determination of Water Flow Characteristics

Differential pressure measurements at set water flow rates have been determined for Pegasus grade SV4 filters. The typical clean water flow rate for a 254 mm (10 inch) filter (part number AB1USV47PH4) is 1.1 L/min at a pressure drop of 2.0 bar (29 psi) at 20 °C (68 °F). These data can be used to assist users in sizing filter systems employing 254 mm (10 inch) Pegasus grade SV4 filters in sanitary AB-style.

Filter Part Number	Typical Water Flow Rate at 2.0 bar (29 psi) Differential Pressure (L/min)
AB1USV47PH4	1.1

Extractables Testing using Water

The typical amount of non-volatile residue (NVR) extracted from Pegasus grade SV4 filter cartridges in sanitary AB-style has been determined after autoclaving (125 °C + 5 °C (257 °F + 9 °F, 2 x 24 hours extraction time at ambient temperature) and after a pre-flush of 10 liters, using water as the extraction fluid. For the 254 mm (10 inch) filter cartridges tested (part number AB1USV47PH4) the aqueous extractable values ranged from 44.1 mg to 87.4 mg in the first extraction (mean ± standard deviation: 66.7 ± 13.5 mg) and from 19.9 mg to 58.9 mg in the second (consecutive) extraction (mean ± standard deviation: 35.3 ± 13.4 mg).

The total NVR values per cartridge were therefore between 71.5 mg and 122.9 mg (mean ± standard deviation: 102.0 ± 19.1 mg) and the NVR values per m^2 of effective filter area were between 32.2 mg and 57.2 mg (mean ± standard deviation: 44.7 ± 8.3 mg).

The FTIR spectra of all extracts indicate the presence of compounds typical for the materials of construction, i.e. the acrylic polymer used to render the PVDF membrane hydrophilic and polyester compounds from the non-woven support and drainage layers. Strong peaks typical for compounds from the PVDF membrane were not detected. Water extractables of polypropylene hardware components are extremely low and were therefore not detected in this test.

Actual service will impose different conditions, such as different steaming conditions, exposure times, temperature, liquid type, etc. Evaluation under process conditions is therefore also recommended.

Biological Reactivity Tests

All of the materials used in Pegasus grade SV4 filters meet the requirements for biological reactivity, *in vivo*, under United States Pharmacopoeia (USP) <88> (for Class VI–121 °C plastics) and *in vitro*, under USP <87>. *In vivo* tests included the Systemic Toxicity Test, the Intracutaneous Tests, and the Implantation Test. *In vitro* testing was per the Minimum Essential Medium (MEM) Elution Cytotoxicity Test.

Copies of the test reports are available by contacting Pall Corporation.

2. Viral (Bacteriophage) Validation Tests

2.1 Introduction

A wide range of pharmaceutical products are produced with cell-culture techniques including vaccines, monoclonal antibodies and other recombinant therapeutic proteins and hormones. The risk of virus contamination is ever-present in such biologic products. Potential sources of virus contamination of biotechnology products include viruses associated with the cell lines (endogenous viruses), or viruses introduced into the bioreactor from culture medium or during the production processes (adventitious viruses). With plasma derivatives viruses could potentially be present in donor plasma.

The incorporation of robust virus inactivation or removal steps into the production process is key to the strategy for preventing viral contamination of the final product.

Virus filtration by size exclusion represents a robust removal method that demonstrates high efficacy for virus removal, and has become a well-accepted orthogonal method for the clearance of infectious viruses from biological API drug products. It is considered as alternative or complementary viral removal technology, and therefore broadens the portfolio of adequate virus contamination-control strategies mandated by regulatory agencies. Filtration is attractive for enhanced virus safety as it has little if any effect on the biological activity of the product, does not require the use of additives (or their subsequent removal), and can typically be readily included into the manufacturing process.

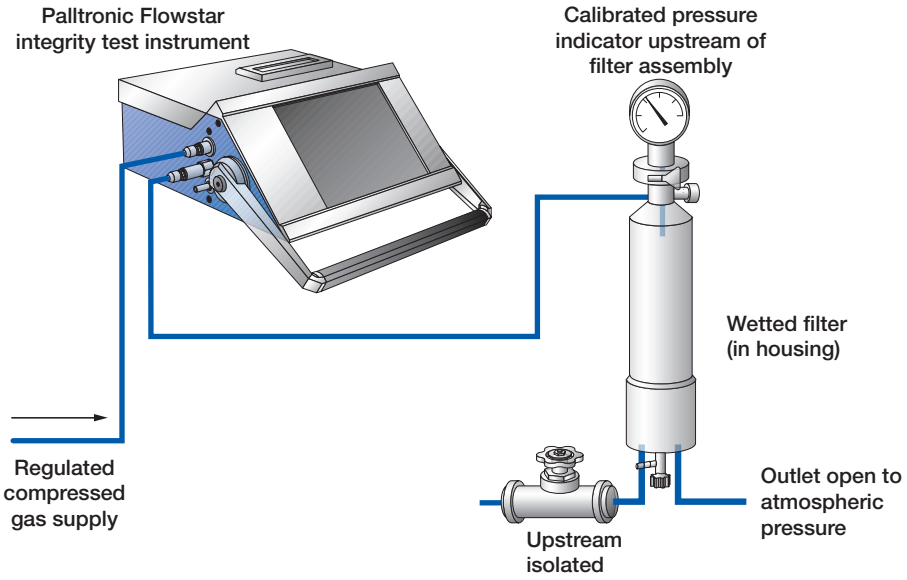
The application of filtration for critical process steps requires demonstration and documentation of the filter's performance through a physical test. The PDA Technical Report No 41-08 "Virus Filtration" states: "These physical tests enable confirmation of filter integrity by the manufacturer prior to shipment and confirmation of performance by the end user."

The correlation between viral retention and a non-destructive integrity test is an important aspect of the validation of filters for virus contamination control The Forward Flow test was the integrity test used during this study. This test is also employed by Pall for the non-destructive integrity testing of each virus removal membrane filter element in manufacturing as part of routine QC testing.

The Forward Flow Test

In the Forward Flow test, a filter is wetted with an appropriate test liquid and a pre-determined gas pressure is applied to the upstream side of the filter assembly. After a stabilization period, the gas flow through the wetted membrane can be measured on the upstream side, using sensitive flow measurement equipment such as the Palltronic® Flowstar filter integrity test instrument (see Figure 2.1).

Figure 2.1
The Automated Integrity Test



The objective of this study was to measure the removal efficiency of typical Pegasus grade SV4 filters for bacteriophage PP7 (sized at 25 nm) and to document the correlation of the integrity test parameters to the bacteriophage removal efficiency.

2.2 Summary of Methods

Pegasus grade SV4 AB-style filter cartridges, part number AB1USV47PH4 (254 mm, 10 inch, mean effective filter area: 2.25 m^2, 24.2 ft^2) were tested using the following procedure:

A) Installing the Filter in the Housing

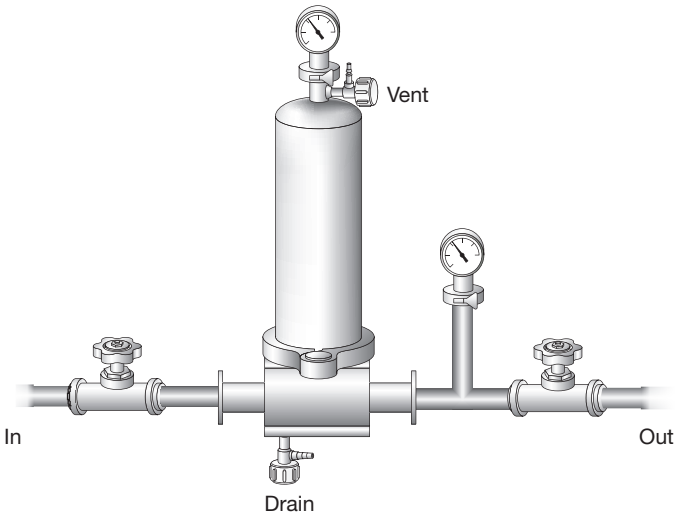
B) Filter Wetting Pre-Autoclaving

The first contact of the filter with wetting liquid was carefully controlled to ensure even and complete wetting. The filling was implemented from bottom to top on a filter being held in a vertical position and bubble free, in a time not faster than 5 minutes for a 254 mm (10 inch) filter. The wetting was generally in accordance with the “Preparation, Use and Integrity Testing of Pall Ultipor® VF DV20 Filter Cartridges and Capsules” (Pall publication USTR 2512) and followed the single steps described below:

1. Install the filter into the filter housing and orientate the Filter Housing as shown in Figure 2.2 below.
2. Connect filter housing to a 0.2 µm pre-filtered de-ionized (DI) water supply.
3. Ensure that the vent valve and downstream valves are open and slowly open the upstream valve to fill the filter housing with 0.2 µm pre-filtered DI water in a time not faster than 5 minutes for a 10 inch high filter.
4. Ensure that all air is purged from the filter housing using the appropriate vent valve, then close the vent valve.
5. Slowly open the upstream valve to generate an inlet pressure of 30 psig (2.07 bar).
6. When the pressure is stable, carefully close the downstream valve to apply back-pressure.
7. Increase the back-pressure until the inlet pressure and back-pressure are approximately 45 psig (3.10 bar) and 15 psig (1.03 bar) respectively.

8. Flow water through the filter for at least 10 minutes, adjusting upstream and downstream valves to maintain the inlet and outlet pressure.
9. After flushing open the downstream valve and wait for the outlet pressure to decay to ~0 psi.
10. Slowly close the upstream valve until the inlet pressure reads 0 psig.
11. Drain the excess fluid from the upstream side of the filter housing.

Figure 2.2
Filter Orientation for Wetting



C) Integrity Testing (Pre-Autoclaving)

Forward Flow integrity testing was performed on the water wetted filters using a Palltronic Flowstar integrity test instrument at a test pressure of 60 psi (4.150 bar) employing a test time of 10 minutes (600 sec).

D) Autoclaving
The water wetted filters were autoclaved at 121 °C for 60 minutes on wet filter and allowed to slowly cool while ensuring that the filters remain fully wetted.

E) Filter Wetting Post-Autoclaving
The filters were flushed with 0.1 micron filtered (or equivalent) sterile DI water for at least 10 minutes at a differential pressure of 30 psi (2.07 bar) following the procedure described above under B).

F) Integrity Test (Post-Autoclaving/Pre Challenge)
Forward Flow integrity testing was performed on the water wetted filters using a Palltronic Flowstar integrity test instrument at a test pressure of 60 psi (4.150 bar) employing a test time of 10 minutes (600 sec).

G) Liquid Bacteriophage Challenge
The bacteriophage challenge test equipment is shown in Figure 2.3. The filters were challenged with PP7 in a carrier fluid at ambient temperature, containing 0.1 % Bovine Serum Albumin (BSA) in Phosphate Buffered Saline (PBS). The bacteriophage spike aimed to contain a final concentration of at least 1.0×10^7 pfu/mL for PP7. Samples were taken from the spiked challenge fluid prior and after the challenge to confirm the actual phage challenge concentration for the respective challenge test and analyzed as described below under K). The challenge pressure was set at 30 psi (2.07 bar). Filtrate samples of 50 mL were collected, respectively, after two (2) liters, ten (10) liters and 23 liters challenge fluid had passed through the test filter. The filtrate samples were analyzed as described below under K).

H) Chemical Sanitization
After challenge the filters were removed from the housing and sanitized with a solution of 1 % sodium hypochlorite (NaOCl) for a minimum of 30 minutes or a maximum of overnight exposure by an overnight soak.

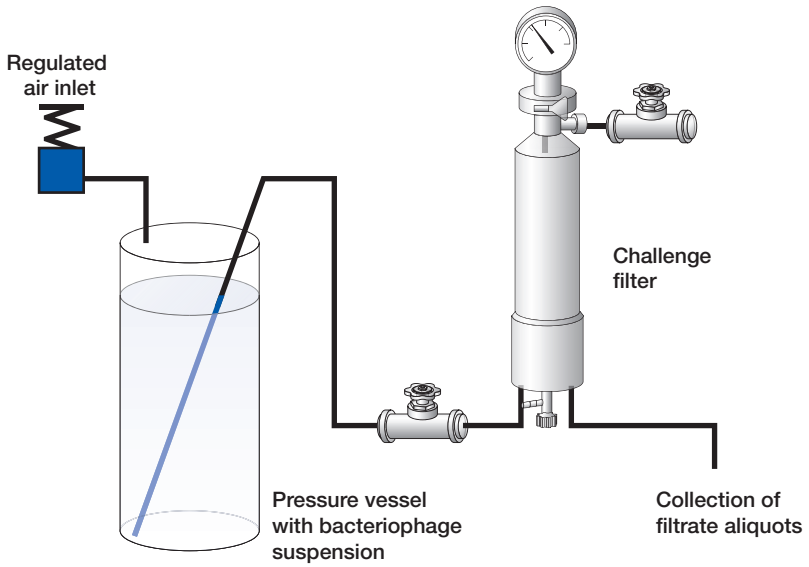
I) Post Sanitization Flushing
The sodium hypochlorite solution was drained from the filter. The filter was installed into a filter housing and flushed with DI water for at least 10 minutes at a differential pressure of 30 psi (2.07 bar) following the procedure described above under B).

J) Integrity Testing (Post Challenge)
Forward Flow integrity testing was performed on the water wetted filters using a Palltronic Flowstar integrity test instrument at a test pressure of 60 psi (4.150 bar) employing a test time of 10 minutes (600 sec).

K) Bacteriophage Assay
All samples were assayed for bacteriophage content using the agar overlay method. For the input challenge fluid of PP7 one (1) mL samples of 10-fold serial dilutions were assayed. For the effluent samples of PP7 one (1) mL samples of 10-fold serial dilutions and also of the undiluted effluent were assayed. The plaque assay plates were incubated at 37 °C ± 2 °C overnight, counted for plaques and the viral (bacteriophage) removal efficiency or Titer Reduction (T_R) of the filter was calculated as follows, using the lowest input challenge concentration determined and the titer determined for the 23rd liter filtrate sample:

$$T_R = \frac{\text{Concentration of challenge bacteriophage in input (pfu/mL)}}{\text{Concentration of challenge bacteriophage in effluent (pfu/mL)}}$$

Figure 2.3
Bacteriophage Challenge Apparatus



2.3 Results

The data from the viral (bacteriophage) retention versus Forward Flow validation study are listed in Table 2.1. The highest of the three Forward Flow values measured (Pre-Autoclaving, Post-Autoclaving/Pre Challenge, Post Challenge) is presented and the data are arranged in order of increasing Forward Flow value.

Pegasus grade SV4 254 mm (10 inch) AB-style filter cartridges, part number AB1USV47PH4 filter cartridges with Forward Flow values from 19.5 mL/min to 42.0 mL/min – when wet with water and tested at 60 psi (4.150 bar) – gave titer reductions for bacteriophage PP7 (sized at 25 nm) of > 4 logs under the test conditions (see section 2.2).

Table 2.1

Results of Forward Flow Values and Bacteriophage PP7 (sized at 25 nm) Retention for 254 mm (10 inch) Pegasus Grade SV4 AB-Style Filter Cartridges, Part Number AB1USV47PH4

Filter Serial Number	Forward Flow* at 60 psi (4.150 bar) (mL/min)	Titer Reduction for Phage PP7 (~ 25 nm)
IS2498-064	19.50	4.00×10^{6}
IS2498-254	19.70	2.50×10^{5}
IS2498-160	19.90	8.00×10^{6}
IS2498-172	20.00	7.83×10^{5}
IS2498-120	20.10	7.58×10^{5}
IS2498-060	20.20	3.57×10^{6}
IS2498-054	20.30	2.73×10^{5}
IS2498-092	20.40	2.27×10^{6}
IS2498-256	20.50	2.20×10^{6}
IS2498-216	20.50	5.09×10^{6}
IS2498-150	20.70	9.23×10^{5}
IS2498-182	20.80	7.06×10^{5}
IS2498-080	20.90	7.14×10^{6}
IS2498-152	21.00	1.30×10^{7}
IS2498-304	21.00	3.43×10^{6}
IS2498-162	21.00	1.60×10^{6}
IS2498-238	21.00	4.40×10^{5}
IS2498-166	21.00	1.19×10^{5}
IS2498-180	21.30	4.42×10^{6}
IS2498-072	21.30	8.00×10^{6}
IS2498-228	21.50	1.45×10^{5}
IS2498-156	21.60	1.00×10^{6}
IS2498-220	21.60	2.00×10^{6}
IS2498-190	21.60	5.09×10^{6}
IS2498-188	21.90	2.80×10^{6}
IS2498-142	22.20	5.00×10^{5}
IS2498-070	22.40	4.59×10^{5}
IS2498-048	22.40	3.50×10^{6}
IS6230-025	22.80	4.40×10^{5}
IS2498-210	23.00	2.16×10^{5}
IS6230-052	23.10	1.90×10^{5}
IS6228-016	23.20	3.80×10^{5}
IS6228-005	23.30	5.95×10^{5}
IS6230-038	23.30	8.75×10^{4}
IS6230-055	23.40	1.22×10^{5}
IS6230-074	23.50	3.70×10^{5}
IS6230-051	23.50	1.29×10^{5}
IS6230-057	23.60	1.44×10^{5}
IS6230-077	23.70	1.45×10^{5}
IS6228-062	24.20	7.06×10^{5}
IS6228-077	24.20	5.16×10^{5}

Table 2.1 Continued

Filter Serial Number	Forward Flow* at 60 psi (4.150 bar) (mL/min)	Titer Reduction for Phage PP7 (~ 25 nm)
IS6228-049	24.30	1.22×10^6
IS6228-065	24.30	3.90×10^5
IS6228-064	24.40	4.71×10^5
IS6230-023	24.40	1.08×10^5
IS6230-022	24.60	1.59×10^5
IS6228-069	24.70	4.92×10^5
IS6230-061	24.70	1.05×10^5
IS6228-028	24.90	4.05×10^5
IS6228-082	25.00	6.29×10^5
IS6228-030	25.20	4.38×10^5
IS6230-047	25.60	1.35×10^5
IS6230-028	26.00	6.90×10^4
IS6229-003	26.80	7.70×10^5
IS6229-001	26.80	2.71×10^5
IS6230-063	27.00	5.77×10^4
IS2498-194	27.10	1.71×10^5
IS6229-002	27.10	4.60×10^5
IS6229-013	27.50	2.64×10^5
IS6230-053	27.50	5.09×10^4
IS6229-037	27.90	2.30×10^5
IS6229-063	28.00	3.30×10^5
IS6229-024	28.30	2.00×10^5
IS6229-004	28.90	1.80×10^5
IS6229-031	29.80	1.30×10^5
IS6229-008	29.90	8.50×10^4
IS6230-026	32.40	4.12×10^4
IS2498-014	33.00	7.62×10^4
IS6228-029	33.80	3.17×10^5
IS2498-232	34.00	8.29×10^4
IS2498-226	38.90	4.55×10^4
IS6230-064	42.00	5.06×10^4

**Forward Flow values wet with water, at 20 °C + 5 °C (68 °F + 9 °F), maximum allowable limit value 30 mL/min at 60 psi (4.150 bar).*

2.4 Conclusions

The Forward Flow integrity test performed at 60 psi (4.150 bar) in water demonstrates that all Pegasus grade SV4 AB-style filter cartridges tested, which displayed Forward Flow values between 19.5 mL/min and 42.00 mL/min at 60 psi (4.150 bar), were retentive for bacteriophage PP7 (sized at 25 nm) with a titer reduction of > 10^4 under the test conditions.

A user Forward Flow limit of 30 mL/min at 60 psi (4.150 bar) test pressure when wetted with water was set for Pegasus grade SV4 AB-style filter cartridges based on the above results of the bacteriophage challenge tests and additional considerations and parameters.

Table 2.2

*Forward Flow Integrity Test Parameters for an AB1USV47PH4 filter cartridge**

Test pressure	60 psi (4.150 bar)
Wetting liquid	Water
Temperature	20 °C ± 5 °C (68 °F ± 9 °F)
Test gas	Air
Maximum allowable Forward Flow limit**	30 mL/min

** See section 2.2 for test procedure.*

*** During the test period the temperature of the filter assembly should not vary more than + 1 °C (2 °F).*

3. Endurance to In-Situ Steaming and Autoclaving

3.1 Introduction

The purpose of these tests was to determine the effects of repeated exposure to in-situ steam cycles and autoclaving on filter integrity using and water wettability on Pegasus grade SV4 AB-style filter cartridges. The tests were used to qualify the steam exposure claims for Pegasus grade SV4 filter cartridges.

3.2 Summary of Methods

In-Situ Steaming Tests Targeting a Steaming Claim of 125 °C (257 °F) under Water Wet Steaming Conditions

Pegasus grade SV4 filters, part number AB1USV47PH4, from two different cartridge batches were used for the tests. Filter manufacturing and sampling considered possible variation in the filter manufacturing conditions.

Twenty-four (24) filter cartridges were submitted to the following test sequence:

A) Installing the Filter in the Housing

B) Filter Wetting Pre-Steaming

The first contact of the filter with wetting agents was carefully controlled to ensure even and complete wetting. The filling was implemented from bottom to top on a filter being held in a vertical position and bubble free, in a time not faster than 5 minutes for a 254 mm (10 inch) filter. The wetting followed the procedure described in section 2.2.

C) Integrity Testing Pre-Steaming

Forward Flow integrity testing was performed on the water wetted filters using a Palltronic Flowstar integrity test instrument at a test pressure of 60 psi (4.150 bar) employing a test time of 10 minutes (600 sec).

D) Wet Steaming and Re-Wetting

The wet filters were subjected to a one-hour in-situ steam cycle at 130 °C (266 °F). During the initial stages of the steam cycle, the wet filter membrane caused the differential pressure to increase across the filter cartridge as steam was introduced. The steam inlet valve was controlled so that the differential pressure across the wetted filter cartridge did not exceed 300 mbar (4.35 psi). At the end of each steam cycle, the upstream and downstream valves were ‘cracked’ slightly open, to allow steam to slowly vent, and to prevent the filter element drying out. The system was allowed to cool to 25 °C ± 5 °C (77 °F ± 9 °F), ensuring that the filter elements remained wetted. After each steam cycle the filters were re-wetted by flushing using the procedure for filter wetting described in section 2.2.

E) Integrity Testing Post-Steaming

Forward Flow integrity testing was performed on the water wetted filters using a Palltronic Flowstar integrity test instrument at a test pressure of 60 psi (4.150 bar) employing a test time of 10 minutes (600 sec). The filters were also visually inspected and checked for any visual damage or change in appearance.

This test sequence was repeated until each filter cartridge had been exposed to four (4) one-hour steam cycles.

Autoclaving Test Targeting an Autoclave Claim of 125 °C (257 °F) under Water Wet Autoclave Conditions

Pegasus grade SV4 filters, part number AB1USV47PH4, from two different cartridge batches were used for the tests. Filter manufacturing and sampling considered possible variation in the filter manufacturing conditions.

Twenty four (24) filter cartridges were submitted to the following test sequence:

A) Installing the Filter in the Housing

B) Filter Wetting and Pre-Autoclaving
The first contact of the filter with wetting agents was carefully controlled to ensure even and complete wetting. The filling was implemented from bottom to top on a filter being held in a vertical position and bubble free, in a time not faster than 5 minutes for a 254 mm (10 inch) filter. The wetting was generally in accordance with USTR 2512 and followed the single steps described in section 2.2.

C) Integrity Testing Pre-Autoclaving
Forward Flow integrity testing was performed on the water wetted filters using a Palltronic Flowstar integrity test instrument at a test pressure of 60 psi (4.150 bar) employing a test time of 10 minutes (600 sec).

D) Wet Autoclaving and Re-Wetting
The wet filters were subjected to a one-hour autoclave cycle at 130 °C (266 °F) using a slow exhaust cycle. The system was allowed to cool to 25 °C ± 5 °C (77 °F ± 9 °F), ensuring that the filter remained fully wetted. After each autoclave cycle filter the filters were re-wetted by flushing using the procedure for filter wetting described in section 2.2.

E) Integrity Testing Post-Autoclave
Forward Flow integrity testing was performed on the water wetted filters using a Palltronic Flowstar integrity test instrument at a test pressure of 60 psi (4.150 bar) employing a test time of 10 minutes (600 sec). The filters were also visually inspected and checked for any visual damage or change in appearance.

This test sequence was repeated until each filter cartridge had been exposed to four (4) one-hour autoclave cycles.

3.3 Results

In-Situ Steaming Tests Targeting a Steaming Claim of 125 °C (257 °F)
The Forward Flow integrity test results for Pegasus grade SV4 AB-style filter cartridges (part number AB1USV47PH4) before and after exposure to 4 one-hour in-situ steam cycles are shown in Table 3.1. All of the filters retained integrity and water wettability following exposure to 4 one-hour cycles at 130 °C (266 °F).

Table 3.1

Effects of In-Situ Steam Exposure at 130 °C (266 °F) on Filter Integrity and Water Wettability for Pegasus Grade SV4 AB-style Filter Cartridges, Part Number AB1USV47PH4

	Forward Flow* (mL/min) pre and after exposure to 4 *in-situ* steam cycles at 130 °C (266 °F)	
Filter Serial Number	**FF (mL/min) pre-steaming**	**FF (mL/min) post-steaming**
IS6228-010	27.6	25.6
IS6228-013	27.4	27.9
IS6228-021	23.8	25.2
IS6228-039	25.9	24.7
IS6228-025	29.0	25.5
IS6228-041	25.1	25.9
IS6228-043	24.4	24.9
IS6228-042	25.8	23.2
IS6228-053	24.7	24.2
IS6228-055	24.9	23.3
IS6228-059	24.8	27.7
IS6228-070	24.9	25.2
IS6228-074	21.9	23.2
IS6228-081	26.2	25.0
IS6230-024	24.1	23.7
IS6230-027	25.9	24.7
IS6230-046	24.6	29.0
IS6230-059	24.9	24.1
IS6230-060	23.8	28.9
IS6230-062	23.9	26.3
IS6230-073	25.5	25.5
IS6230-075	26.8	24.0
IS6230-081	22.7	26.7
IS6230-085	29.6	27.6

** Forward Flow values wet with water, at 20 °C + 5 °C (68 °F + 9 °F), maximum allowable limit value 30 mL/min at 60 psi (4.150 bar).*

Autoclaving Tests Targeting an Autoclave Claim of 125 °C (257 °F)

The Forward Flow integrity test results for Pegasus grade SV4 AB-style filter cartridges (part number AB1USV47PH4) before and after exposure to 4 one-hour autoclave cycles are shown in Table 3.2. All of the filters retained integrity and water wettability following exposure to 4 one-hour cycles at 130 °C (266 °F).

Table 3.2

Effects of Autoclave Exposure at 130 °C (266 °F) on Filter Integrity and Water Wettability for Pegasus Grade SV4 AB-style Filter Cartridges, Part Number AB1USV47PH4

	Forward Flow* (mL/min) pre and after exposure to 4 autoclave cycles at 130 °C (266 °F)	
Filter Serial Number	**FF (mL/min) pre-autoclaving**	**FF (mL/min) post-autoclaving**
IS6228-001	24.2	22.0
IS6228-003	27.8	29.6
IS6228-015	25.6	26.8
IS6228-037	27.8	24.5
IS6228-044	23.5	21.1
IS6228-051	25.5	24.4
IS6228-054	27.4	25.9
IS6228-066	23.8	23.0
IS6228-056	28.3	25.2
IS6228-068	24.7	25.3
IS6228-071	24.2	22.2
IS6228-073	24.4	24.6
IS6228-075	28.7	22.9
IS6228-086	25.7	25.5
IS6230-043	27.6	27.3
IS6230-045	24.4	24.7
IS6230-048	26.4	25.8
IS6230-058	27.5	29.8
IS6230-066	26.8	25.5
IS6230-072	26.4	24.6
IS6230-080	26.8	25.4
IS6230-082	29.5	26.9
IS6230-083	27.7	27.3
IS6230-084	25.7	29.6

** Forward Flow values wet with water, at 20 °C + 5 °C (68 °F + 9 °F), maximum allowable limit value 30 mL/min at 60 psi (4.150 bar).*

3.4 Conclusions

Pegasus grade SV4 AB-style filter cartridges (part number AB1USV47PH4) have been demonstrated to be capable of withstanding multiple *in-situ* steaming and autoclave cycles while water wet. Tests performed demonstrate that the Pegasus grade SV4 filters are robust and capable of withstanding differential pressures up to 300 mbar (4.35 psi) in the forward direction during steaming. Test conditions involved exposure to 130 °C (266 °F) temperature during in-situ steaming and autoclave testing, to cover possible temperature variation in actual use when targeting 125 °C (257 °F) temperature exposure.

The data presented in this section support the following product claims for in-situ steaming and autoclaving Pegasus grade SV4 AB-style filter cartridges (part number AB1USV47PH4).

Filter Part Number	Steam Conditions	Steam Life Claim
AB1USV47PH4	In-situ steam cycles at 125 °C (257 °F)	2 one-hour cycles
AB1USV47PH4	Autoclave cycles at 125 °C (257 °F)	2 one-hour cycles

The above claim is supported by data with a safety margin of 100 % or above.

4. Determination of Water Flow Characteristics

4.1 Introduction

The objective of these tests was to determine the typical differential pressure across Pegasus grade SV4 filter cartridges at set water flow rates.

4.2 Summary of Methods

The tests were performed on twelve (12) Pegasus grade SV4 filter cartridges, part number AB1USV47PH4, from three different cartridge batches. Filter manufacturing and sampling considered possible variation in the filter manufacturing conditions.

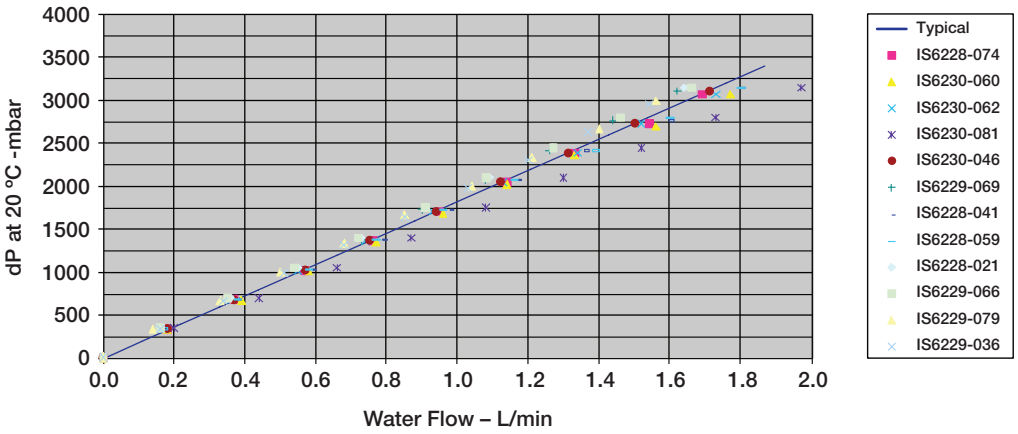
Prior to water flow measurement the filters were wetted and Forward Flow tested using the methods described under section 2.2. For water flow measurements, pre-filtered DI water was pumped through the filters in the normal flow ('out to in') direction. Pressure readings from transducers on the upstream and downstream sides of the test assembly were monitored to calculate the differential pressure at set water flow rates.

The results were corrected for viscosity variation at different temperatures in order to normalize performance to a standard temperature of 20 °C (68 °F).

4.3 Results

Figure 4.1 shows the clean water flow rates of the twelve (12) Pegasus grade SV4 filter cartridges, part number AB1USV47PH4, that were tested.

Figure 4.1
Water Flow/Differential Pressure Values of Pegasus Grade SV4 Filter Cartridges, Part Number AB1USV47PH4



4.4 Conclusions

Water flow rates at set differential pressures have been determined. The results support that the typical clean water flow rate for a 254 mm (10 inch) Pegasus grade SV4 filter cartridge (part number AB1USV47PH4) at 2.0 bar (29 psi) pressure drop is 1.1 L/min at 20 °C (68 °F). These data can be used to assist users in sizing filter systems employing Pegasus grade SV4 filter cartridges.

Filter Part Number	Typical Water Flow Rate at 2.0 bar (29 psi) Differential Pressure (L/min)
AB1USV47PH4	1.1

5. Extractables Testing Using Water

5.1 Introduction

The objective of this series of tests was to quantify and characterize the material that can be extracted from Pegasus grade SV4 filters using water. Pegasus grade SV4 filter cartridges in sanitary AB-style are constructed from a hydrophilic methacrylate-modified polyvinylidene fluoride (PVDF) filter membrane, polyester non-woven support and drainage layers, and polypropylene molded components.

5.2 Summary of Methods

Twelve (12) Pegasus grade SV4 filter cartridges of part number AB1USV47PH4 (mean effective filter area: 2.25 m^2, 24.2 ft^2) from three different cartridge batches were used for the tests. Filter manufacture and sampling considered possible variation in the filter manufacturing process.

Preparation of Filter Samples

The filters were flushed with 20 liters of 0.2 µm pre-filtered DI water at a differential pressure of 30 psi (2.07 bar) and then autoclaved at a temperature of 125 °C ± 5 °C (257 °F ± 9 °F) for 60 min in order to mimic a standard sterilization cycle performed by a customer prior to filter use. Autoclaving will maximize the quantity of extractable material present in a polymeric material. After autoclaving the filters were flushed with 10 liters of 0.2 µm pre-filtered DI water at a differential pressure of 30 psi (2.07 bar) and submitted to extraction.

Extraction Procedure for Filter Cartridges and Determination of Non-volatile Residue (NVR)

Dynamic extraction tests were performed in a known volume of water at ambient temperature. The test filters were immersed in the extraction fluid in a clean measuring cylinder, as shown in Figure 5.1. For twenty four (24) hours the filter was gently moved up and down. This movement created flow through the filter membrane as a result of the pressure head that was created each time the element was partially lifted out of the liquid.

The filter cartridges were submitted to a second (consecutive) dynamic extraction cycle under the same extraction conditions as described above. Blank samples were determined as appropriate for method and result controls.

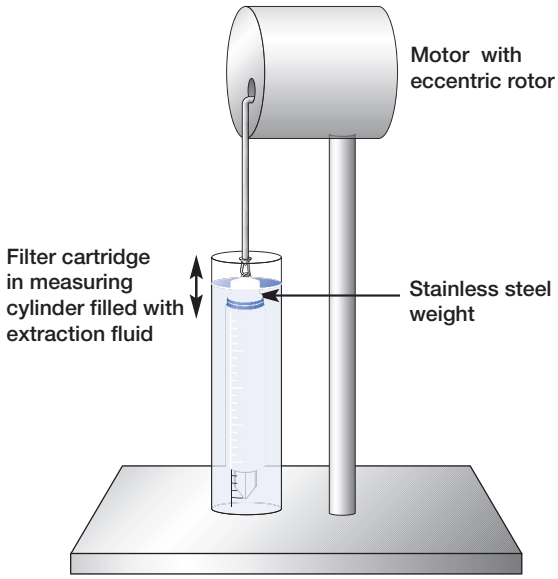
Following the extraction period, a measured volume of the extraction liquid was evaporated to dryness and the non-volatile residue (NVR) was determined gravimetrically. A correction was made to the NVR value to account for the total extraction volume used.

Analysis by Fourier Transform Infra Red Spectroscopy (FTIR)

The dry NVR of some filter cartridges was analyzed by Fourier Transform Infra Red Spectroscopy (FTIR) to provide information on the nature of its organic compounds. The analysis included first and also second (consecutive) extracts.

Figure 5.1

Filter Extraction Apparatus



5.3 Results of NVR and FTIR

Table 5.1 shows the levels of aqueous extractables obtained from the twelve (12) Pegasus grade SV4 filter cartridges, part number AB1USV47PH4, that were tested. The NVR values in the first extraction ranged from 44.1 mg to 87.4 mg in the first extraction and from 19.9 mg to 58.9 mg in the second (consecutive) extraction.

Table 5.1

Non-volatile Aqueous Extractables Obtained Using Pegasus Grade SV4 Filter Cartridges after Autoclaving at 125 °C ± 5 °C (257 °F ± 9 °F) and 10 Liter Pre-Flush (24 Hours Extraction Time at Ambient Temperature)

Cartridge Serial Number	Effective Filter Area (EFA) (m^2)	Non-Volatile Residue (NVR) (mg) in 1st Extraction	Non-Volatile Residue (NVR) (mg) in 2nd Extraction	Total Non-Volatile Residue (NVR) (mg) per Cartridge	Total Non-Volatile Residue (NVR) (mg) per m^2 of Effective Filter Area
IS6228-006	2.31	87.4	44.7	132.1	57.2
IS6228-017	2.31	71.7	30.2	101.9	44.1
IS6228-018	2.31	80.1	27.0	107.1	46.4
IS6228-079	2.31	86.1	36.8	122.9	53.2
IS6229-009	2.31	55.4	20.0	75.4	32.6
IS6229-030	2.31	58.1	23.0	81.1	35.1
IS6229-034	2.31	44.1	58.9	103.0	44.6
IS6229-068	2.31	61.6	44.0	105.6	45.7
IS6230-035	2.22	72.0	41.5	113.5	51.1
IS6230-041	2.22	51.6	19.9	71.5	32.2
IS6230-050	2.22	64.9	54.3	119.2	53.7
IS6230-078	2.22	66.9	23.3	90.2	40.6
	Mean	**66.7**	**35.3**	**102.0**	**44.7**
	StDev	**13.5**	**13.4**	**19.1**	**8.3**

Typical infrared spectra of the aqueous NVR from Pegasus grade SV4 filter cartridges (part number AB1USV47PH4) are shown in Figure 5.2 and Figure 5.3. Figure 5.2 shows the Infrared spectrum of the NVR from a first extraction of a filter cartridge. Figure 5.3 shows the infrared spectrum of the NVR from the second (consecutive) extraction of that cartridge.

Figure 5.2

Typical Infra Red Spectrum of the Aqueous NVR from Pegasus Grade SV4 Filter Cartridges from the First Extraction

AB1USV47PH4 Serial Number IS6228079 Infrared Spectrum of NVR from 1st Extraction

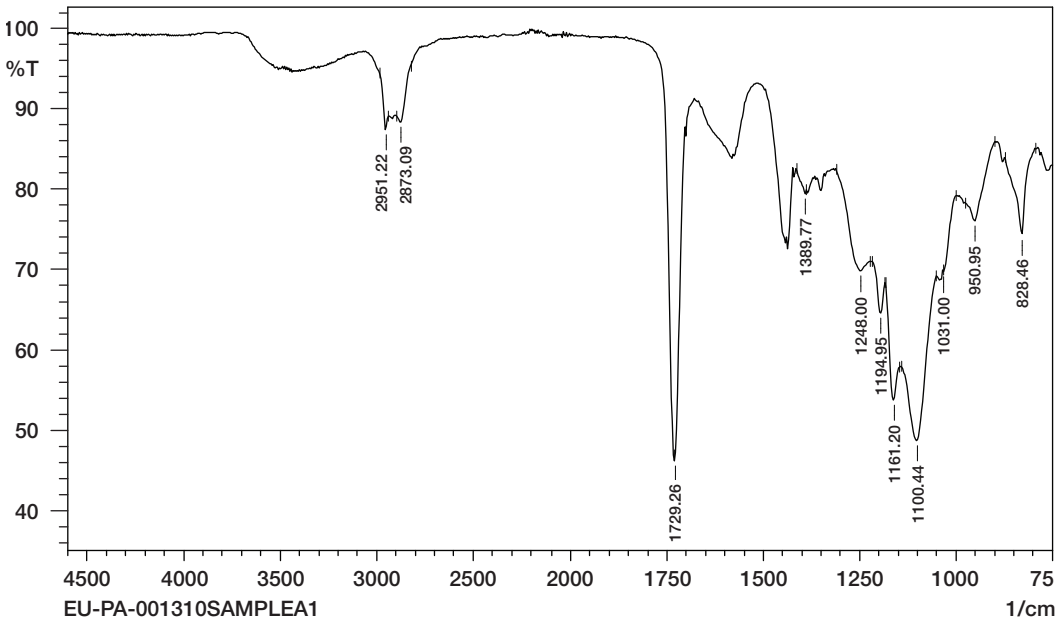
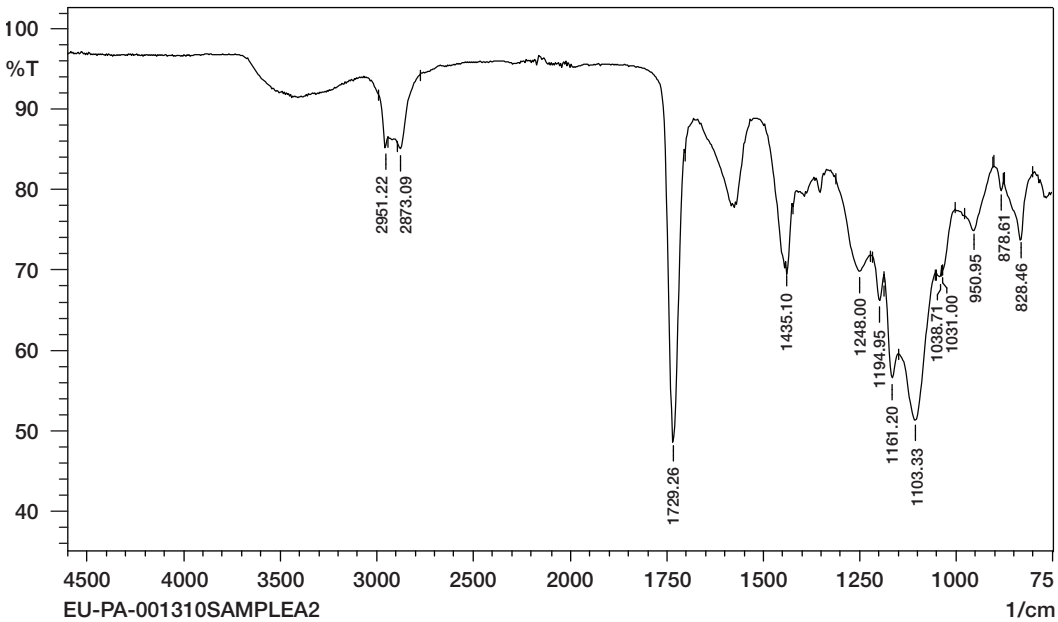


Figure 5.3

Typical Infra Red Spectrum of the Aqueous NVR from Pegasus Grade SV4 Filter Cartridges from the Second Extraction

AB1USV47PH4 Serial Number IS6228079 Infrared Spectrum of NVR from 2nd Extraction



5.4 Conclusions

The typical amount of non-volatile residues (NVR) extracted from Pegasus grade SV4 filter cartridges in AB-style has been determined using water as the extraction fluid. For the 254 mm (10 inch) filter cartridges tested (part number AB1USV47PH4) the aqueous extractable values ranged from 44.1 mg to 87.4 mg in the first extraction (mean ± standard deviation: 66.7 ± 13.5 mg) and from 19.9 mg to 58.9 mg in the second (consecutive) extraction (mean ± standard deviation: 35.3 ± 13.4 mg).

This results in total NVR values per cartridge between 71.5 mg and 122.9 mg (mean ± standard deviation: 102.0 ± 19.1 mg) and NVR values per m^2 of effective filter area between 32.2 mg and 57.2 mg (mean ± standard deviation: 44.7 ± 8.3 mg).

The FTIR spectra of all extracts indicate the presence of compounds typical for the materials of construction, i.e. the acrylic polymer used to render the PVDF membrane hydrophilic, and polyester compounds from the non-woven support and drainage layers. Strong peaks typical for compounds from the PVDF membrane were not detected. Water extractables of polypropylene hardware components are extremely low and were therefore not detected in this test.

Actual service will impose different conditions, such as different steaming conditions, exposure times, temperature, liquid type, etc. Evaluation under process conditions is therefore also recommended.

6. Biological Reactivity Tests on the Materials of Construction

6.1 Introduction

The aim of this study was to evaluate the biological suitability of the materials of construction of Pegasus grade SV4 filter cartridges in AB-style. The materials of construction of the filters are as follows:

Table 6.1

Materials of Construction for Pegasus Grade SV4 Filters in AB-style

Membrane:	Pall hydrophilic polyvinylidene fluoride membrane rendered hydrophilic by a methacrylate co-polymer
Membrane support and drainage layers:	Polyester
Core and end caps:	Polypropylene
Adapter:	Polypropylene with internal reinforcing ring
Filter cage:	Polypropylene with titanium dioxide
O-rings:	Silicone elastomer for 'H4' option

6.2 Summary of Methods

The tests on the respective material of construction were performed in accordance with the <88> Biological Reactivity Tests (*in vivo*) for Class VI Plastics (121 °C) as described in the current *United States Pharmacopeia* (USP).

The testing procedures described in the *United States Pharmacopeia* include:

- Injection of extracts of plastic materials
- Implantation of the solid material into animal tissue

The four extracting media listed in the *United States Pharmacopeia* simulate parenteral solutions and body fluids. These include:

- Sodium Chloride Injection
- 1 in 20 Solution of Alcohol in Sodium Chloride Injection
- Polyethylene Glycol 400
- Vegetable Oil (sesame or cottonseed oil)

The *United States Pharmacopeia* <88> states that extracts may be prepared at one of three standard conditions: 50 °C (122 °F) for 72 hours, 70 °C (158 °F) for 24 hours or 121 °C (250 °F) for 1 hour. The most stringent condition not resulting in physical changes in the plastic is recommended, therefore the filter materials were extracted at 121 °C (250 °F) for 1 hour.

Acute Systemic Injection Tests

An Acute Systemic Injection Test was performed to evaluate the potential of a single injection of an extract to produce systemic toxicity. Sodium Chloride Injection and 1 in 20 Solution of Alcohol in Sodium Chloride Injection were injected intravenously. Vegetable oil extract and Polyethylene Glycol 400 extract were injected intraperitoneally.

Intracutaneous Tests

An Intracutaneous Test was performed to evaluate the potential of a single injection of an extract to produce tissue irritation. All four of the extracts listed above were used for these tests.

Implantation Tests

Implantation tests were also performed, in order to subject the materials of construction to the most stringent conditions included in the United States Pharmacopoeia. Each of the materials of the Pegasus grade SV4 filter cartridges was implanted separately.

Under USP <87>, several procedures are defined. An Elution Test was carried out to determine the biological reactivity of mammalian cell cultures following contact with extracts of the polymeric materials of construction.

6.3 Results

No unacceptable biological response was observed in any of the tests performed and therefore the materials used in Pegasus grade SV4 filter cartridges passed all of the tests specified.

6.4 Conclusions

The materials used in Pegasus grade SV4 filter cartridges in sanitary AB-style met the requirements of the *United States Pharmacopoeia* (USP) Biological Reactivity Tests (*in vivo*) for Class VI-121 °C plastics. The tests included the Systemic Injection test, the Intracutaneous test and the Implantation test.

Copies of the test reports are available by contacting Pall Corporation.

7. Transmissible Spongiform Encephalopathy (TSE) / Bovine Spongiform Encephalopathy (BSE) Statement

Pegasus SV40 filter cartridges do not contain materials of construction that are considered TSE or BSE-risk materials according to current legislation and guidance in both Europe and the United States:

1. The European CPMP Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathies via human and veterinary medicinal products. (EMA/410/01).
3. The U.S. Code of Federal Regulations, Title 21 of part 189.5, which defines specified risk materials obtained from cattle.

Pall has an established program with our raw material suppliers to assess whether animal derived products (e.g. bovine / ovine / caprine) are present in the materials employed for our pharmaceutical grade products. We have identified that polypropylene resins, used to manufacture plastic components of the referenced products, contain trace levels of additives, which may be derived from bovine tallow. Tallow derivatives are not considered specified BSE risk materials according to the current revision of Title 21, of the U.S. Code of Federal Regulations, part 189.5. Furthermore, the CPMP's Note for guidance (EMA 410/01) gives specific consideration to tallow derivatives and states they are unlikely to be infectious due to the rigorous processing steps used (an example of which is trans-esterification, or hydrolysis, at not less than 200 °C (392 °F) under pressure for not less than 20 minutes). The raw materials we purchase have been processed under these conditions. Additionally, during the conversion of polypropylene resin into plastic components further high temperature steps are performed.

PALL Life Sciences

Corporate Headquarters
Port Washington, NY, USA
+1 800 717 7255 toll free (USA)
+1 516 484 5400 phone
biopharm@pall.com e-mail

European Headquarters
Fribourg, Switzerland
+41 (0)26 350 53 00 phone
LifeSciences.EU@pall.com e-mail

Asia-Pacific Headquarters
Singapore
+65 6389 6500 phone
sgcustomerservice@pall.com e-mail

Filtration. Separation. Solution.SM

Visit us on the Web at www.pall.com/biopharm

E-mail us at biopharm@pall.com

International Offices
Pall Corporation has offices and plants throughout the world in locations such as: Argentina, Australia, Austria, Belgium, Brazil, Canada, China, France, Germany, India, Indonesia, Ireland, Italy, Japan, Korea, Malaysia, Mexico, the Netherlands, New Zealand, Norway, Poland, Puerto Rico, Russia, Singapore, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, the United Kingdom, the United States, and Venezuela. Distributors in all major industrial areas of the world. To locate the Pall office or distributor nearest you, visit **www.pall.com/contact**.

The information provided in this literature was reviewed for accuracy at the time of publication. Product data may be subject to change without notice. For current information consult your local Pall distributor or contact Pall directly.

May 2013, PDF, GN13.5743

USTR 2839(1)