PALL Life Sciences

Validation Guide

USTR 2399[1]

Pegasus™ LV6 Virus Removal Filter Cartridges

*Filtration. Separation. Solution.*SM

Contents

1. Overview

1.1 Scope

This document contains validation data applicable to Pegasus LV6 (large virus > 6 log reduction) virus removal filters in AB-style cartridge configurations. Pegasus LV6 AB-style Filter Cartridges are designed to very efficiently and economically remove large viruses (e.g. retroviruses) with > 6 log from biological solutions. The following validation data have demonstrated robust > 6 log titer reduction for PR772 bacteriophage (model for retroviruses and other large viruses). The 254 mm (10 in.) Pegasus LV6 AB-style Filter Cartridges are manufactured with Ultipleat™ laid-over pleated filter construction, the next generation of membrane pleating technology. Ultipleat laid-over pleated filter construction offers increased filter flow rates and reduced hold-up volume due to a significantly higher filter area per filter cartridge. Pegasus LV6 AB-style Filter Cartridges are made from a hydrophilic acrylate-modified polyvinylidene fluoride (PVDF) filter membrane, polyethylene/polypropylene support and drainage layers and polypropylene molded components. Pegasus LV6 AB-style Filter Cartridges are intended for installation in suitable stainless steel filter housing assemblies.

Table 1
Product Tested

	Part Number	Height	Filter Area per 254 mm (10 in.)
Pegasus LV6 Virus Removal Filter	AB1ULV67PJ	254 mm (10 in.)	1.65 m^2 (17.8 ft^2)

The tests that were performed to qualify the performance of Pegasus LV6 Filter Cartridges under a range of test conditions were as follows:

- Viral (bacteriophage) retention tests correlated with Forward Flow integrity testing
- Determination of water flow characteristic
- Determination of aqueous extractables testing out-of-box and post autoclaving
- Biological Reactivity Tests

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 are subject to stringent quality control including in-process controls and testing of the filter elements as follows:

1. Forward Flow Test is performed on a 100% basis
2. Total Organic Carbon (TOC), Conductivity and pH Tests
3. Effluent Cleanliness Test
4. Limulus Amebocyte Lysate Test
5. Viral (bacteriophage) Challenge Tests.

1.2 Summary of Conclusions

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

Pegasus LV6 AB-style Filter Cartridges were tested using viral (bacteriophage) challenge tests with bacteriophage PR772 (53 – 82 nm). The results support a claim of > 6 log titer reduction for this test organism. The Forward Flow integrity test was shown to be a suitable non-destructive integrity test for Pegasus LV6 AB-style Filter Cartridges, and test parameters have been set as follows for 254 mm (10 in.) filters, part number AB1ULV67PJ.

Integrity Tests	
Test Pressure	4.150 bar (60 psi)*
Wetting Liquid	Water
Temperature	20 °C ± 5 °C
Test Gas	Air
Maximum Allowable Forward Flow Limit**	40 mL/min

** Bar value rounded up to nearest 0.025 bar.*
*** During the test period the temperature of the filter assembly should not vary more than ±1 °C.*

1.2.2 Determination of Water Flow Characteristics

Differential pressure measurements at set water flow rates have been determined for Pegasus LV6 AB-style Filter Cartridges, part number AB1ULV67PJ (254 mm [10 in.]). These data can be used to assist users in sizing filter systems employing Pegasus LV6 AB-style Filter Cartridges and support the following claim:

Table 2
Typical Water Flow Rate

Pall Filter Part Number	Typical Water Flow Rate at 2.1 bar (30 psi) Differential Pressure (L/min)
AB1ULV67PJ	12

1.2.3 Extractables Testing Using Water

The level of aqueous extractables was determined for Pegasus LV6 AB-style Filter Cartridges, part number AB1ULV67PJ (254 mm [10 in.]). Tests were performed with filters both out-of-box and after autoclaving at 125 °C ± 5 °C (4 hours extraction time at ambient temperature). Actual service in a pharmaceutical application may impose different conditions, such as different steaming conditions, exposure times, temperature, liquid type, etc. Evaluation under actual process conditions is therefore also recommended.

Table 3
Extractables

Pall Filter Part Number	Extraction Fluid	Level of Non-Volatile Extractables Out-of-Box (mg)		Level of Non-volatile Extractables after Autoclaving (mg)	
		Mean	Standard Deviation	Mean	Standard Deviation
AB1ULV67PJ	Water	32	3.6	56	3.6

1.2.4 Biological Reactivity Tests

All of the materials used in Pegasus LV6 AB-style Filter Cartridges filters meet the requirements of the 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.

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. These biological expression systems bear the risk of product contamination by viruses.

Potential sources of virus contamination include viruses associated with the cell lines (endogenous viruses) or viruses introduced into the cell line of product during the production process (adventitious viruses). 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, which is considered as alternative or complementary viral removal technology, and therefore broadens the portfolio of adequate virus contamination-control strategies mandated by regulatory agencies. Multiple virus inactivation and removal methods are recommended to ensure complementary levels of protection (ICH Harmonized Tripartite Guideline, 1997). 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 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. PDA Technical Report No 41 "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."

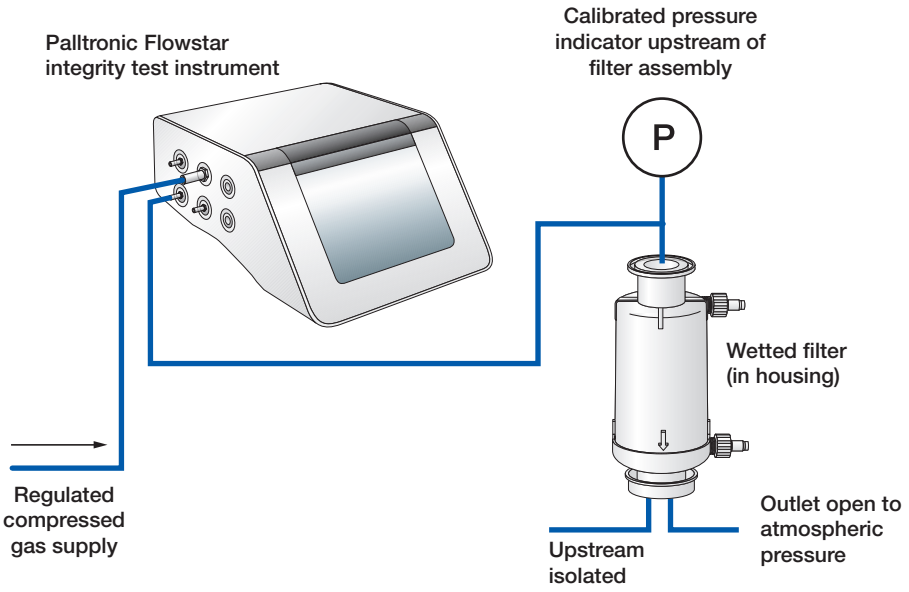
Pall uses the Forward Flow Test method on a 100% production basis for the non-destructive integrity testing of membrane filter elements.

Viral validation tests for Pegasus LV6 AB-style Filter Cartridges are made with PR772 bacteriophage. PR772 belongs to the Tectiviridae family of icosahedral double-stranded DNA bacteriophages. The Parenteral Drug Association virus filter task force has chosen PR772 as the model bacteriophage to standardize nomenclature for large-pore-size virus-retentive filters.

2.1.1 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 (Figure 1).

Figure 1
The Automated Integrity Test



The objective of this study was to measure the removal efficiency of typical Pegasus LV6 AB-style Filter Cartridges for bacteriophage PR772 and to document the correlation of the integrity test parameters to the bacteriophage removal efficiency.

2.2 Summary of Method

Pegasus LV6 AB-style Filter Cartridges, part number AB1ULV67PJ (254 mm [10 in.]) effective filter area: 1.65 m^2 [17.8 ft^2]) were tested using the following procedure:

1. Install the filter in the housing.
2. Flush with 0.1 µm filtered de-ionized water for 5 minutes at a differential pressure of 2.1 bar (30 psi).
3. Sterilize the wet filter, in the housing, by autoclaving at 121 °C for 60 minutes on a slow exhaust cycle.
4. Perform a post-autoclave, pre-challenge integrity test of the filter as follows:
   (i) Flush the with 0.1 µm filtered de-ionized water for 5 minutes at a differential pressure of 2.1 bar (30 psi).
   (ii) Perform aseptic Forward Flow tests at a test pressure of 4.150 bar (60 psi) using a Palltronic Flowstar integrity test instrument with a test time of 10 minutes.
   (iii) Record the integrity test value in mL/min.
5. Perform the viral (bacteriophage) challenge as follows (see Figure 2: Bacteriophage Challenge Apparatus):
   (i) Fill a sterile pressure vessel with a pre-filtered carrier fluid (BSA/PBS [15 mg/mL]) inoculated with bacteriophage PR772 at a concentration of approximately 10^6 plaque-forming units (PFU/mL).
   (ii) Aseptically remove a sample of the challenge suspension from the pressure vessel for assay.
   (iii) Connect the outlet port of the pressure vessel to the inlet port of the filter housing, and the inlet port of the pressure vessel to a regulated air source.
   (iv) Adjust the air pressure to the pressure vessel via the pressure regulator to produce 0.34 bar (5 psi) differential pressure across the filter.
   (v) Allow 1 liter of effluent to pass through the filter. Collect an aliquot for bacteriophage assays.
   (vi) Adjust the air pressure to maintain 0.69 bar (10 psi) for the remainder of the challenge.
   (vii) Collect aliquots for bacteriophage assays after 5 liter and 10 liter of effluent.
   (viii) Stop the challenge flow by clamping the downstream tubing and releasing the pressure from the pressure vessel.
   (ix) Remove the filter from the housing and sanitize by submerging in 1% sodium hypochlorite overnight.
6. Assay the input and effluent samples for bacteriophage content using the agar overlay method. Prepare serial dilutions of the input samples to confirm the initial concentration of PR772. Assay ten 1 mL aliquots of the undiluted effluent to determine any low level PR772 transmission.
7. Incubate the plaque assay plates at 37 °C ± 2 °C overnight, count for plaques and calculate the viral (bacteriophage) removal efficiency or Titer Reduction (T_R) of the filter as follows:

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

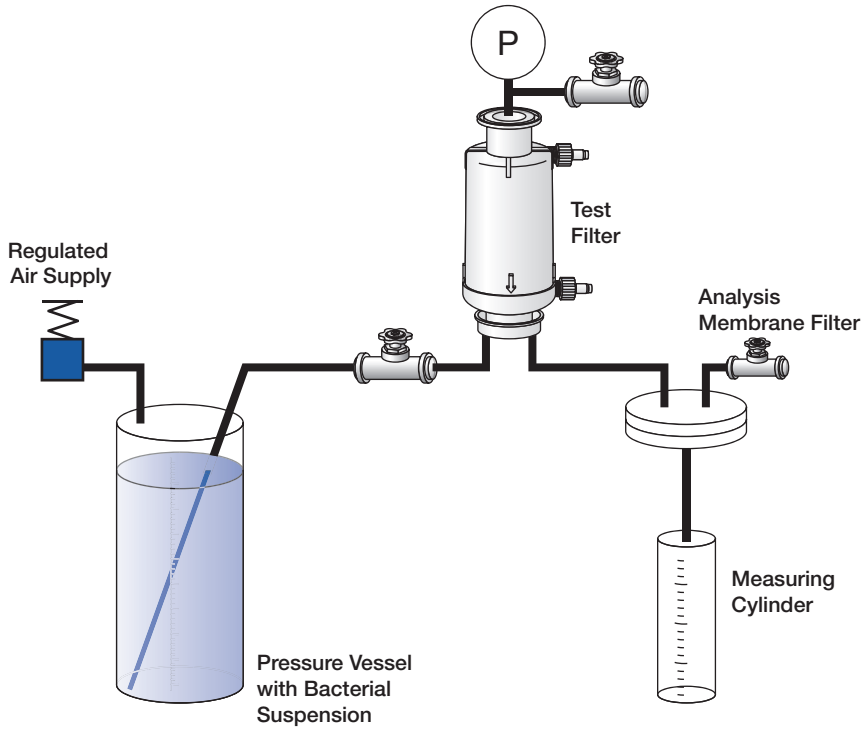
8. Perform a post-challenge integrity test of the filter as follows:
   (i) Flush the filter with 0.1 µm filtered de-ionized water for 5 minutes at a differential pressure of 2.1 bar (30 psi).

(ii) Perform Forward Flow integrity tests at a test pressure of 4.150 bar (60 psi) using a Palltronic Flowstar integrity test instrument with a test time of 10 minutes.

(iii) Record the integrity test value in mL/min.

Figure 2

Bacteriophage Challenge Apparatus



2.3 Results

The data from the viral (bacteriophage) retention versus Forward Flow validation study are listed in Table 4: Results of Forward Flow Values and Bacteriophage PR772 (sized at 53 – 82 nm) Retention for Typical Pegasus LV6 AB-style Filter Cartridges, Part Number AB1ULV67PJ on page 5. The higher of the Forward Flow values (pre- and post-challenge) is presented and the data are arranged in order of increasing Forward Flow value.

Pegasus LV6 AB-style Filter Cartridges, part number AB1ULV67PJ (254 mm [10 in.]), with Forward Flow values from 28.5 mL/min to 54.2 mL/min — when wet with water and tested at 4.150 bar (60 psi) — gave titer reductions for bacteriophage PR772 (53 – 82 nm) of > 6 log under the test conditions. The test conditions included challenges with bacteriophage PR772 at concentrations of 10^6 PFU/mL in an isotonic protein solution (phosphate buffered saline +15 mg/mL bovine serum albumin) with an applied differential pressure of 0.69 bar (10 psi). Effluent was sampled and analyzed post-filtration after a 1-liter, 5-liter and 10-liter challenge throughput volume.

Table 4

Results of Forward Flow Values and Bacteriophage PR772 (sized at 53 – 82 nm) Retention for Typical Pegasus LV6 AB-style Filter Cartridges, Part Number AB1ULV67PJ

Filter Serial Number	Forward Flow* at 4.150 bar (60 psi)* mL/min	Titer Reduction for Phage PR772 (53 – 82 nm)
IJ6989026	28.5	> 10^6
IJ6988036	29.1	> 10^6
IJ6988040	29.5	> 10^6
IJ6989021	29.6	> 10^6

Table 4 *Continued*

Results of Forward Flow Values and Bacteriophage PR772 (sized at 53 – 82 nm) Retention for Typical Pegasus LV6 AB-style Filter Cartridges, Part Number AB1ULV67PJ

Filter Serial Number	Forward Flow* at 4.150 bar (60 psi)* mL/min	Titer Reduction for Phage PR772 (53 – 82 nm)
IJ6988070	30.0	$> 10^6$
IJ6988071	30.3	$> 10^6$
IJ6547001	30.4	$> 10^6$
IJ6988009	30.7	$> 10^6$
IJ6988057	30.9	$> 10^6$
IJ6547022	31.1	$> 10^6$
IJ6988041	31.2	$> 10^6$
IJ6989072	31.7	$> 10^6$
IJ6547002	32.5	$> 10^6$
IJ6988058	34.1	$> 10^6$
IJ6547078	35.3	$> 10^6$
IJ6989035	37.6	$> 10^6$
IJ6989070	37.7	$> 10^6$
IJ6547026	38.8	$> 10^6$
IJ6989050	51.4	$> 10^6$
IJ6547040	54.2	$> 10^6$

** Forward Flow values wet with water, at 20 °C ± 5 °C, maximum allowable limit value 40 mL/min at 4.150 bar (60 psi)*
*** Bar value rounded up to nearest 0.025 bar.*

2.4 Conclusions

The Forward Flow integrity test performed at 4.150 bar (60 psi) in water demonstrates that Pegasus LV6 AB-style Filter Cartridges, with Forward Flow values at or below the limit of 40 mL/min at 4.150 bar (60 psi), are retentive for bacteriophage PR772 (53 – 82 nm) with a titer reduction of $\geq 10^6$ under the test conditions, thus demonstrating the correlation between Forward Flow and Bacteriophage PR772 $\geq 10^6$ removal performance. Forward Flow parameters are set for Pegasus LV6 AB-style Filter Cartridges based on the above results of the bacteriophage challenge tests and additional considerations and parameters.

Table 5

*Forward Flow Integrity Test Parameters for an AB1ULV67PJ Filter Cartridge**

Integrity Tests	
Test Pressure	4.150 bar (60 psi)**
Wetting Liquid	Water
Temperature	20 °C ± 5 °C
Test Gas	Air
Maximum Allowable Forward Flow Limit***	40 mL/min

** See Section 2.2: Summary of Method for the test procedure.*
*** Bar value rounded up to nearest 0.025 bar.*
**** During the test period the temperature of the filter assembly should not vary more than ± 1 °C.*

3 Repeated Steam Exposure Cycles

3.1 Introduction

The purpose of these tests was to determine the effects of repeated exposure to *in-situ* steam cycles on filter integrity using standard Pegasus LV6 AB-style Filter Cartridges from production. The tests were used to qualify the steam exposure claims for Pegasus LV6 AB-style Filter Cartridges.

3.2 Summary of Method

3.2.1 *In-Situ* Steaming at 125 °C

Typical Pegasus LV6 AB-style Filter Cartridges from three manufacturing batches (part number AB1ULV67PJ) were used for the tests. The filters were flushed with de-ionized water for 5 minutes at a differential pressure of 2.1 bar (30 psi) and Forward Flow integrity tested using an air test pressure of 4.150 bar (60 psi).
The filters were then subjected to an one-hour *in-situ* steam cycle at 125 °C.

During the initial stages of the steam cycles, the wet filter membrane caused the differential pressure to increase across the filter as steam was introduced. The steam inlet valve was controlled so that the differential pressure across the wetted filter did not exceed 0.3 bar (4.4 psi).

Immediately after each one-hour steam cycle had finished, the upstream and the downstream valve were slightly opened to allow steam to slowly vent and to prevent the filter element drying out. The system was allowed to cool down to 25 °C ± 5 °C. The filters were then flushed with de-ionized water for 5 minutes at a differential pressure of 2.1 bar (30 psi) prior to starting the next steam cycle.

This sequence of

1. Water flush for 5 minutes at 2.1 bar (30 psi) differential pressure
2. Forward Flow Integrity testing at 4.150 bar (60 psi) test pressure
3. *In-situ* steaming for 1 hour at 125 °C was repeated until each filter had been exposed to 6 one-hour steam cycles.

On completion of the 6 steam cycles, the filters were flushed with de-ionized water for 5 minutes at a differential pressure of 2.1 bar (30 psi) and Forward Flow integrity tested again as described above.

3.3 Results

3.3.1 *In-Situ* Steaming at 125 °C

The Forward Flow integrity test results for Pegasus LV6 AB-style Filter Cartridges (part number AB1ULV67PJ) before and after exposure to 6 one-hour *in-situ* steam cycles are shown in Table 6: Effects of *In-Situ* Steam Exposure at 125 °C on Filter Integrity and Water Wettability for Pegasus LV6 AB-style Filter Cartridges, Part Number AB1ULV67PJ. All of the filters retained integrity and water wettability following exposure to 6 one-hour cycles at 125 °C.

Table 6

Effects of In-Situ Steam Exposure at 125 °C on Filter Integrity and Water Wettability for Pegasus LV6 AB-style Filter Cartridges, Part Number AB1ULV67PJ

Filter Serial Number	Forward Flow (mL/min) pre and after exposure to 6 *in-situ* steam cycles at 125 °C*	
	FF (mL/min) Pre steaming	FF (mL/min) Post Steaming
IJ6547034	Not performed	29.9
IJ6547042	31.4	21.2
IJ6989048	31.3	29.9
IJ6989071	33.0	33.1
IJ6989075	31.7	26.9
IJ6989090	31.0	29.4
IJ6988029	33.2	27.5
IJ6988066	30.0	29.4
IJ6988087	36.3	27.4
IJ6988091	29.1	23.2

** Forward Flow values wet with water, at 20 °C ± 5, maximum allowable limit value 40 mL/min at 4.150 bar (60 psi).*

3.4 Conclusions

Pegasus LV6 AB-style Filter Cartridges have maintained integrity after multiple *in-situ* steam-sterilization cycles while water wet.

The data presented in this section support the following product claim (Table 7) for *in-situ* steaming Pegasus LV6 AB-style Filter Cartridges:

Table 7

*Product Claim for In-situ Steaming**

Part Number	Steam Conditions	Maximum Recommended Steam Life Claim
AB1ULV67PJ	*In-situ* steam cycles at 125 °C	3 one-hour cycles**

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

*** The claim for 3 one-hour cycles of steam sterilization at 125 °C can also be applied to steam sterilization in an autoclave, as an in-situ steaming process presents the same thermal and an even higher mechanical challenge to a filter than an autoclaving process.*

4 Determination of Water Flow Characteristics

4.1 Introduction

The objective of these tests was to determine the typical differential pressure across Pegasus LV6 AB-style Filter Cartridges at set water flow rates.

4.2 Summary of Method

The tests were performed on filters of part number AB1ULV67PJ from three different manufacturing batches. Pre-filtered DI water was pumped through the filters in the normal flow (out to in) direction. Pressure readings from transducers upstream and downstream of the test assembly were monitored to calculate the differential pressure at set water flow rates.

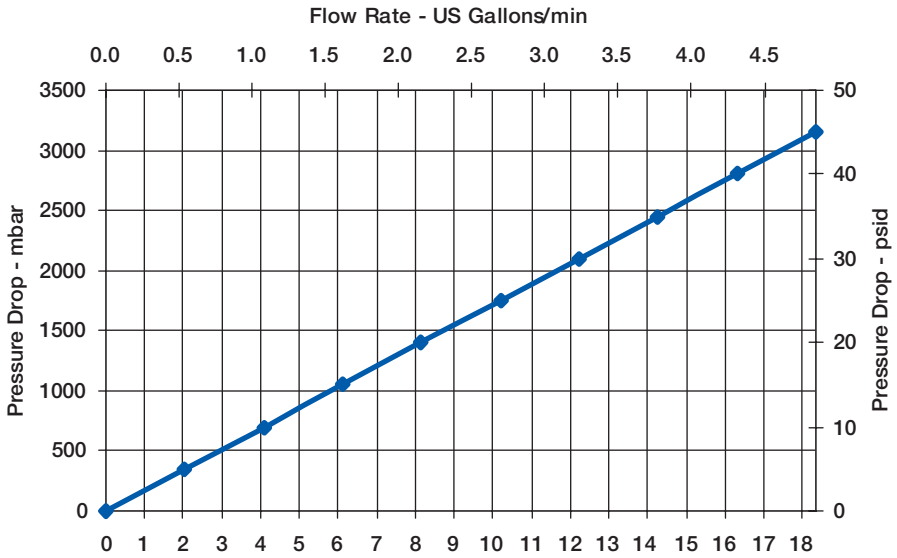
Additional flow measurements were taken with the test rig with no filter cartridge installed so that the pipework/housing losses could be measured and then subtracted from the filter assembly results. All data were corrected to a standard temperature of 20 °C.

4.3 Results

The water flow/differential pressure data obtained using typical Pegasus LV6 AB-style Filter Cartridges are shown in Figure 3. The data in the graph represent average values from measurements taken using six different filters.

Figure 3

Water Flow/Differential Pressure Characteristics of Pegasus LV6 AB Style Filters Part Number AB1ULV67PJ



4.4 Conclusions

Differential pressure measurements at set water flow rates have been determined for Pegasus LV6 AB-style Filter Cartridges, part number AB1ULV67PJ. The data presented in this section support the following product claim for water flow characteristics of Pegasus LV6 AB-style Filter Cartridges.

Table 7

Product Claim for Water Flow Characteristics

Pall Filter Part Number	Typical Water Flow Rate at 2.1 bar (30 psi) Differential Pressure (L/min)
AB1ULV67PJ	12

5 Extractables

5.1 Introduction

The objective of this series of tests was to quantify and characterize the material that can be extracted from Pegasus LV6 filter cartridges using water. Pegasus LV6 Filter Cartridges are made from a hydrophilic acrylate-modified polyvinylidene fluoride (PVDF) filter membrane, polyethylene/ polypropylene support and drainage layers, and polypropylene molded components.

5.2 Summary of Method

Filters of part number AB1ULV67PJ (effective filter area: 1.65 m^2 [17.8 ft^2]) from 3 production lots were used for the tests.

5.2.1 Preparation of Filter Samples

Extractables Testing Out-of-Box

The filters were taken out of the water-filled primary packaging (FEP bag, with FEP being a co-polymer of tetrafluoroethylene and hexafluoropropylene), excess water was allowed to drain, the protective cap and O-rings were removed from the bomb fin and the filters were submitted to the extraction procedure.

Extractables Testing Employing an Autoclave Cycle

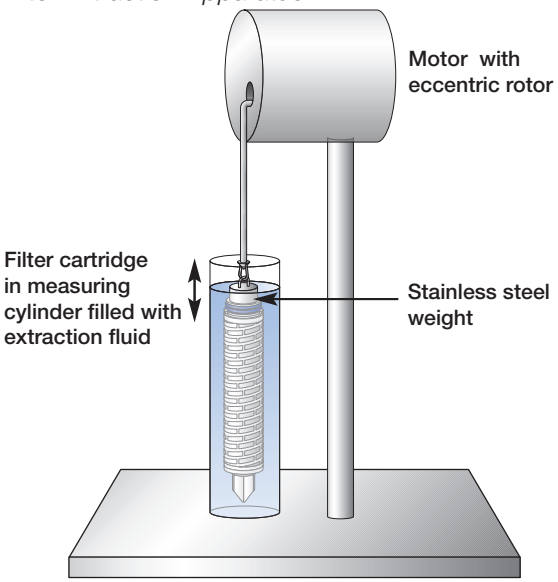
The filter samples were autoclaved in order to mimic a standard sterilization cycle performed by a customer prior to filter use. Autoclaving will maximize the quantity of extractable material present in a polymeric material. The water-filled primary packaging of the filters was cut open, the water was drained, the protective cap was removed from the bombfin, and the filters were autoclaved in their primary packaging for one hour at 125 °C ± 5 °C, using a slow exhaust cycle. The filters were then submitted to the extraction procedure. Visible droplets of water remaining on the filter elements were allowed to drain away at room temperature before the extraction was performed.

5.2.2 Extraction Procedure for Filter Cartridges

Dynamic extraction tests of the filter cartridges were performed in a known volume of water. The test filters were immersed in the extraction fluid in a clean measuring cylinder, as shown in Figure 4: Filter Extraction Apparatus. The filter was gently moved up and down for four hours. This movement caused 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. Following the extraction period, a measured volume of the extraction liquid was evaporated to dryness and the non-volatile extractables were determined gravimetrically. A correction was made to the NVR value to account for the total extraction volume used. The extractables were also analyzed by Fourier Transform Infrared Spectroscopy (FTIR).

Figure 4

Filter Extraction Apparatus



5.3 Results

Tables 8 and 9 show the typical levels of aqueous extractables obtained from the Pegasus LV6 AB-style Filter Cartridges that were tested.

Table 8

Typical levels of Non-volatile Aqueous Extractables Out-of-Box

Part Number	Extraction Fluid	Filter Serial Number	Non-Volatile Residue (mg)*
AB1ULV67PJ	DI water	IJ8258017	26
		IJ8258031	30
		IJ8259015	35
		IJ8259032	36
		IJ8260016	32
		IJ8260026	33

** Obtained using Pegasus LV6 Filter Cartridges (4 Hours Extraction Time at Ambient Temperature). The data in Table 8 result in a mean of 32 mg with a standard deviation of 3.6 mg.*

Table 9

Typical levels of Non-volatile Aqueous Extractables after Autoclaving at 125 °C ± 5 °C

Part Number	Extraction Fluid	Filter Serial Number	Non-Volatile Residue (mg)*
AB1ULV67PJ	DI water	IJ8258015	57
		IJ8258033	53
		IJ8259009	61
		IJ8260006	52
		IJ8260030	57

** Obtained using Pegasus LV6 Filter Cartridges after autoclaving at 125 °C ± 5 °C (4 Hours Extraction Time at Ambient Temperature). The data in Table 9 result in a mean of 56 mg with a standard deviation of 3.6 mg.*

Typical infrared spectra of the aqueous extracts from Pegasus LV6 AB-style Filter Cartridges (part number AB1ULV67PJ) out-of-box and after autoclaving are shown in Figure 5 and Figure 6.

Figure 5

Typical Infrared Spectrum of the Aqueous Extractables from Pegasus LV6 AB-style Filter Cartridges Out-of-Box

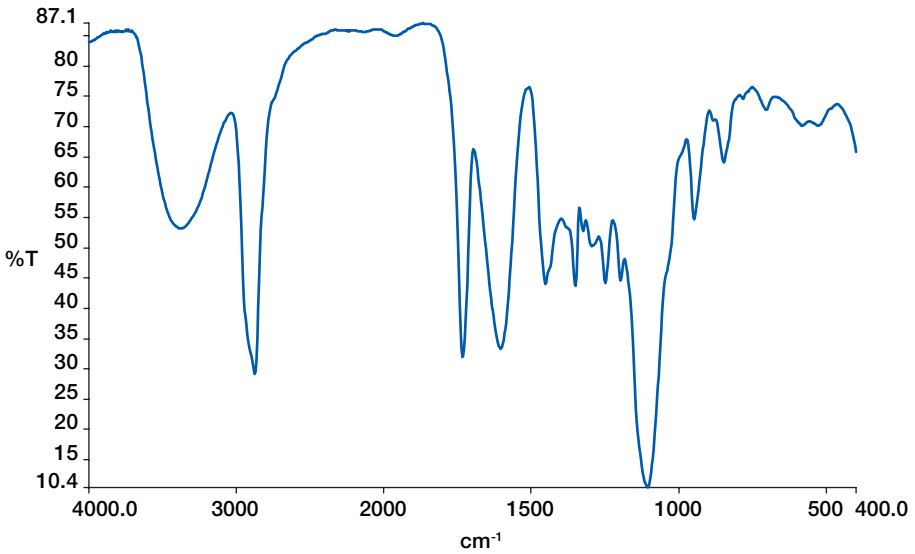
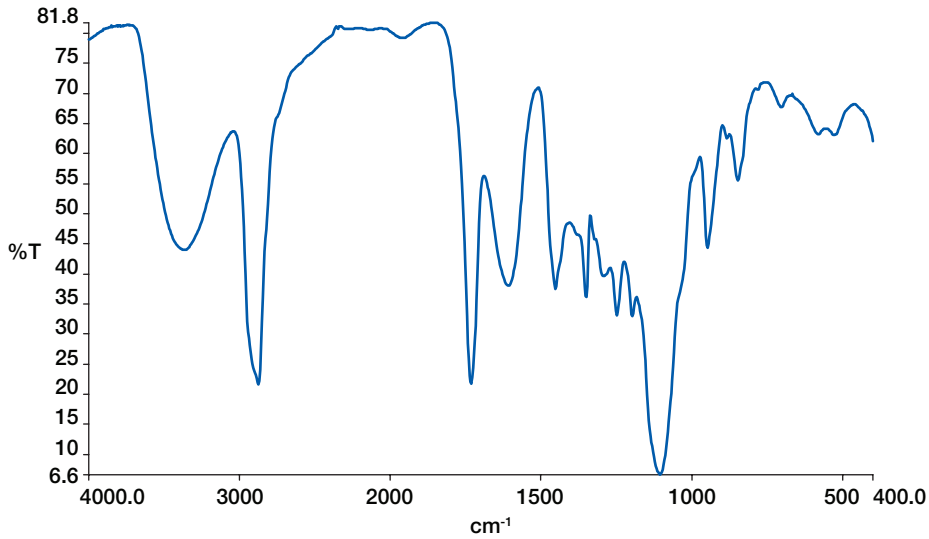


Figure 6

Typical Infrared Spectrum of the Aqueous Extractables from Pegasus LV6 AB-style Filter Cartridges Post-autoclaving



5.4 Conclusions

The levels of aqueous extractables was determined for Pegasus LV6 AB-style Filter Cartridges (part number AB1ULV67PJ). The FTIR spectra of the extracts prepared from extracted filters out-of-box and from filters extracted after autoclaving indicate the presence of the acrylates used to render the polyvinylidene fluoride membrane hydrophilic. Actual service may impose different conditions, such as different steaming conditions, exposure times, temperature, liquid type etc. Evaluation under process conditions is therefore also recommended.

During the investigation performed the following mean values (Table 10) for aqueous non-volatile extractables (4 hours extraction time at ambient temperature) from Pegasus LV6 AB-style Filter Cartridges were measured both out-of-box and after autoclaving at 125 °C ± 5 °C.

Table 10

Mean Values for Aqueous Non-volatile Extractables

Pall Filter Part Number	Extraction Fluid	Level of Non-Volatile Extractables Out-of-Box (mg)	Level of Non-Volatile Extractables after Autoclaving (mg)
AB1ULV67PJ	Water	Mean 32	Mean 56
		Standard Deviation 3.6	Standard Deviation 3.6

6 Biological Reactivity Tests on the Materials of Construction

6.1 Introduction

The objective of this study was to evaluate the biological suitability of the materials of construction of Pegasus LV6 AB-style Filter Cartridges. The materials of construction of the filters are as follows:

Table 11
Materials of Construction

Components	Materials
Membrane	Acrylate-modified polyvinylidene fluoride (PVDF)
Membrane Support and Drainage Layers	Polyethylene/polypropylene
Core and Endcaps	Polypropylene
Filter Cage	Polypropylene with encapsulated reinforcing ring
O-rings	Ethylene propylene

6.2 Summary of Method

The tests were performed in accordance with the Biological Reactivity Tests *in vivo* for Class VI Plastics (121 °C) as described in the current United States Pharmacopeia.

The testing procedures described in the USP include:

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

The four extracting media listed in the USP simulate parenteral solutions and body fluids. These include:

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

The USP states that extracts may be prepared at one of three standard conditions: 50 °C for 72 hours, 70 °C for 24 hours, or 121 °C for 1 hour. The most stringent condition not resulting in physical changes in the plastic is recommended; therefore the filter materials were extracted at 121 °C.

6.2.1 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 extract and 1 in 20 Solution of Alcohol in Sodium Chloride Injection extract were injected intravenously. Vegetable oil extract and Polyethylene Glycol 400 extract were injected intraperitoneally.

6.2.2 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.

6.2.3 Implantation Tests

Implantation tests were also performed, in order to subject the materials of construction to the most stringent conditions included in the USP. Each of the materials of the Pegasus LV6 filter was implanted separately.

6.3 Results

No biological response was observed in any of the tests performed and therefore the materials used in Pegasus LV6 AB-style Filter Cartridges passed all of the tests specified.

6.4 Conclusions

The materials used in Pegasus LV6 AB-style Filter Cartridges met the requirements of the 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.

7 References

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