Biotech

Validation Guide

USTR 3374

Acro® 50 0.2 µm Vent Filter

Part Number 6074270L

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Contents

1 Overview

Sterilizing filtration of air, gases, and liquids used in the manufacture of sterile pharmaceuticals is of paramount importance. All aspects of the aseptic manufacturing process may be validated to ensure the safety and efficacy of the final pharmaceutical and biological product. This validation guide is designed to assist the filter user in meeting the validation requirement of regulatory authorities within the pharmaceutical industry for the use of Acro 50 0.2 µm vent filter capsules, part number 6074270L, henceforth referred to as Acro 50 filter capsules.

These filter capsules have been specifically designed for the sterilizing filtration of compatible gases in vent and gas supply applications. They incorporate a single-layer (0.2 µm sterilizing grade) membrane made of inherently hydrophobic polytetrafluoroethylene (PTFE), which is supported by a polypropylene non-woven material. All hardware components are manufactured from polypropylene.

The materials of construction of these filter capsules have met the requirements for biological reactivity, in vivo, specified in United States Pharmacopoeia (USP) <88> (for Class VI–121°C plastics) [(1)] and a MEM Elution test (USP <87> [(2)]). Although not intended for food contact use, the materials of construction of this product are made in line with the listings given in U.S. FDA 21 CFR Parts 170-199 [(3,4)].

The tests that were performed to qualify the performance under a range of test conditions include the following:

- Bacteria retention and bubble point tests
- Air flow characterization
- Autoclaving
- Isopropanol liquid pressure
- Extractables in 50/50 (v/v) % ethanol/water
- Biological reactivity tests on filter components

Acro 50 filter capsules may be used in conformance with current Good Manufacturing Practices (cGMP) per Title 21 of the U.S. Code of Federal Regulations (21 CFR Part 210) [(4)] and cGMP for Finished Pharmaceuticals (21 CFR Part 211) [(5)].

They are manufactured in accordance with an ISO 9001 [(6)] certified Quality Management System in a controlled environment that meets the air quality standards of an ISO Class 8 room [(7)] with respect to viable and non-viable particulate and positive pressure and are subject to stringent quality control including in-process controls and testing of the filter capsules as follows:

1. 100% fabrication integrity
2. Bacterial retention (*Brevundimonas diminuta*, American Type Culture Collection (ATCC♦) 19146)
3. Effluent cleanliness
4. Oxidizable substances
5. pH
6. Endotoxin

This guide may be complemented by other documentation for Acro 50 filter capsules, namely:

- Datasheet for Acro 50 0.2 µm vent filter capsules part number 6074270L.
- Certificate of Test for Acro 50 filter capsules (included in each filter packaging; see Section 6 for a sample certificate). This document substantiates the product specification and quality control standards applied to Acro 50 filter capsules.

Note: The units of pressure quoted in this document are “mbar” 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

Note: Air flow rates are quoted in standard liters per minute (sL/min). Conversions to standard cubic feet per minute (SCFM) and standard cubic centimeter per minute (SCCM) are shown below:

- 1 sL/min = 0.0353 SCFM
- 1 sL/min = 1000 SCCM

2 Summary of Qualification Tests

2.1 Microbial Retention Testing

Acro 50 filter capsules were tested for bacterial retention using *Brevundimonas diminuta* (American Type Culture Collection (ATCC) 19146), following procedures described in this validation guide and American Society for Testing and Materials (ASTM) Standard Test Method F838-15a [8], in accordance with the applicable requirements of the FDA Guidance for Industry: Sterile Drug Products Produced By Aseptic Processing - Current Good Manufacturing Practice (2004) [5]. These tests demonstrated that filter capsules, with a minimum bubble point of 1000 mbar (14.5 psi) when wetted with 60/40 (v/v) isopropanol/water will retain >10^7 colony forming units (cfu) of *Brevundimonas diminuta* per cm^2 of effective filtration area (cfu/cm^2) in liquid (water) and produce a sterile effluent. This bubble point value is thus validated to be a suitable non-destructive integrity test limit for Acro 50 filter capsules.

Table 1.

Summary of microbial retention testing

Filter Part Number	Challenge Organism	Challenge Description	Microbial Recovery	Titer Reduction*
6074270L	*Brevundimonas diminuta* (ATCC 19146)	Liquid challenge level >10^7 cfu/cm^2 of effective filtration area	0	>10^8

** Calculated based on the minimum total challenge level for 6074270L filter capsules (19.6 cm^2 effective filtration area, >10^7 cfu/cm^2)*

2.2 Autoclave Resistance

Acro 50 filter capsules have been demonstrated to retain integrity and the bacterial retention capabilities of a sterilizing grade filter after repeated autoclave cycles under the conditions listed below.

Table 2.

Summary of autoclave testing conditions

Cycle Type	Temperature Setting	Cycle Time	Cycle Number
Liquid cycle	125 °C	30 minutes	5

Warning: This product must not be sterilized *in-situ* by passing steam through under pressure. Integrity should be verified after each autoclave cycle before use.

2.3 Integrity Test Limit Values

The user integrity (bubble point) limit value for Acro 50 filter capsules has been defined as follows, based on correlation to bacterial retention testing:

Table 3.

Bubble point limit value for 60/40 (v/v) isopropanol/water

Bubble Point Limit Value	Wetting Liquid	Temperature
1000 mbar (14.5 psi)	60/40 (v/v) isopropanol/water	20 °C ± 5 °C*

** During the test period the temperature of the filter assembly should not vary more than ± 1 °C.*

The user integrity test (bubble point) limit value for Acro 50 filter capsules for the alternative wetting fluid 70/30 (v/v) isopropanol/water has been defined as follows based on surface tension ratio and actual bubble point measurements with both fluids.

Table 4.

Bubble point limit value for 70/30 (v/v) isopropanol/water

Bubble Point Limit Value	Wetting Liquid	Temperature
1000 mbar (14.5 psi)	70/30 (v/v) isopropanol/water	20 °C ± 5 °C*

** During the test period the temperature of the filter assembly should not vary more than ±1 °C.*

The user integrity test (bubble point) limit value for Acro 50 filter capsules for the alternative wetting fluid 100% isopropanol has been defined as follows based on surface tension ratio and actual bubble point measurement with both fluids.

Table 5.

Bubble point limit value for 100% isopropanol

Bubble Point Limit Value	Wetting Liquid	Temperature
900 mbar (13.0 psi)	100% isopropanol	20 °C ± 5 °C*

** During the test period the temperature of the filter assembly should not vary more than ± 1 °C.*

2.4 Air Flow

Acro 50 filter capsules were evaluated for air flow at a set upstream pressure and differential pressure of 0.2 bar (3 psi). One hundred and fifty (150) test units from three (3) production batches (50 test units per batch) were tested. Airflow results are expressed as standard liters per minutes (sL/min). The lowest air flow measured was 15 sL/min. The highest air flow measured was 17 sL/min, while the mean of three batches amounted to 16 sL/min, 15.88 sL/min and 16.04 sL/min. The results show that Acro 50 filter capsules consistently exceed the minimum air flow specification of 8.0 sL/min at 0.2 bar (3 psi) differential pressure. This data can be used for system sizing calculations.

2.5 Liquid Pressure Test

Acro 50 filter capsules were subjected to liquid (isopropanol) pressure testing at a set upstream pressure of 4.1 barg (60 psig) and observed for leakage. Three hundred and seventy-five (375) test units from three (3) production batches, one hundred and twenty-five (125) test units per batch were tested. No leakage or sudden increase in flow was observed. Additional sample of fifteen (15) test units were also evaluated for filter integrity using bubble point testing and demonstrated values that exceed the minimum bubble point. These test results demonstrate that Acro 50 filter capsules are robust and withstand a maximum operating pressure of 4.1 bar (60 psi).

2.6 Water Breakthrough Test

Acro 50 filter capsules from three (3) production batches were subjected to a water breakthrough test at 2.1 barg (30 psig) upstream pressure. Three hundred and seventy-five (375) capsules were tested, one hundred and twenty-five (125) from each batch. No water breakthrough or leak was observed. The test results demonstrate that Acro 50 filter capsules feature a hydrophobic filter media and withstand a water column pressure of 2.1 bard (30 psid) without water breakthrough or leak.

2.7 Extractables (Non-Volatile Residue)

Two (2) Acro 50 filter capsules were connected in series, representing a total of 39.2 cm^2 of effective filtration area (EFA). These were autoclaved (125 °C, 1 hour) and extracted in 50/50 (v/v) ethanol/water at 40 °C for 24 hours. The non-volatile gravimetric residue (NVR) was determined. Three (3) filter device batches were evaluated following this method. Total non-volatile residue for the two filters in this extraction fluid was found to be less than 0.2 mg of NVR. Fourier Transform Infrared (FTIR) spectra could not be obtained due to the extremely low residue levels.

2.8 Biological Reactivity Test

The materials of construction of Acro 50 filter capsules were tested and found to meet the requirements for biological reactivity, *in vivo*, under United States Pharmacopeia (USP) <88> (for Class VI – 121°C plastics) [(1)], and *in vitro* cytotoxicity testing. *In vivo* tests included the Systemic Toxicity Test, the Intracutaneous Test, and the Implantation Test. *In vitro* cytotoxicity was assessed determining the biological reactivity of mammalian cell cultures following contact with extracts of the polymeric materials of construction (Minimum Essential Medium (MEM) Elution testing). The test method followed USP <87> [(2)].

3 Validation Testing

3.1 Bacterial Retention Testing and Bubble Point Determination

3.1.1 Background

FDA's Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice (2004) states: "A sterilizing filter should be validated to reproducibly remove viable microorganisms from the process stream, producing a sterile effluent". The guideline also states: "the microorganism *Brevundimonas diminuta* (ATCC 19146), when properly grown, harvested and used, is a common challenge organism for 0.2 µm rated filters because of its small size (0.3 µm mean diameter)".

Although FDA's 'Guidance for Industry' document was updated in 2004, the minimum bacterial challenge level for a sterilizing grade filter, remains as defined in the previous version (1987) [(9)]. The minimum bacterial challenge level of ≥1.0 x 10^7 colony forming units (cfu) per cm^2 of effective filtration area (EFA) is the industry standard for performance characterization of a sterilizing grade filter.

Acro 50 filter capsules were tested for retention of *B. diminuta* (ATCC 19146) using bacterial challenge tests using procedures described in this document and ASTM Standard Test Method F838-15a [(8)], in accordance with the applicable recommendations of the FDA's Guidance for Industry - Sterile Drug Products Produced By Aseptic Processing - Current Good Manufacturing Practice (September 2004) [(10)] using a minimum of 1 x 10^7 cfu/cm^2 of effective filtration area.

The FDA guideline further states: "After a filtration process is properly validated for a given product, process and filter, it is important to ensure that identical filters (e.g. of identical polymer construction and pore size rating) are used in production runs....Integrity testing of the filter(s) can be performed prior to processing, and should be routinely performed post-use. Forward Flow and bubble point tests, when appropriately employed, are two integrity tests that can be used. A production filters' integrity test specification should be consistent with the data generated during bacterial retention validation studies."[(10)]

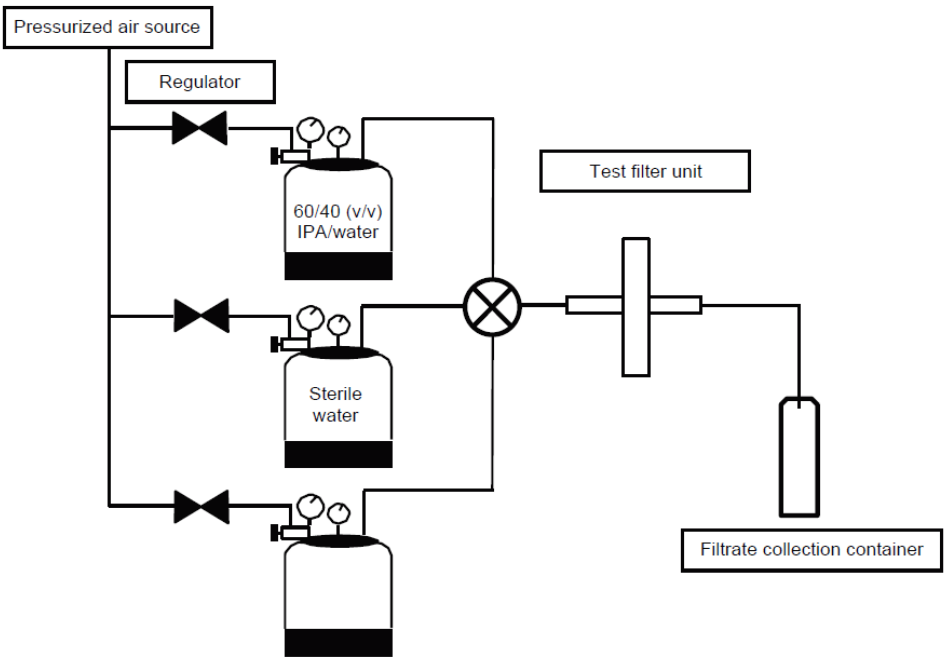
The correlation between *B. diminuta* bacterial retention and a non-destructive integrity test is an essential aspect of the validation of sterilizing grade filters. The integrity test employed in this study was the bubble point test. This test was employed due to the small filter area (19.6 cm^2) of the capsules, which hinders the use of the Forward Flow or Water Intrusion tests, as the gas and evaporative water flows during testing are extremely small and prevent a reliable user integrity test with this device type.

3.1.2 Bacterial Challenge Testing

Filter capsules from three (3) different batches were subjected to bacterial challenge tests using an aqueous suspension of *B. diminuta* (ATCC 19146). Twenty-six (26) filter capsules from three different batches were tested. A schematic representation of the challenge test rig used is shown in Figure 1.

Figure 1

Bacterial Challenge Testing Set-up



The *B. diminuta* suspension was prepared in accordance with standard test method ASTM F838-15a [8] and diluted to achieve a cell count of >4 x 10^6 cfu/mL. Prior to challenge, the test filter units were flushed with a 60/40 (v/v) isopropanol/water mixture to wet out the hydrophobic membrane and allow passage of the aqueous challenge suspension. After flushing with isopropanol/water, the filters were flushed with water before the challenge suspension was passed through the test filter units.

A total volume of 100 mL containing >4 x 10^6 cfu/mL of *B. diminuta* was passed through each test filter to achieve a minimum challenge level of >1.0 x 10^7 cfu/cm^2 of EFA of the test filter. The challenge was carried out at 1.4 barg (20 psig) differential pressure. The filtrate was collected in a sterile container. After the bacterial challenge test, the entire filter effluent was passed through a 0.2 µm rated recovery membrane. Following the challenge test, the recovery membrane was aseptically removed from the filter housing in a laminar flow cabinet and placed onto Tryptic Soy Agar (TSA) plates. All agar plates were incubated at 30 ± 2 ºC, for 48 hours.

After incubation, the recovery membranes were examined for growth, to determine if bacteria had passed through the test filter during the challenge. The titer reduction (TR) ratio for each filter was determined as follows:

$$TR = \frac{\text{Total number of bacteria influent to the filter (cfu)}}{\text{Total number of colonies recorded on the downstream recovery disc (cfu)}}$$

When no colonies were detected downstream, the titer reduction was expressed as: greater than the total number of bacteria influent to the filter (e.g. $>1 \times 10^8$).

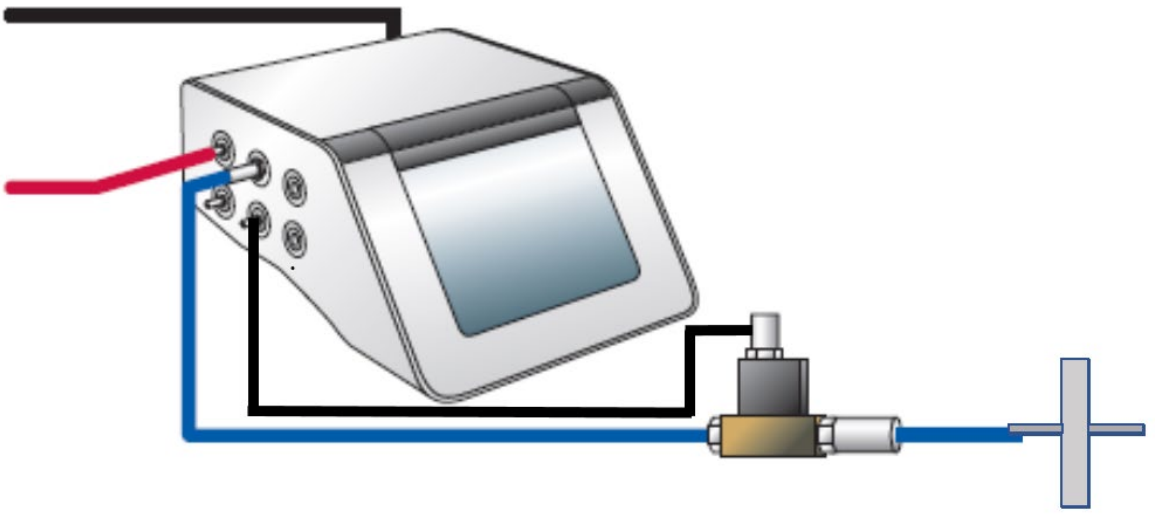
On completion of the challenge, the test filter units were flushed with 60/40 (v/v) isopropanol/water and bubble point tests were performed as per method described below.

3.1.3 Bubble Point Integrity Testing

The bubble point test is based on measuring the gas flow across a completely wetted membrane at increasing gas pressure on the upstream side. Air pressure was applied to the pressure vessel, to transfer the wetting fluid from the pressure vessel to the test filter capsule. The wetting fluid was flushed through the test filter unit to achieve complete wetting (about 5 minutes at 0.7 bar [10 psi]). The bubble point was then measured using Palltronic® Flowstar filter integrity test instrument.

Figure 2

Test configuration for bubble point testing



3.1.4 Results

The data from the bacterial retention test versus the bubble point integrity test is listed in Table 6. The data are arranged by filter batch number.

Table 6.
Brevundimonas diminuta retention and bubble point measurement results for Acro 50 filter capsules

Test Unit*	Lot Number	Total Challenge per Test Filter (cfu)	Challenge per cm^2 of Effective Filtration Area (cfu/cm^2)	Number of cfu in Effluent	Titer * Reduction	Bubble Point Wetted with 60/40 IPA/Water (psi)
1	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	18.13
2	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	18.13
3	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	18.13
4	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	18.13
5	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	18.13
6	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	17.40
7	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	18.13
8	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	17.40
9	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	18.13
10	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	17.40
11	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	16.68
12	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	15.95
13	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	17.40
14	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	15.95
15	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	16.68
16	FE8215	$2.3x10^8$	$1.2x10^7$	0	$>2.3x10^8$	14.50
17	FE8216	$4.1x10^9$	$2.19x10^8$	0	$>4.1x10^9$	17.40
18	FE8216	$4.1x10^9$	$2.19x10^8$	0	$>4.1x10^9$	15.95
19	FE8216	$4.1x10^9$	$2.19x10^8$	0	$>4.1x10^9$	17.40
20	FE8216	$4.1x10^9$	$2.19x10^8$	0	$>4.1x10^9$	17.40
21	FE8216	$4.1x10^9$	$2.19x10^8$	0	$>4.1x10^9$	17.40
22	FE8217	$4.1x10^9$	$2.19x10^8$	0	$>4.1x10^9$	17.40
23	FE8217	$4.1x10^9$	$2.19x10^8$	0	$>4.1x10^9$	17.40
24	FE8217	$4.1x10^9$	$2.19x10^8$	0	$>4.1x10^9$	17.40
25	FE8217	$4.1x10^9$	$2.19x10^8$	0	$>4.1x10^9$	17.40
26	FE8217	$4.1x10^9$	$2.19x10^8$	0	$>4.1x10^9$	18.13

**Titer reduction calculated based on total challenge per test filter unit*

3.1.5 Conclusion

The results in this chapter demonstrate that Acro 50 filter capsules are retentive for *B. diminuta* at a challenge level of $>10^7$ cfu/cm^2. The lowest bubble point value of a test filter measured in this study was 1000 mbar (14.5 psi) when wetted with 60/40 (v/v) isopropanol/water. The bacteria retention performance fulfils the requirements of a sterilizing grade filter.

3.2 Autoclave Resistance and Bacterial Retention Testing

3.2.1 Introduction

Heat and steam exposure of filters during autoclave sterilization cycles create a substantial stress and can alter the physical structure of some filters or cause them to lose integrity. These tests were performed to evaluate the ability of Acro 50 filter capsules to withstand one or multiple autoclave cycles. The bacterial retention capabilities of the test filter units were assessed after exposure to the autoclave cycles.

3.2.2 Summary of Methods

A total of thirty (30) filter capsules were tested. The first set of twenty (20) filter capsules, from three batches were exposed to a single 125 °C, 30 min autoclave cycle. The filter capsules were allowed to cool to ambient temperature and wetted with 60/40 (v/v) isopropanol/water. The filter capsules were then flushed with water and subjected to bacterial retention testing. Fifteen (15) of the filter capsules were challenge tested in the forward direction and five (5) of the filter capsules were challenge tested in the reverse direction. The test in reverse direction was performed at 1030 mbard (15 psid) so that the filter would not be exposed to undue reverse differential pressure. It should be noted that this filter device does not have a filter support layer on the upstream side.

A second set of ten (10) filter capsules, from three (3) filter device batches were exposed to five (5) autoclave cycles of 125 °C, 30 minutes as indicated. The filter capsules were allowed to cool to ambient temperature and wetted with 60/40 (v/v) isopropanol/water. The filter capsules were then flushed with water and subjected to bacterial retention testing in the forward direction as described in previous chapters.

Table 7 shows the bacteria retention and bubble point test results of the twenty (20) filter capsules after exposure to one (1) autoclave cycle (test units 1-20) and the ten (10) filter capsules after exposure to five (5) autoclave cycles (test units 20-30). All filter capsules delivered a sterile filtrate independent of the flow direction for the challenge (forward or reverse).

Table 7.

Brevundimonas diminuta retention and bubble point results after autoclave cycles of 125 °C, 30 minutes. Filter capsules were exposed to one autoclave cycle (test units 1-20) or five autoclave cycles (test units 21-30).

Test Unit*	Lot Number	Flow Direction for Challenge**	Total Challenge per Test Filter Unit (cfu)	Challenge per cm^2 of Effective Filtration Area (cfu)	Number of cfu in Effluent	Titer Reduction***	Bubble Point After Challenge (psi)****
1	FE8215	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
2	FE8215	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.23
3	FE8215	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
4	FE8215	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.23
5	FE8215	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
6	FE8216	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
7	FE8216	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.23
8	FE8216	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
9	FE8216	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
10	FE8216	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.23
11	FE8217	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	16.68
12	FE8217	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	16.68
13	FE8217	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	16.68
14	FE8217	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	17.40
15	FE8217	Forward	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	17.40
16	FE8215	Reverse	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	15.95
17	FE8216	Reverse	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	15.23
18	FE8216	Reverse	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	15.95
19	FE8217	Reverse	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
20	FE8217	Reverse	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	16.68
21	FE8215	Forward	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
22	FE8215	Forward	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
23	FE8215	Forward	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
24	FE8216	Forward	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	18.13
25	FE8216	Forward	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
26	FE8216	Forward	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
27	FE8217	Forward	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
28	FE8217	Forward	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
29	FE8217	Forward	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
30	FE8217	Forward	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40

**Filters were exposed to one autoclave cycle (test units 1-20) or five autoclave cycles (test units 21-30).*
*** Flow direction for challenge:*
Forward testing: Test fluid enters device through inlet port Reverse testing: Test fluid enters device through outlet port
**** Titer reduction calculated based on total challenge per test filter unit*
***** Wetted with 60/40 (v/v) isopropanol/water*

3.2.3 Conclusions

The results in this chapter demonstrate that Acro 50 filter capsules are fully resistant to up to five (5) autoclave cycles at a temperature of 125 °C and a duration of 30 minutes per cycle. All test units delivered sterile filtrate in bacterial challenge testing using *B. diminuta* as the test organism after exposure to the autoclave cycle(s), irrespective of the applied flow direction for the challenge (forward or reverse). The filter capsules were thus confirmed as having maintained their sterilizing grade filter bacterial retention performance. The lowest bubble point value of a test filter measured in this study was 15.23 psi when wetted with 60/40 (v/v) isopropanol/water.

3.3 Integrity Test (Bubble Point) Limit Values for User

3.3.1 User Integrity Test (Bubble Point) Limit Values for 60/40 (v/v) Isopropanol/Water

During validation testing for Acro 50 filter capsules several studies were performed that included bubble point testing as a non-destructive test and bacterial challenge testing employing an aqueous suspension of *B. diminuta* (ATCC 19146). The methods and results of these studies are reported in Sections 3.1 and 3.2. The results demonstrate that integral capsules fully retain *B. diminuta* from aqueous suspension.

The integrity of the test filters was always assessed with the non-destructive integrity test method, the bubble point test after wetting with 60/40 (v/v) isopropanol/water. Table 8 lists the bacterial retention and bubble point test results from the above studies arranged by decreasing bubble point. The lowest bubble point value of a test filter that fully retained *B. diminuta* during bacteria retention testing in these studies was 1000 mbar (14.5 psi) wetted with 60/40 (v/v) isopropanol/water.

Table 8.

Bubble point results and B.diminuta retention for Acro 50 filter capsules

Test Unit*	Lot Number	Total Challenge per Test Filter (cfu)	Challenge per cm^2 of Effective Filtration Area (cfu/cm^2)	Number of cfu in Effluent	Titer ** Reduction	Bubble Point When Wetted With 60/40 IPA/Water (psi)
1	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	18.13
2	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	18.13
3	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	18.13
4	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	18.13
5	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	18.13
6	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	18.13
7	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	18.13
8	FE8217	$4.1x10^9$	$2.2x10^8$	0	$>4.1x10^9$	18.13
9	FE8216	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	18.13
10	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	17.40
11	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	17.40
12	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	17.40
13	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	17.40
14	FE8216	$4.1x10^9$	$2.2x10^8$	0	$>4.1x10^9$	17.40
15	FE8216	$4.1x10^9$	$2.2x10^8$	0	$>4.1x10^9$	17.40
16	FE8216	$4.1x10^9$	$2.2x10^8$	0	$>4.1x10^9$	17.40

Test Unit*	Lot Number	Total Challenge per Test Filter (cfu)	Challenge per cm^2 of Effective Filtration Area (cfu/cm^2)	Number of cfu in Effluent	Titer ** Reduction	Bubble Point When Wetted With 60/40 IPA/Water (psi)
17	FE8216	$4.1x10^9$	$2.2x10^8$	0	$>4.1x10^9$	17.40
18	FE8217	$4.1x10^9$	$2.2x10^8$	0	$>4.1x10^9$	17.40
19	FE8217	$4.1x10^9$	$2.2x10^8$	0	$>4.1x10^9$	17.40
20	FE8217	$4.1x10^9$	$2.2x10^8$	0	$>4.1x10^9$	17.40
21	FE8217	$4.1x10^9$	$2.2x10^8$	0	$>4.1x10^9$	17.40
22	FE8217	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	17.40
23	FE8217	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	17.40
24	FE8215	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
25	FE8215	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
26	FE8215	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
27	FE8216	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
28	FE8216	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
29	FE8217	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
30	FE8217	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
31	FE8217	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
32	FE8217	$3.8x10^9$	$1.9x10^8$	0	$>3.8x10^9$	17.40
33	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	16.68
34	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	16.68
35	FE8217	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	16.68
36	FE8217	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	16.68
37	FE8217	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	16.68
38	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	15.95
39	FE8215	$2.8x10^8$	$1.4x10^7$	0	$>2.8x10^8$	15.95
40	FE8216	$4.1x10^9$	$2.2x10^8$	0	$>4.1x10^9$	15.95
41	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
42	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
43	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
44	FE8216	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
45	FE8216	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
46	FE8216	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.95
47	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.23
48	FE8215	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.23
49	FE8216	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.23
50	FE8216	$2.4x10^8$	$1.2x10^7$	0	$>2.4x10^8$	15.23
51	FE8215	$2.3x10^8$	$1.2x10^7$	0	$>2.3x10^8$	14.50

**Test filters arranged in descending order of bubble point value*

*** Titer reduction calculated based on total challenge per test filter unit*

As indicated in Table 8 all filters with a bubble point value of 1000 mbar (14.5 psi) or greater produced a sterile filtrate in bacteria challenge testing. Based on this data, the minimum bubble point for user integrity testing using 60/40 (v/v) isopropanol/water can be set to 1000 mbar (14.5 psi). This limit value is also supported by similar studies with other filter formats that use identical capsule hardware and PTFE membrane, where sterile filtrates were produced down to a bubble point value of 1000 mbar (14.5 psi) or greater.

Based on these studies Pall defined the minimum bubble point value for Acro 50 filter capsule when wetted with 60/40 (v/v) isopropanol/water as 1000 mbar (14.5 psi) (Table 9).

Table 9.

Bubble point limit values for 60/40 (v/v) isopropanol/water

Bubble Point Limit Value (Minimum Bubble Point Pressure)	Wetting Liquid	Temperature
1000 mbar (14.5 psi)	60/40 (v/v) isopropanol/water	20 °C ± 5 °C*

**During the test period, the temperature of the filter assembly should not vary by more than ± 1 °C.*
See Section 3.1.3 for test procedure

3.3.2 User Integrity (Bubble Point) Limit Value for 70/30 (v/v) Isopropanol/Water and 100% Isopropanol

3.3.2.1 Ratio of Measured Bubble Point Values

The alternative wetting fluids to perform a bubble point test on Acro 50 filter capsules are 70/30 (v/v) isopropanol/water solution and 100% isopropanol. Actual bubble point measurement studies with twenty-four (24) filter capsules, wetted with 60/40 and 70/30 /(v/v) isopropanol/water and 100% isopropanol were performed for the determination of the corresponding bubble point ratios of these liquids. Each test filter was wetted with the three wetting fluids using the procedure as described in Section 3.1.3. At the end of each wetting the bubble point was determined as described in previous sections.

The results of the bubble point measurements are shown in Tables 9 and 10. The mean bubble point ratio for 70/30 (v/v) isopropanol/water and 60/40 (v/v) isopropanol/water was 0.9563 (Table 10). The mean bubble point ratio for 100% isopropanol and 60/40 (v/v) isopropanol/water was 0.8901 (Table 11).

Table 10.

Bubble point measurement results on Acro 50 filter capsules, when wetted with 70/30 (v/v) isopropanol/water and 60/40 (v/v) isopropanol/water

Test Unit Number	Lot Number	Bubble Point When Wetted with 70/30 (v/v) IPA/Water (psi)	Bubble Point When Wetted with 60/40 IPA/Water (psi)	Bubble Point Ratio
1	FE8215	15.23	15.95	0.9549
2	FE8215	15.23	15.95	0.9549
3	FE8215	15.23	15.95	0.9549
4	FE8215	15.23	15.23	1.0000
5	FE8215	15.23	15.95	0.9549
6	FE8215	14.50	15.23	0.9521
7	FE8215	13.78	14.50	0.9503
8	FE8215	15.23	15.95	0.9549
9	FE8216	15.23	15.95	0.9549
10	FE8216	15.23	16.68	0.9131
11	FE8216	15.95	16.68	0.9562
12	FE8216	15.00	15.95	0.9404
13	FE8216	14.50	15.23	0.9521
14	FE8216	15.95	16.68	0.9562
15	FE8216	14.50	15.23	0.9521
16	FE8216	15.23	15.95	0.9549
17	FE8217	15.23	15.95	0.9549
18	FE8217	16.68	16.68	1.0000
19	FE8217	16.68	17.40	0.9586
20	FE8217	15.95	16.68	0.9562
21	FE8217	15.23	15.95	0.9549
22	FE8217	16.68	17.40	0.9586
23	FE8217	16.68	17.40	0.9586
24	FE8217	14.50	15.23	0.9521
	Minimum	13.78	14.50	0.9131
	Maximum	16.68	17.40	1.0000
	Mean	15.37	16.07	0.9563
	Standard Deviation	0.77	0.76	0.0163

Table 11.

Bubble point measurement results on Acro 50 filter capsules when wetted with 100% isopropanol and 60/40 (v/v) isopropanol/water

Test Unit Number	Lot Number	Bubble Point When Wetted with 100% IPA (psi)	Bubble Point When Wetted with 60/40 IPA/Water (psi)	Bubble Point Ratio
1	FE8215	13.78	15.95	0.8639
2	FE8215	14.50	15.95	0.9091
3	FE8215	13.78	15.95	0.8639
4	FE8215	13.78	15.23	0.9048
5	FE8215	14.50	15.95	0.9091
6	FE8215	13.78	15.23	0.9048
7	FE8215	13.78	14.50	0.9503
8	FE8215	13.78	15.95	0.8639
9	FE8216	13.78	15.95	0.8639
10	FE8216	13.78	16.68	0.8261
11	FE8216	14.50	16.68	0.8693
12	FE8216	14.50	15.95	0.9091
13	FE8216	13.78	15.23	0.9048
14	FE8216	13.78	16.68	0.8261
15	FE8216	13.78	15.23	0.9048
16	FE8216	13.78	15.95	0.8639
17	FE8217	15.23	15.95	0.9549
18	FE8217	15.23	16.68	0.9131
19	FE8217	15.23	17.40	0.8753
20	FE8217	14.50	16.68	0.8693
21	FE8217	14.50	15.95	0.9091
22	FE8217	15.23	17.40	0.8753
23	FE8217	15.23	17.40	0.8753
24	FE8217	14.50	15.23	0.9521
	Minimum	13.78	14.50	0.8261
	Maximum	15.23	17.40	0.9549
	Mean	14.29	16.07	0.8901
	Standard Deviation	0.58	0.76	0.0347

3.3.2.2 *Surface Tension Ratio – Literature Data*

If a filter is wetted with two different wetting fluids (wetting fluid A and wetting fluid B), the ratio of the bubble points measured with wetting fluid A and wetting fluid B is expected to correspond to the surface tension ratio of the two wetting fluids [11,12]. Surface tension information for aqueous mixtures of isopropanol (2-propanol) in increasing concentrations by mass percent can be found in the reference cited [13]. The surface tension for 60/40 (v/v) isopropanol /water solution, 70/30 (v/v) isopropanol/water solution or 100% isopropanol calculated based on this literature reference are as follows:

Table 12.

Surface tension of 60/40 (v/v) isopropanol/water, 70/30 (v/v) isopropanol/water and 100% isopropanol*

Fluid	Surface Tension	Surface Tension Ratio for Fluids (Basis Surface Tension of Isopropanol/Water 60/40 (v/v)
60/40 (v/v) isopropanol/water	24.47 mN/m	1.0000
70/30 (v/v) isopropanol/water	23.63 mN/m	0.9657
100% isopropanol	21.74 mN/m	0.8884

**At a temperature of 20 °C*

The mean bubble point ratio shown in Tables 10 and 11 for 100% isopropanol and 60/40 (v) isopropanol/water was 0.8901 and that of 70/30 (v/v) isopropanol/water and 60/40 (v) isopropanol/water was 0.9563. Both ratios are comparable to the theoretical calculated ratios (Table 12).

Bubble point limits for the alternative wetting liquids were set based on a worst-case approach, i.e. using the higher ratio for calculation of the bubble point limits of each alternative wetting liquid, leading to a higher bubble point limit.

Based on the surface tension ratio (0.9657) at 20 °C, a bubble point value of 1000 mbar (14.5 psi) for 60/40 (v/v) isopropanol/water will correspond to a bubble point value of 965 mbar (14.0012 psi) for 70/30 (v/v) isopropanol with the same filter type. This results in a user bubble point limit value of 1000 mbar (14.5 psi) based on Pall's rounding rules for user limit integrity test data.

Based on the measured bubble point ratio (0.8901) at 20 °C a bubble point value of 1000 mbar (14.5 psi) for 60/40 (v/v) isopropanol/water will correspond to a bubble point value of 890 mbar (12.91 psi) for 100% isopropanol with the same filter type. This results in a user bubble point limit value of 900 mbar (13 psi) based on Pall's rounding rules for user limit integrity test data.

3.3.3 Conclusion

A comparison of the ratios obtained in these validation studies with the surface tension data/ratios available in the literature for these alternative wetting liquids confirm that the ratios are comparable. The higher ratio for both alternative wetting liquids were used which reflects a worst-case approach. In addition, Pall´s rounding rules applied for setting the bubble point limits adds an additional safety margin.

Table 13.

Bubble point limit value for 70/30 (v/v) isopropanol/water

Bubble Point Limit Value (Minimum Bubble Point Pressure)	Wetting Liquid	Temperature*
1000 mbar (14.5 psi)	70/30 (v/v) isopropanol/water	20 °C ± 5 °C*

**During the test period the temperature of the filter assembly should not vary more than ± 1°C.*

Table 14.

Bubble point limit value for 100% isopropanol

Bubble Point Limit Value (Minimum Bubble Point Pressure)	Wetting Liquid	Temperature*
900 mbar (13.0 psi)	100 % isopropanol	20 °C ± 5 °C*

**During the test period the temperature of the filter assembly should not vary more than ± 1°C.*

3.4 Air Flow

3.4.1 Introduction

The objective of these tests was to determine the air flow of Acro 50 filter capsules at a set pressure differential.

3.4.2 Summary of Methods

Fifty (50) filter capsules from three (3) batches were measured, resulting in a total of fifty (150) test units. The test filter units were installed in a suitable air flow test rig with the pressure regulator and flow measurement installation upstream of the test filter unit and its outlet open to atmosphere. Air flow was measured at a set upstream system pressure of 0.2 barg (3 psig), which also represented a differential pressure of 0.2 bar (3 psi) in this test installation. The measured gas flow is reported as standard liters per minute (sL/min).

3.4.3 Results

The results of the air flow tests are shown in Table 15. The lowest air flow measured was 15.0 sL/min. The highest air flow measured was 17.0 sL/min, while the mean of three batches amounts to 16.00 sL/min, 15.88 sL/min and 16.04 sL/min.

Table 25.

Air flow test results

Filter Lot	Number of Test Units	Minimum Flow (sL/min)	Maximum Flow (sL/min)	Mean Flow (sL/min)	Standard Deviation
FE8215	50	15	17	16.00	0.40
FE8216	50	15	17	15.88	0.52
FE8217	50	15	17	16.04	0.28

3.4.4 Conclusions

The test results in this section confirm that Acro 50 filter capsules consistently meet the minimum air flow of 8.0 sL/min at 0.2 bard (3 psid) differential pressure for this part number.

3.5 Isopropanol Liquid Pressure Test to Confirm Maximum Operating Pressure Conditions

3.5.1 Introduction

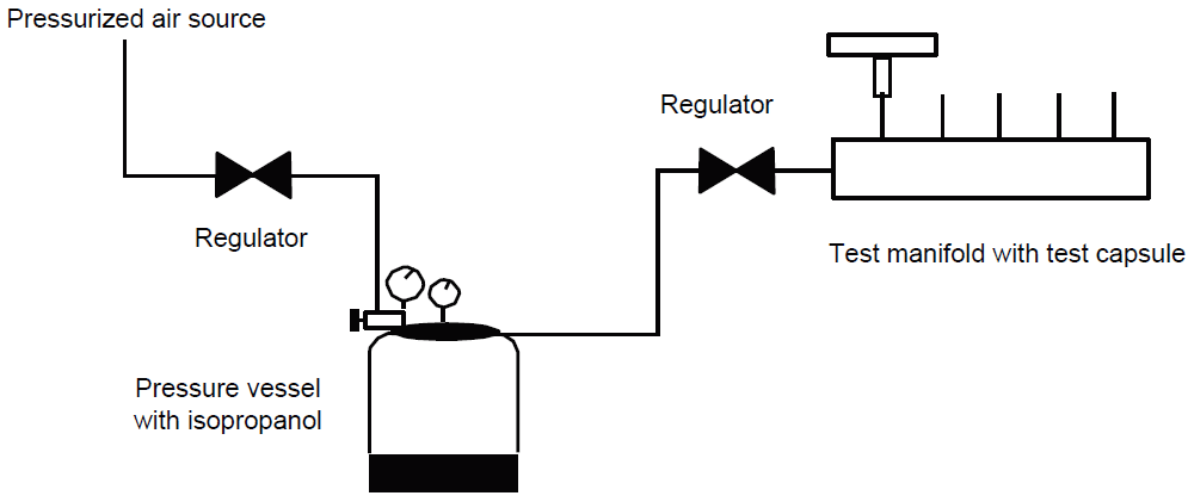
The mechanical robustness of a filter device is important for its safe and reliable operation and use and drives the operating and differential pressure specification. Acro 50 filter capsules were subjected to a liquid (isopropanol) pressure test at ambient temperature to assess their mechanical robustness and demonstrate tolerance up to 4.1 barg (60 psig) operating pressure.

3.5.2 Summary of Methods

Filter capsules from three (3) batches were subjected to a liquid (isopropanol) pressure test at 4.1 barg (60 psig) upstream pressure. Three hundred and seventy-five (375) capsules were tested, one hundred and twenty-five (125) from each batch. The test setup is shown in Figure 3. The pressure vessel was filled with isopropanol and the test filter unit installed on the test manifold. The pressure was set to 4.1 barg (60 psig) and the valve to the test manifold slowly opened until 4.1 bard (60 psid) was reached at the test unit, thus equating to 4.1 bard (60 psid) between upstream and downstream. Isopropanol was flushed through the test unit and the unit observed for any leaks and sudden flow increase while the pressure of 4.1 bard (60 psid) was maintained. After testing, the flow valve was closed and the system depressurized. Five (5) filter capsules from each lot which had pre-challenge “pass” bubble point test results were also integrity tested by bubble point at the end of the tests.

Figure 3.

Test setup for isopropanol liquid pressure test



3.5.3 Results

The results of the liquid pressure test carried out at 4.1 barg (60 psig) are shown in Table 16.

Table 16.

Liquid pressure test results

Filter Lot	Number of Test Units	Test Type	Leak Observed	Pass/Fail
FE8215	125	Liquid pressure at 60 psig forward pressure	No	Pass
FE8216	125		No	Pass
FE8217	125		No	Pass

Table 17.

Liquid pressure challenge and bubble point test results

Filter Lot	Liquid Challenge Pressure (psig)	Post Challenge Bubble Point (psi)
FE8215	60	15.95
FE8215	60	16.68
FE8215	60	15.95
FE8215	60	16.68
FE8215	60	15.95
FE8216	60	15.23
FE8216	60	14.50
FE8216	60	15.23
FE8216	60	15.23
FE8216	60	15.23
FE8217	60	15.95
FE8217	60	15.95
FE8217	60	15.95
FE8217	60	15.95
FE8217	60	15.95

3.5.4 Conclusions

The results of the liquid pressure test demonstrate that Acro 50 filter capsules are mechanically robust and withstand an operating pressure of 4.1 barg (60 psig) in the forward direction without leak and membrane rapture, and maintaining filter integrity as demonstrated by bubble point testing.

3.6 Water Breakthrough

3.6.1 Introduction

In some applications the resistance to fluid penetration is a relevant feature of a vent filter. Acro 50 filter capsules contain an inherently hydrophobic PTFE membrane that can repel water and other fluids with higher surface tension and withstand the intrusion and breakthrough of a liquid phase for such fluids. A water breakthrough test confirms the hydrophobic nature of the filter media. It can also serve as a structural integrity test, confirming the correct seal of the filter media and absence of gross leaks.

3.6.2 Summary of Methods

Filter capsules from three (3) batches were subjected to a water breakthrough test at 2.1 barg (30 psig) upstream pressure. Three hundred and seventy-five (375) capsules were tested, one hundred and twenty-five (125) from each batch.

Test procedure for water breakthrough testing:

1. A pressure vessel was filled with a solution consisting of 0.6% colored dye in filtered water. The dye serves to increase the optical contrast during visual assessment.
2. Using a regulated pressurized air source, pressure was introduced to force the dyed water to the inlet of the test unit. Visual checks ensured that the water phase reached the filter media surface.
3. The upstream pressure was slowly increased to reach 2.1 barg (30 psig) and held for at least 15 seconds, thus exposing the filter to 2.1 bard (30 psid) from upstream to downstream.
4. The test units were visually inspected for water penetration through the filter media and for leaks.
5. Absence of water penetration and leaks were considered a “pass”.

3.6.3 Results

The results of the water breakthrough tests carried out at 2.1 barg (30 psig) are shown in Table 18.

Table 18.

Water breakthrough test results

Filter Lot	Number of Test Units	Water Breakthrough of Leak Observed at 2.1 bard (30 psid)	Test Results
FE8215	125	No	Pass
FE8216	125	No	Pass
FE8217	125	No	Pass

3.6.4 Conclusions

The results of the water breakthrough test demonstrate that Acro 50 filter capsules can withstand a water column pressure of 2.1 bard (30 psid) without breakthrough of the liquid phase and without leaking.

3.7 Extractables

3.7.1 Introduction

The objective of these tests was to quantify the material that can be extracted from Acro 50 filter capsules under challenging extraction conditions. The conditions chosen were above ambient

(extraction temperature of 40 °C) with a non-aqueous extraction fluid (50/50 (v/v) ethanol/water) and for a prolonged extraction duration (24 hours). To maximize the amount of extractables and mimic the actual conditions of use, the test filter units were autoclaved at 125 °C for 1 hour prior to extraction. These aggressive extraction conditions aim to present worst-case conditions for extractables release and thus allow users to assess the maximum amount of extractables released by these filters into their drug product.

3.7.2 Summary of Methods

Filter capsules were autoclaved at 125 °C for 1 hour. For each extraction, two (2) filter capsules were connected in series using PTFE-lined tubing and filled with 50/50 (v/v) ethanol/water. The extraction took place for 24 hours at 40 °C. At the end of the extraction time, the extraction liquid in the filters was pushed out with fresh 50/50 (v/v) ethanol/water, the liquid collected and evaporated to dryness. The non-volatile residue (NVR) was determined gravimetrically. Fourier Transform Infrared (FTIR) spectra of the NVRs could not be prepared due to extremely low amount of residue.

3.7.3 Results

Table 19 shows the levels of the extractables obtained from two batches of filter capsules that were tested. The NVR values were below 0.2 mg (limit of detection) for the two filter capsules extracted together.

Table 19.

Non-volatile residue (19.6 cm^2 effective filtration area per filter capsule) with 50/50 (v/v) ethanol/water as extraction fluid

Filter Lot	Number of Test Units Extracted Together	Total NVR (mg per 2 Test Filter Units)
FE5896	2	<0.2*
FE6001	2	<0.2
FE6002	2	<0.2

**0.2 mg limit of detection*

3.7.4 Conclusions

The level of extractables obtained from Acro 50 filter capsules, even under aggressive extraction conditions (after autoclaving at 125 °C for 1 hour, extraction with 50/50 (v/v) ethanol/water at 40 °C for 24 hours), was extremely low (<0.2 mg NVR). While actual service will impose different conditions, the extractables conditions chosen will typically represent worst-case conditions for use of these filter capsules.

4 Biological Reactivity Tests on the Materials of Construction

4.1 Introduction

The aim of these studies was to evaluate the biological suitability of the materials of construction of Acro 50 filter capsules. The materials of construction of the filter capsules are as follows:

Table 20.

Materials of construction

Membrane	Hardware Parts
Hydrophobic polytetrafluorethylene (PTFE) backed with a polypropylene non-woven support	Polypropylene

4.2 Summary of Methods

The tests on the respective material of construction were performed in accordance with the USP <88> Biological Reactivity Tests (*in vivo*) for Class VI-121 °C plastics as described in the current United States Pharmacopeia (USP) [(1)].

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

- Sodium chloride Injection
- 1:20 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 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 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, to subject the materials of construction to the most stringent conditions included in the United States Pharmacopoeia. Each of the materials of the Acro 50 filter capsules was implanted separately.

In vitro cytotoxicity was assessed determining the biological reactivity of mammalian cell cultures following contact with extracts of the polymeric materials of construction (MEM Elution testing). The test method followed USP <87> [2].

4.3 Results

All materials of construction used in the capsule passed all of the tests specified under USP <88> Biological Reactivity Tests (*in vivo*) for Class VI Plastics (121 °C) [1] and a MEM Elution test assessing cytotoxicity [2,3].

4.4 Conclusions

The materials of construction used in Acro 50 filter capsules meet the requirements of the USP Biological Reactivity Tests (*In vivo*) for Class VI-121°C plastics [1] and a MEM Elution test [2,3] assessing cytotoxicity. The tests under USP Biological Reactivity Tests (*In vivo*) for Class VI-121 °C included the Systemic Injection Test, the Intracutaneous Test and the Implantation Test. The results demonstrate that the materials of construction of Acro 50 filter capsules are biologically safe and fit for use in manufacturing of drug products under cGMP.

5 Transmissible Spongiform Encephalopathy (TSE) / Bovine Spongiform Encephalopathy (BSE)

Acro 50 0.2 µm vent filter capsules, part number 6074270L, are assembled from components using polymeric resin materials and elastomeric materials. While some of the materials may contain chemicals produced from animal material substances, they are not considered a TSE/BSE risk based on their source (sourcing takes into consideration animal species, tissue and country of origin) and/or exposure to processing conditions known to inactivate infectious agents associated with TSE/BSE diseases. See below for further information on polymeric chemical additives produced from 'tallow'.

Tallow-Derivatives:

Some polymeric resin manufacturers employ trace levels of additives in the resin formulation. These additives may be manufactured using animal tallow as a starting substance ('tallow-derivatives'). The tallow may have been sourced from bovine species or, less commonly, from non-TSE relevant species. Please be advised that bovine tallow-derivatives are not considered risk material for TSE/BSE according to the current revision of the U.S. Code of Federal Regulations, Title 21 Part 189.5 Prohibited cattle material [(3)]. Furthermore, the European CPMP's Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathies via human and veterinary medicinal products (EMA410/01 current version), and other international guidelines, gives specific consideration to tallow-derivatives and states that they are unlikely to be infectious due to the rigorous processing steps used during their manufacture (for example, transesterification or hydrolysis, at not less than 200 °C under pressure for not less than 20 minutes). Our suppliers have stated that these raw materials have been processed under conditions at least as rigorous as these.

The above information reflects Pall's current knowledge based on supplier's information and Pall process controls. The statements provided are subject to change as new information from our suppliers becomes available. Pall will notify customers if any new information is received that would impact TSE/BSE safety; however, we also recommend that users periodically confirm this information.

Shelf Life Statement:

Pall Corporation does not assign specific expiration dates to non-sterile pharmaceutical grade filters. Given the stable nature of our filter materials of construction, we have not seen any deterioration of filter performance over time. However, in order to assist our biopharmaceutical customers who, require a defined shelf life, we conservatively recommend usage within five years. To achieve satisfactory performance, it is advised that the following storage conditions are maintained:

1. Store at a temperature of 0-30 °C in dry conditions.
2. Do not expose filters to direct sunlight, radiation or direct weather conditions.
3. Store filters in original shipping bag and boxing.
4. Exercise care during filter handling to avoid physical damage. Ensure shipping bag and any seals are intact prior to use. Plastics can be damaged if roughly handled, particularly at sub-zero temperatures. Thermal shock by quickly raising the temperature from sub-zero conditions should also be avoided.
5. Inspection and integrity testing is recommended prior to use.

6 Certificate of Test Acro 50 0.2 µm Vent Filter Capsules, Part Number 6074270L

Pall Corporation

Certificate of Test

For Pharmaceutical-Grade Sterilizing Filters

We hereby certify that

Pall® : ACRO® 50 VENT FILTER

Rated: 0.2 µm

Part Number: 6074270L

Lot Number: Sample

was manufactured in a controlled environment. **These filters are not supplied sterile.**

Fabrication Integrity

Each filter in this lot successfully passed a bubble point integrity test as per internal manufacturing specifications. The bubble point test parameters have been validated for bacterial removal by correlation with a microbiological challenge test. Recommended test values for integrity testing of Pall filters as installed must be obtained from Pall.

Bacterial Retention

Finished product has been sampled and successfully tested for retention of *Brevundimonas diminuta* (ATCC 19146), using procedures described in Pall Validation Guides and ASTM Standard Test Method F838-15, in conformance with the applicable requirements of the FDA **Guidance for Industry**: **Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice** (September 2004).

Materials of Construction

Representative filter components have met the requirements for biological reactivity, *in vivo,* under USP <88> (for Class VI - 121 °C plastics).

These filters also are made from materials listed in Title 21 of the U.S. **Code of Federal Regulations** (CFR) parts 170-199.

This product does not contain materials of construction that are considered specified TSE or BSE risk materials according to current legislation and guidelines (reference European CPMP EMA/410/01 and Title 21 of the U.S. Code of Federal Regulations (CFR), part 189.5).

Effluent Quality

Filter samples from this manufacturing lot underwent the following tests and the lot was released by Quality Control when it was verified that their respective criteria were met:

Cleanliness

Meets with adequate safety margin after flushing, current limits under USP <788> Particulate Matter in Injections, with effluent counts determined microscopically. Counts serve to document conformance with the requirements for a non-fiber-releasing filter per Title 21 of the U.S. **Code of Federal Regulations** (CFR) parts 211.72 and 210.3 (b)(6).

Oxidizable Substances

Meets the current USP requirements after flushing under Sterile Purified Water, as determined by a Potassium Permanganate test.

pH

Meets internal specifications after flushing, upstream versus downstream differential not to exceed +/- 0.5 pH units, when tested in accordance with USP <791> pH.

Endotoxins

Meets the current requirements under USP Water for Injection, 0.25 EU/ml, when an aliquot from a soak solution is tested using Limulus Amoebocyte Lysate (LAL) reagent in accordance with USP <85> Bacterial Endotoxins Test.

In addition to the above tests, this product met manufacturing inspection standards and requirements for full traceability. This product is manufactured under a Quality System certified to ISO 9001. Consider only unopened, undamaged packages for use. Further information is available by contacting Pall.

Jose Cubero

7/February/2020

Jose Cubero, Quality Manager, Pall Life Sciences Puerto Rico
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CoT0104E rev 01

Date of Manufacture

www.pall.com

Filtration. Separation. Solution. *SM*

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2. United States Pharmacopoeia (USP) Chapter <87> Biological Reactivity Tests *In Vitro* USP 42-NF 37 (2019) Rockville, MD

3. Code of Federal Regulations Title 21 Foods and Drugs, Parts 170 to 199: Food and Drugs: FDA-Food for Human Consumption

4. Code of Federal Regulations Title 21 Foods and Drugs, Part 210: Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs

5. Code of Federal Regulations Title 21 Foods and Drugs, Part 211: Current Good Manufacturing Practice for Finished Pharmaceuticals

6. International Organization for Standardization (ISO) 9001: Quality Management Systems – Requirements

7. International Organization for Standardization (ISO) 14644-1: Cleanrooms and associated controlled environments

8. American Society for Testing and Materials (ASTM) Standard Test Method F838-15a Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration

9. US Federal Drug Administration (FDA), Center for Drug Evaluation and Research: Guidance for Industry - Guidelines on Sterile Products Produced by Aseptic Processing (1987)

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