Life Sciences

Validation Guide

USTR 2431

Pall® Kleenpak™ Water Filter

Disposable Water Filter for Use in Cleanroom Airlocks in Pharmaceutical Production and Related Applications

Filtration. Separation. Solution.SM

CONTENTS

Part I Overview

1. Introduction

This report contains data applicable to Pall **Kleenpak** Water Filters, Part Number KAQ14F1S.

Table 1 Pall Kleenpak Water Filters Variant Identification

Product	Outlet Configuration	Capsule Length	Product Code
Pall **Kleenpak** Water Filter	Smooth outlet	71 mm	KAQ14F1S
Pall **Kleenpak** Water Filter	Shower rose outlet	85 mm	KAQ14F1R

This product is supplied sterile, ready for use to provide water for hand washing in cleanroom airlocks in pharmaceutical production and related applications. Following manufacture, sterilization is achieved by gamma irradiation. The conditions used ensure a minimum Sterility Assurance Level of 10^{-6}. The sterilization process has been validated and routinely controlled in compliance with the following standards:

BS EN 552 (1994) 'Sterilisation of Medical Devices Validation and Routine Control of Sterilisation by Irradiation'.

ISO 11137 (2006) 'Sterilization of Healthcare Products - Radiation'.

Please contact Pall if further information about the gamma sterilization process is required.

This product is validated by:

- Liquid microbial challenge tests using *Brevundimonas diminuta* (ATCC 19146) using an industry standard method for 0.2 µm sterilizing grade filters and in simulated intermittent use for 14 days.
- Typical flow rate measurements at various inlet water pressures
- Maximum operating temperature and pressure rating measurements
- Extractables testing as per the British Standard for Testing for Non-metallic Materials for use with Drinking Water (BS 6920:2000) and Commission Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs
- Biological reactivity tests to US Pharmacopoeia Class IV <88> *(in vivo)* specification
- Evaluation of shelf life

Please contact Pall if more detailed information on the test methods is required.

2. Summary of Conclusions

2.1 Verification of 0.2 µm Sterilizing Grade Filter Performance

Pall **Kleenpak** Water Filters retain *Brevundimonas diminuta* when tested by industry standard laboratory liquid microbial challenge tests used for validating 0.2 µm sterilizing grade filters, to ≥ 10^7 CFU/cm^2 of effective filtration area.

2.2 Microbial Retention in Intermittent Use

Pall **Kleenpak** Water Filters retain *Brevundimonas diminuta* during intermittent use for 14 days in laboratory liquid challenge tests at ≥ 10^7 CFU/cm^2 of effective filtration area.

2.3 Evaluation of Bacteriostatic Additive

Pall **Kleenpak** Water Filters contain a bacteriostatic additive incorporated within the plastic housing to reduce external microbial contamination by greater than 99% after 24 hours contact.

2.4 Typical Flow Rates at Nominal Water Pressures

Pall **Kleenpak** Water Filters typically deliver flow rates at inlet water pressures as detailed in Table 2:

Table 2 Typical Mean Clean Water Flow Rates, Liters/minute (L/min) Water Temperature 20 °C (68 °F)

Product Type	**Water Pressure, bar (approx psi)**				
Pall **Kleenpak** Water Filter	**1 (15)**	**2 (30)**	**3 (45)**	**4 (60)**	**5 (75)**
Typical Water flow (L/min)	5.0	8.2	11.3	13.7	16.1

2.5 Maximum Operating Temperature and Pressure

Pall **Kleenpak** Water Filters have been qualified to operate at up to 60 °C (140 °F) and 5 bar (72.5 psi) inlet pressure.

2.6 Extractables Testing

Pall **Kleenpak** Water Filters materials meet the requirements of BS 6920:2000 for chlorinated and unchlorinated water.

Pall **Kleenpak** Water Filters meet the requirements of European Commission Directive 2002/72/EC .

2.7 Biological Reactivity Tests

Pall **Kleenpak** Water Filters meet the requirements of United States Pharmacopoeia <88> *(in vivo)* specification.

2.8 Shelf Life

Pall **Kleenpak** Water Filters can be used at up to 36 months from the date of sterilization.

Part II Microbial Validation

1. Microbial Challenge Testing for Verification of 0.2 µm Sterilizing-Grade Filter Performance

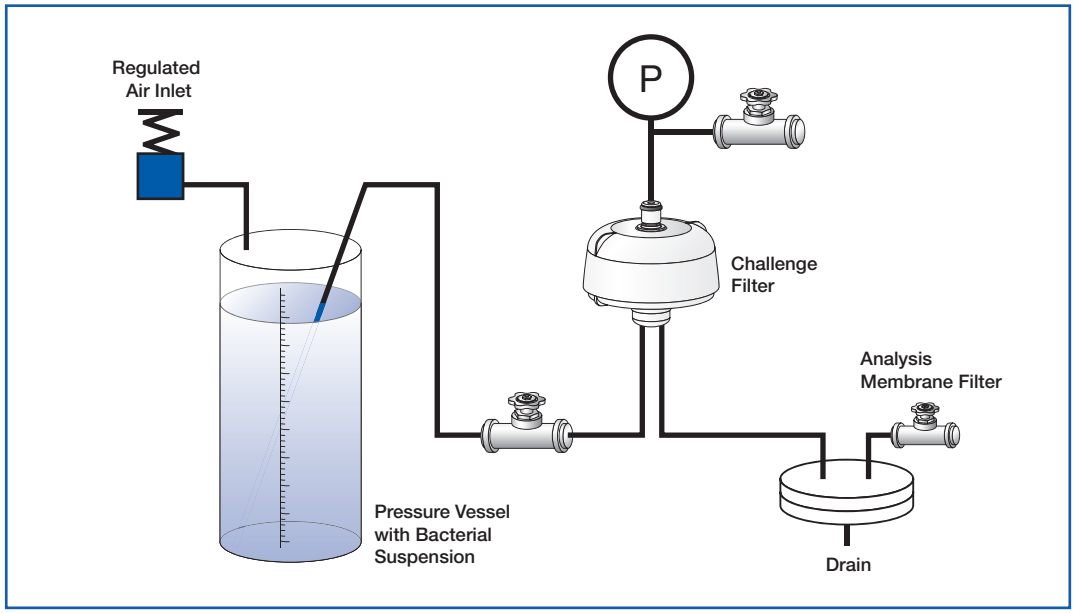
1.1 Introduction

The aim of this series of tests was to confirm that Pall **Kleenpak** Water Filters retain the test organism *Brevundimonas diminuta* following the industry standard methods defined for validating 0.2 µm sterilizing grade filters.

1.2 Summary of Methods

This method follows the principles of ASTM F838-05 Standard for validating 0.2 µm sterilizing grade filters. The apparatus was assembled as shown in Figure 1. A number of Pall **Kleenpak** water filters were subjected to a liquid microbial challenge test after gamma irradiation. The sterile pressure vessel was filled with 10 L of 0.2 µm filtered sterile deionised water. A single bolus inoculum of *Brevundimonas diminuta* (ATCC 19146) was added to the vessel and mixed thoroughly to give a challenge level of greater than or equal to 1 x 10^7 colony forming units (CFU)/cm^2 media area. This was sampled aseptically to confirm the challenge level. The complete challenge solution was passed through the test filters at 500 mL/minute and the filtrate passed through 0.2 µm analysis membrane filter discs placed in a holder downstream of the filter. The analysis membranes were incubated on Tryptone Soya Agar at 30 °C (86 °F) for at least 48 hour and examined for microbial growth.

Figure 1 Microbial Challenge Test Rig for Verification of Sterilizing-Grade Filter Performance



1.3. Results

A summary of results is shown in Table 3. All samples of water collected from the test filters were found to be free of the test organism.

Table 3 Retention of *B. diminuta* Liquid Challenge by Pall Kleenpak Water Filters

Serial Number	Total Challenge CFU	Total Challenge CFU/cm²	Recovery
IJ1159 0006	1.95 x 1010	4.24 x 10^{7}	0
IJ1159 0021	1.95 x 10^{10}	4.24 x 10^{7}	0
IJ1159 0022	1.46 x 10^{10}	3.17 x 10^{7}	0
IJ1159 0031	1.46 x 10^{10}	3.17 x 10^{7}	0
IJ1159 0032	2.10 x 10^{10}	4.57 x 10^{7}	0
IJ1159 0071	2.10 x 10^{10}	4.57 x 10^{7}	0
IJ1159 0100	1.90 x 10^{10}	4.13 x 10^{7}	0
IJ1159 0110	1.90 x 10^{10}	4.13 x 10^{7}	0
IJ1159 0118	1.94 x 10^{10}	4.22 x 10^{7}	0
IJ1159 0121	1.94 x 10^{9}	4.22 x 10^{7}	0
IJ1146 0020	6.00 x 10^{9}	1.30 x 10^{7}	0
IJ1146 0041	6.00 x 10^{9}	1.30 x 10^{7}	0
IJ1146 0043	7.15 x 10^{9}	1.55 x 10^{7}	0
IJ1146 0054	7.15 x 10^{9}	1.55 x 10^{7}	0
IJ1146 0061	7.40 x 10^{9}	1.61 x 10^{7}	0
IJ1146 0070	7.40 x 10^{9}	1.61 x 10^{7}	0
IJ1146 0088	7.55 x 10^{9}	1.64 x 10^{7}	0
IJ1146 0092	7.55 x 10^{9}	1.64 x 10^{7}	0
IJ1146 0095	8.70 x 10^{9}	1.89 x 10^{7}	0
IJ1146 0099	8.70 x 10^{9}	1.89 x 10^{7}	0
IJ1147 0003	8.25 x 10^{9}	1.79 x 10^{7}	0
IJ1147 0029	8.25 x 10^{9}	1.79 x 10^{7}	0
IJ1147 0034	8.40 x 10^{9}	1.83 x 10^{7}	0
IJ1147 0036	8.40 x 10^{9}	1.83 x 10^{7}	0
IJ1147 0042	7.70 x 10^{9}	1.67 x 10^{7}	0
IJ1147 0054	7.70 x 10^{9}	1.67 x 10^{7}	0
IJ1147 0060	8.20 x 10^{9}	1.78 x 10^{7}	0
IJ1147 0073	8.20 x 10^{9}	1.78 x 10^{7}	0
IJ1147 0082	9.40 x 10^{9}	2.04 x 10^{7}	0
IJ1147 0085	9.40 x 10^{9}	2.04 x 10^{7}	0

1.4 Conclusion

Pall **Kleenpak** Water Filters retained *Brevundimonas diminuta* in laboratory liquid challenge tests following the industry standard for 0.2 µm sterilizing grade filters, at a level of $\geq 10^7$ CFU/cm^2 of effective filtration area.

1.5 References

1. American Standard Test Method (ASTM) F838-05 "Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration"

2. Microbial Retention in Intermittent Use

2.1 Introduction

The aim of this series of tests was to confirm that Pall **Kleenpak** Water Filters retain the microbial challenge organism *Brevundimonas diminuta* in typical intermittent use for a period of fourteen days.

2.2 Summary of Methods

The sterile test apparatus was assembled as shown in Figure 2. Two filters which had been gamma irradiated were each subjected to a liquid microbial challenge test.

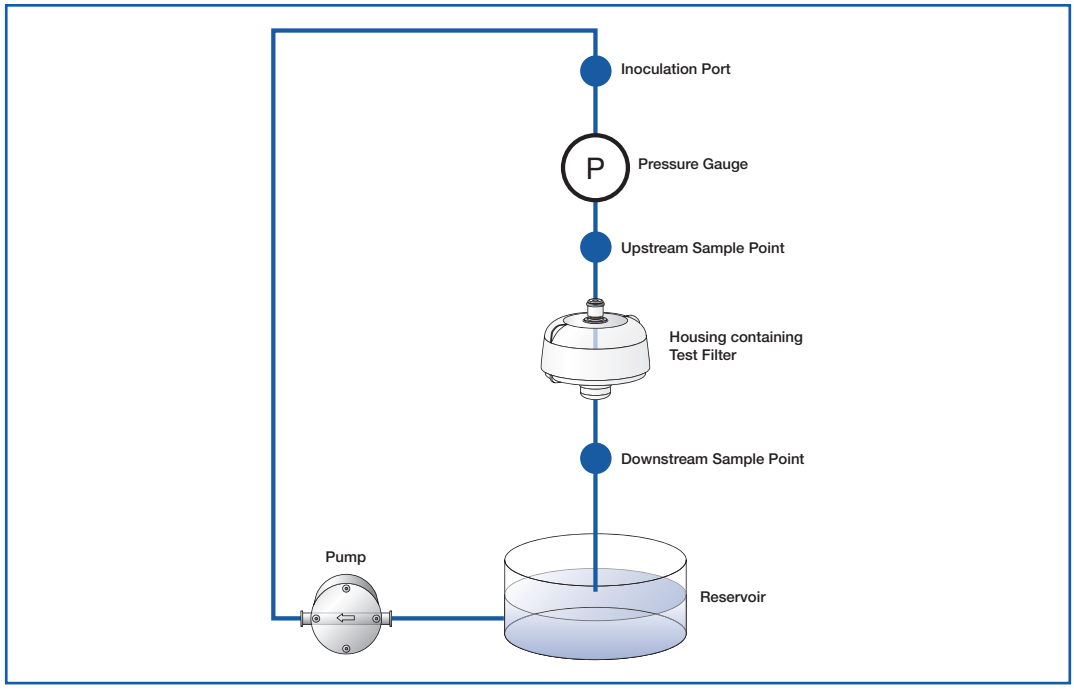
Filters were tested with intermittent water flow, each for a total duration of 14 days.

The pump was set to deliver a flow of approximately 5 L/min, which was delivered for 50 minutes each hour continuously during the working day. On Day 1 and Day 8, a bolus *Brevundimonas diminuta* challenge suspension was added via the inoculation port and a 100 mL sample was taken immediately downstream of the test filter.

The 100 mL water samples were filtered through 0.2 µm analysis membranes, which were placed onto Petri dishes of Tryptone Soya Agar (TSA). A viable cell count was performed on the challenge suspension. All plates were incubated for up to 48 hr at 30 °C (86 °F).

One further microbial challenge was delivered at the beginning of the 15th day (i.e. end of simulated life) to complete the assessment. A positive control was then performed by delivering one bolus challenge via the test rig inoculation port but with no filter in place.

Figure 2 Microbial Challenge Test Apparatus for Simulated Intermittent Use



2.3 Results

A summary of results is shown in Table 4. All samples of water collected from the test filters were found to be free of the test organism.

Table 4 Retention of *B. diminuta* Liquid Challenge by Pall Kleenpak Water Filters During Intermittent Use

	***B. diminuta* challenge (CFU)**			**Total challenge (CFU)**	**Total challenge (CFU/cm²)**	***B. diminuta* recovered downstream (CFU/100 mL sampled)**		
Serial Number	**Day 1**	**Day 8**	**Day 15**			**Day 1**	**Day 8**	**Day 15**
IJ11590114	4.8×10^9	3.0×10^9	3.6×10^{10}	4.4×10^{10}	9.5×10^7	0	0	0
IJ11590112	4.8×10^9	3.9×10^9	3.6×10^{10}	4.4×10^{10}	9.5×10^7	0	0	0
Positive Control (CFU)			4.2×10^{10}					1.5×10^{10}

2.4 Conclusions

Pall **Kleenpak** Water Filters retain $>10^7$ CFU/cm² *Brevundimonas diminuta* over an extended challenge period of 14 days.

3. Evaluation of Bacteriostatic Additive

3.1 Introduction

The aim of this series of tests was to assess the efficacy of the silver based bacteriostatic additive incorporated within the plastic housing of Pall **Kleenpak** Water Filters even after repeated sterilization throughout a 16-day period. These tests were performed independently by an external laboratory.

3.2 Summary of Methods

Samples of the plastic housing incorporating the bacteriostat used for Pall **Kleenpak** Water Filters were inoculated with *Staphylococcus aureus* and *Pseudomonas aeruginosa* on Day 0. The number of viable cells on the surface of the samples after 24 hours contact was then assessed before they were re-sterilized and re-inoculated. The samples were then repeatedly sterilized and inoculated every other day.* Although inoculated every other day, the number of viable cells on the surface after 24 hours contact was assessed on Day 16. Control samples of plastic housing without the bacteriostatic additive were also evaluated in the same way.

* The sterilization step demonstrates that the bacteriostatic additive was not leached from the surface and maintained its effectiveness.

3.3 Results

A summary of the results is shown in Table 5.

Table 5 Evaluation of Bacteriostatic Additive

	24 hours			Day 16		
Test Organism	**Control**	**Test**	**% Reduction**	**Control**	**Test**	**% Reduction**
Ps. aeruginosa	2.3×10^7	2.0×10^5	99.1	1.7×10^8	7.9×10^5	99.5
S. aureus	Not done	Not done	Not done	9.9×10^6	1.3×10^4	99.9

Not done = laboratory did not perform test at this time point.

3.4 Conclusion

The bacteriostatic additive within the housing of Pall **Kleenpak** Water Filters is capable of inhibiting growth of externally introduced microbial contamination by greater than 99% within 24 hours. Its effectiveness is maintained even after repeated sterilization and re-inoculation throughout a 16 day period.

Part III Validation of Physical Characteristics

1. Typical Flow Rate at Various Inlet Water Pressures

1.1 Introduction

The aim of this test was to illustrate typical water flow rates that might be expected at different inlet water pressures.

1.2 Summary of Methods

A number of filters equivalent to KAQ14F1S, sterilized by gamma irradiation, were subjected to water flow pressure drop testing as follows:

The test rig consists of a recirculation loop containing pre-filtered deionised water. The test filters were pre-wetted and inserted into the circuit. After a short period of recirculation to allow the system to stabilise, readings were taken of flow rate at different pressure levels (1 bar increments).

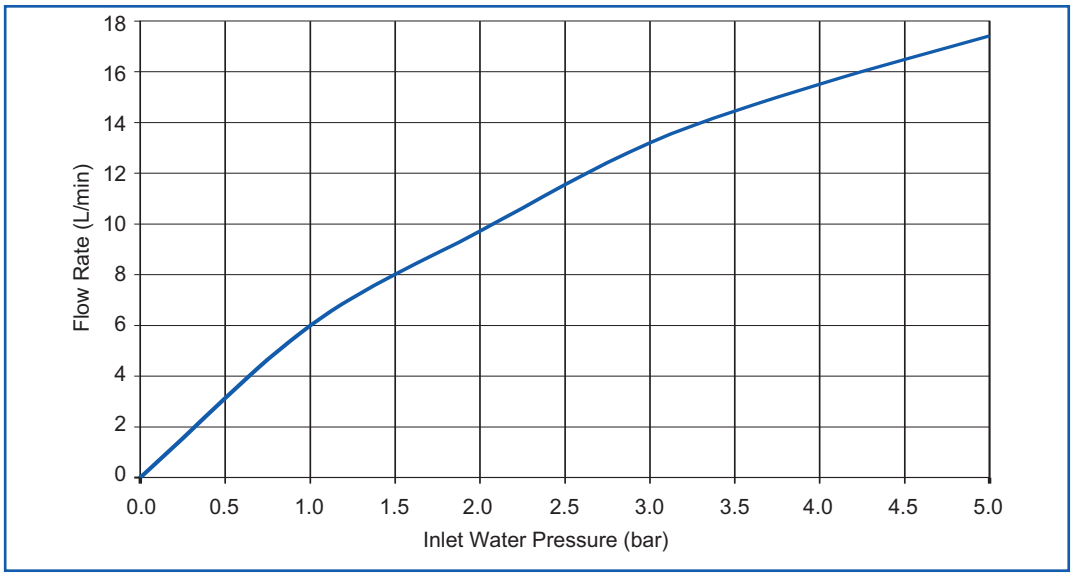
1.3 Results

A summary of results is shown in Table 6 and represented graphically in Figure 3.

Table 6 Typical Clean Water Flow Rates at Various Inlet Water Pressures, Water Temperature 20 °C (68 °F)

Filter Serial Number	Water Flow (L/min) at the following inlet pressures, bar (approx. psi)				
	1 (15)	2 (30)	3 (45)	4 (60)	5 (75)
IJ1159 0061	5.1	8.3	11.4	14.1	16.5
IJ1159 0090	4.9	8.3	11.4	13.7	16.0
IJ1159 0124	4.9	8.1	11.0	13.4	15.8
Average:	**5.0**	**8.2**	**11.3**	**13.7**	**16.1**

Figure 3 Flow Rate Versus Inlet Water Pressure for the Pall Kleenpak Water Filter



1.4 Conclusions

At 1-5 bar (15-75 psi) water pressure, Pall **Kleenpak** Water Filters typically deliver 5.0 - 16.1 L/min.

NOTE: Water flow rates during use will be dependent on the contamination levels of the influent water supply. In heavily contaminated water systems, flow rates will drop as the filters remove contaminants and eventually may become blocked. Pall can recommend suitable pre-filtration for heavily contaminated influent water qualities or under other operating conditions.

2. Maximum Operating Temperature and Pressure Rating

2.1 Introduction

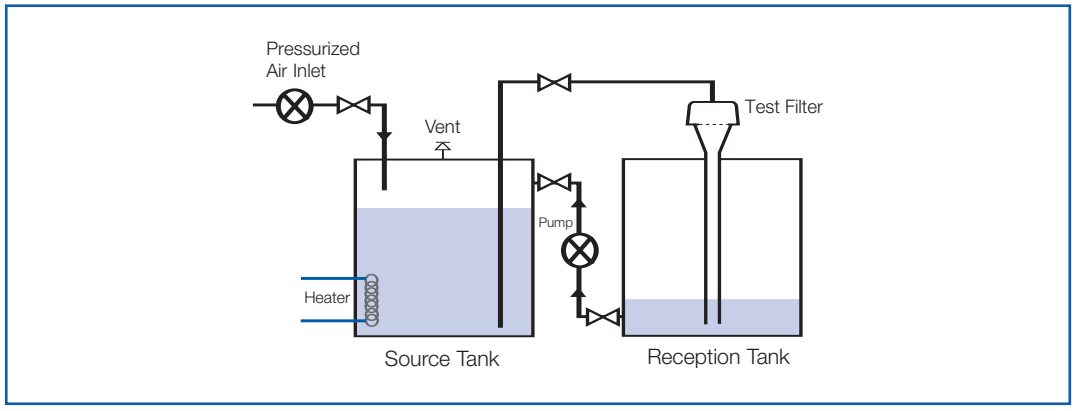
The aim of this series of tests was to confirm that Pall **Kleenpak** Water Filters are capable of operating at a continuous maximum temperature of 60 °C (140 °F) combined with a maximum inlet pressure of 5 bar (72.5 psi) and also able to withstand 70 °C (158 °F) for a total cumulative period of 30 minutes over the life of the filter.

2.2 Summary of Methods

Two sterile filters equivalent to KAQ14F1S from manufacturing batches were integrity tested using the Diffusive Forward Flow non-destructive integrity test method at an applied air pressure of 2760 mbar (40 psi) water wet* and then placed into a purpose-built rig designed to re-circulate hot water under high pressure in defined cycles (see Figure 4). The filters were subjected to 504 x 5 minute cycles of hot water at 60 °C (140 °F) and at an inlet pressure of 5 bar, cycling on and off to simulate 36 x 5 minute episodes per day (3 hours cumulative use per day) for an equivalent period of 14 days use. Each filter was integrity tested again after the cycling was complete before being subjected to 6 x 5 minute cycles at 70 °C (158 °F) and 5 bar (72.5 psi). A final integrity test was then performed on each filter.

* This test is directly correlated with bacteria removal efficiency.

Figure 4 Hot Water Test Rig



2.3 Results

A summary of results is shown in Table 7. Filters tested retained their integrity after hot water (60 °C [140 °F]) testing at 5 bar inlet pressure.

Table 7 Maximum Operating Temperature and Pressure Rating

	Forward Flow Integrity Test Results		
Serial Number	**Pre-exposure**	**Post-exposure 60 °C (140 °F)***	**Post-exposure 70 °C (158 °F)**
IJ1159 0044	-	PASS	PASS
IJ1159 0039	-	PASS	PASS
IJ1159 0033	PASS	PASS	PASS
IJ1159 0131	PASS	PASS	PASS

* 504 cycles x 5 minutes equivalent to 42 hours total exposure = 3 hours continuous use per day for 14 days or 36 x 5 minute periods of use per day.

2.4 Conclusions

Pall **Kleenpak** Water Filters have been demonstrated to operate continually at a maximum recommended water pressure of 5 bar (approx 75 psi) at 60 °C (140 °F) and maintain their integrity over their simulated service life. The filters are also able to withstand a temperature of 70 °C (158 °F) for a total cumulative period of 30 minutes over the life of the filter, at 5 bar (72.5 psi) inlet pressure as may be used during some thermal sanitization regimes.

Part IV Extractables Testing and Biological Reactivity Testing

1. Extractables Testing

1.1 Introduction

The aim of these tests was to evaluate the material that can be extracted from Pall **Kleenpak** Water Filter using water as extraction fluid.

1.2 Summary of Methods

Testing was performed on products equivalent to Pall **Kleenpak** Water Filters.

1.2.1 BS 6920: 2000

Filter samples that had been sterilized by gamma irradiation were subjected to five tests, as described in the British Standard for Testing for Non-Metallic Materials for use with Drinking Water (BS 6920:2000) by an independent organisation. This included testing for the impact of the filter device on the odor and flavor of drinking water. The test filters were held in water at the specified temperature for the specified test duration. Extracts were tested as described and conformance to the standard was assessed by comparison with control samples of the test water.

1.2.2 2002/72/EC

Filters (sterilized by gamma irradiation) were subjected to extractables testing by an independent organisation.

1.3 Conclusions

1.3.1 BS 6920: 2000 Compliance

Filters meet the limits specified in the Testing for Non-Metallic Materials for use with Drinking Water (BS 6920:2000 standard). Data is on file, silver levels were below the limit of detection (less than 1 µg/L).

1.3.2 2002/72/EC Compliance

The samples of Pall **Kleenpak** Water Filters meet the requirements of European Directive 2002/72/EC. Data is on file.

2. Biological Reactivity Testing

2.1 Introduction

The purpose of this study was to evaluate the biological suitability of the materials of construction (see Table 8) of Pall **Kleenpak** Water Filters as previously performed for other Pall products composed of identical materials of construction.

Table 8 Pall Kleenpak Water Filters - Materials of Construction

Component	Material
Filter Membrane	Polyethersulfone
Support/drainage Layers Closed End-cap	Polyester
Inlet and Outlet Housing	Polyester, pigment, bacteriostat, opacifier
Membrane Core and Cage	Polypropylene
O-ring Seal	Ethylene polypropylene elastomer

2.2 Summary of Methods

Biological Reactivity Tests *(in vivo)* were independently performed as described in United States Pharmacopoeia (USP) <88> *(in vivo)* specification.

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).

2.2.1 Acute Systemic Toxicity Injection Tests

An Acute Systemic Toxicity 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.

2.2.2 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 previously were used for these tests.

2.2.3 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 material components of the filter capsules in contact with the fluid pathway was implanted separately.

2.3 Results

Pall **Kleenpak** Water Filter passed all of the tests specified.

2.4 Conclusions

All materials of construction of Pall **Kleenpak** Water Filters that are in contact with the fluid pathway meet the requirements of the US Pharmacopoeia Class VI <88> *(in vivo)* specification.

Part V Evaluation of Shelf Life

1.1 Introduction

The purpose of this evaluation was to confirm the shelf-life of Pall **Kleenpak** Water Filters following gamma irradiation. Results of analyses from other Pall products of identical material components were used.

1.2 Summary of Methods

A number of filter capsules that had been gamma irradiated were stored in their original packaging at room temperature for a set real time and then subjected to a range of tests in order to confirm that the performance of the product had not degraded over time.

The tests performed on samples of the stored products were as follows:

- Integrity of packaging
- Liquid bacterial challenge testing using *Brevundimonas diminuta* (ATCC 19146)
- Burst pressure testing

The packaging of the stored product was inspected prior to other tests being performed. Other tests performed on stored product were conducted as described previously.

1.3 Results

1.3.1 Integrity of Packaging

The packaging used for Pall **Kleenpak** Water Filters was found to be intact on product that had been stored for up to 36 months.

1.3.2 Bacterial Challenge

Following gamma irradiation, filter capsules retained *B.diminuta* after 36 months storage.

1.3.3 Burst Pressure Testing

Following gamma irradiation, all filter capsules retained integrity and maintained a safety margin over maximum operating pressure after 36 months storage.

1.4 Conclusions

Pall **Kleenpak** Water Filters can be stored for up to 36 months following gamma irradiation before use.

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