Life Sciences

Validation Guide

USTR 1650(2)

Pall® 0.1µm-rated Fluorodyne® II DJL Filters

Filtration. Separation. Solution.SM

CONTENTS

Part I. Overview

1. Introduction

This report contains validation data applicable to **Pall** microbially-rated 'P' grade **Fluorodyne** II DJL membrane filter cartridges. It is designed to assist the filter user in meeting the validation requirements of regulatory authorities within the pharmaceutical industry.

The validation program included:

- **Correlation of Forward Flow integrity test values with the removal of *Brevundimonas diminuta* (ATCC 19146)**
- **Liquid challenge tests with mycoplasma using *Acholeplasma laidlawii* (ATCC 23206)**
- **Determination of the effects of steam sterilization on filter integrity**
- **Testing temperature and pressure limits**
- **Extractables testing using water and ethanol**
- **Biological reactivity tests**
- **Determination of protein transmission characteristics**

2. Summary of Conclusions

(I) Bacterial Removal and Integrity Test Parameters

Fluorodyne II DJL filters were demonstrated to be sterilizing grade, as determined by liquid bacterial challenge tests using *B. diminuta,* in accordance with the FDA guidelines on Sterile Products produced by Aseptic Processing (1987).

Forward Flow integrity test parameters, correlated to liquid bacterial challenge tests, were set as follows for **Fluorodyne** II DJL filters (part number AB1DJL7PH4):

Test pressure	4475 mbar (65 psi)
Wetting liquid	Water
Temperature	20°C (68°F) ± 5°C
Test gas	Air
Maximum allowable Forward Flow limit	29 mL/min

(II) Removal of Mycoplasma

Fluorodyne II DJL filters have been shown to retain high levels of mycoplasma, as validated using *A. laidlawii*. Typical titer reductions were found to be in the order of 10^8.

(III) Steam Sterilization Tests

Fluorodyne II DJL filters have been shown to retain integrity after repeated one hour steam in place cycles at 125°C (257°F) and 142°C (288°F), as follows:

125°C (257°F)	60 hours
142°C (288°F)	10 hours

(IV) Maximum Temperature and Pressure Ratings

Fluorodyne II DJL filters (part number AB1DJL7PH4) were found to retain integrity after exposure to the following conditions:

Flow Direction	Differential Pressure at Temperature
Forward	5.3 bar (77 psi) at 50°C (122°F) 3.4 bar (49 psi) at 80°C (176°F)
Reverse	1 bar (15 psi) at 40°C (104°F)

(V) Extractables Testing using Water and Ethanol

Aqueous extractables were found to be extremely low for **Fluorodyne** II DJL filters, in the range of 1.2 - 2.1 mg per 25 cm (10") module (part number AB1DJL7PH4). Ethanol extractables were found to be in the range of 131 - 166 mg per 25 cm (10") module.

(VI) Biological Reactivity Tests

Fluorodyne II DJL filters were found to meet the requirements of the USP for Class VI (121°C) Plastics.

(VII) Protein Transmission Characteristics

Protein transmission studies were performed by passing dilute solutions of radio-labelled IgG through discs of **Fluorodyne** II DJL filter membrane. In all cases, the transmission of IgG was found to be in excess of 98%.

Part II. Microbial Validation Studies

1. Microbial Validation using *Brevundimonas diminuta*

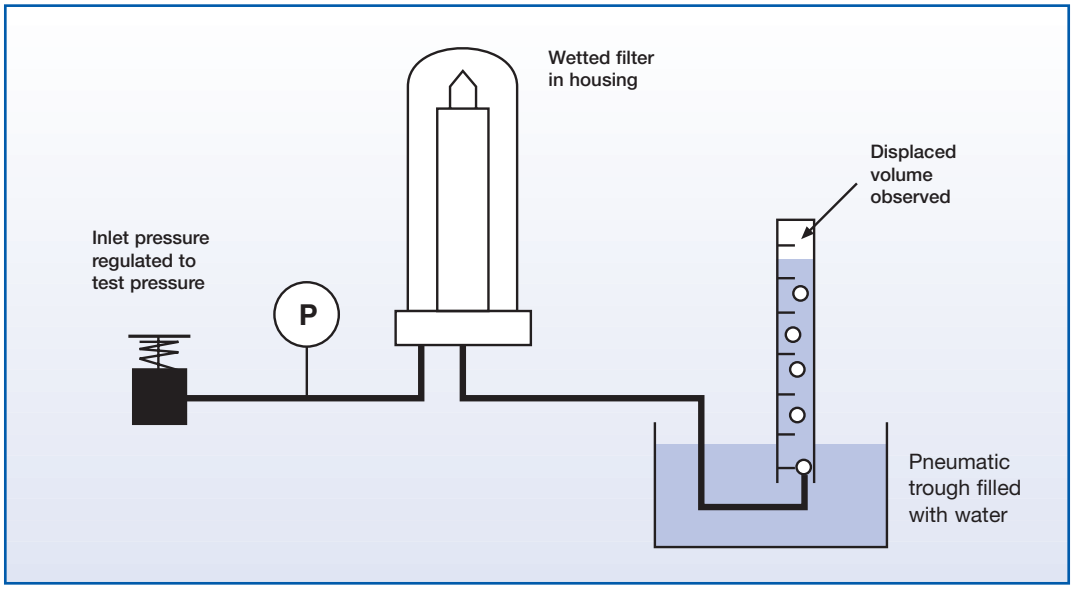
1.1 Introduction

Liquid challenge tests using *B. diminuta* (ATCC 19146) were performed with **Fluorodyne** II DJL filter cartridges using a minimum of 1 x 10^7 colony forming units (CFU)/cm^2 of effective filtration area.

The correlation between microbial retention and a non-destructive integrity test is an important aspect of the validation of sterilizing grade filters. The FDA guidelines on Sterile Products Produced by Aseptic Processing (1987) state, 'After a filtration process is properly validated for a given product, process and filter, it is important to assure that identical filter replacements (membrane or cartridge) used in production runs will perform in the same manner. One way of achieving this is to correlate filter performance data with filter integrity testing data'. The integrity test used during this validation study was the Forward Flow integrity test.

In order to perform the Forward Flow test, a filter is wetted with a suitable 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 downstream side (as shown in Figure II-1) or on the upstream side, using sensitive flow measurement equipment such as the Palltronic® TruFlow or **Palltronic** Flowstar filter integrity test devices.

Figure II-1 The Manual Forward Flow Integrity Test



In summary, the aims of this stage of the validation were to:

- **Determine the microbial removal efficiency of Fluorodyne II DJL filters in liquid challenge tests**
- **Correlate non-destructive integrity tests with destructive challenge tests**
- **Determine integrity test parameters**

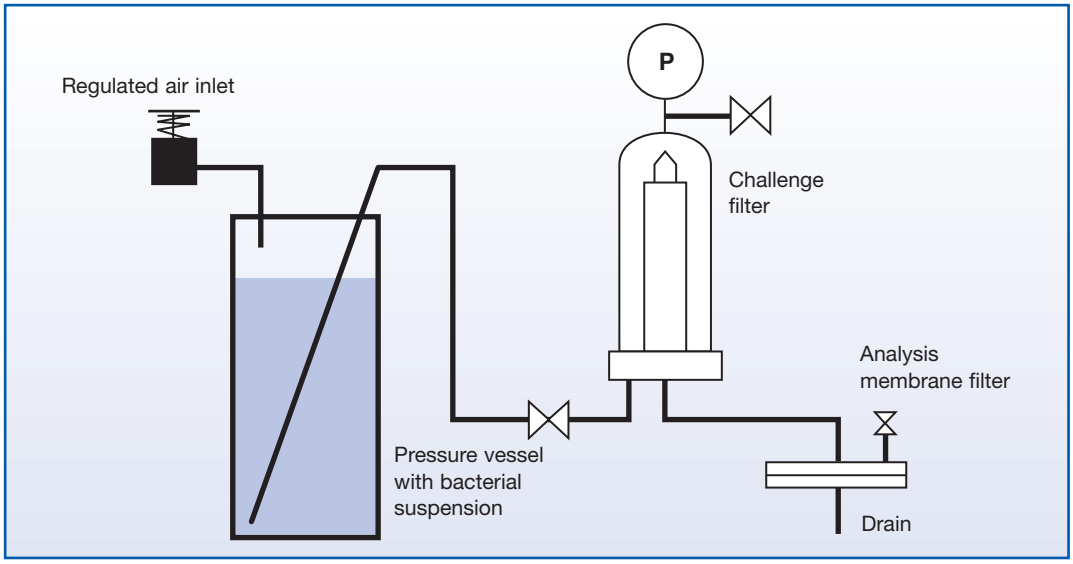
1.2 Summary of Methods

Fluorodyne II DJL filters (part number AB1DJL7PH4) with a range of Forward Flow values were selected from manufacturing lots and subjected to microbial challenge tests using an aqueous suspension of *B. diminuta.*

Each filter sample was installed in a housing and tested for integrity by the Forward Flow method, prior to being autoclaved at 121°C (250°F) for 60 minutes. The filter assembly was then aseptically connected to the pre-sterilized challenge apparatus, as shown in Figure II-2.

An aqueous suspension of *B. diminuta* was passed through the filter to achieve a challenge level of ≥ 1 x 10^7 colony forming units (CFU) per cm^2 of effective filtration area. A total challenge per filter of > 1 x 10^{10} CFU was achieved in all tests. On completion of the challenge a second Forward Flow test was performed.

Figure II-2 Microbial Challenge Apparatus



During the challenge test the entire filter effluent was passed through a 0.2 µm-rated analysis disc on the downstream side of the test filter assembly. The filter disc was incubated on agar and, following incubation, the disc was examined to determine if any colonies had grown, indicating whether or not bacteria had passed through the test filter during the challenge. The titer reduction (T_R) for each filter was determined as follows:

$$T_R = \frac{\text{Total number of organisms influent to the filter}}{\text{Number of colonies recorded on the downstream analysis disc}}$$

When no colonies were detected downstream, the titer reduction was expressed as: > total number of organisms influent to the filter (e.g. >1 x 10^{10}).

Please contact Pall if a more detailed description of the test methods is required.

1.3 Results

The data obtained from this validation study are shown in Table II-1 in order of increasing Forward Flow. The higher of the two Forward Flow values are presented in the table.

All of the filters tested, with Forward Flow values up to 37.1 mL/min, gave sterile effluent when challenged with *B. diminuta* at a challenge level of > 1 x 10^7 CFU per cm^2 of effective filter area.

Table II-1 Correlation of Forward Flow with *B. diminuta* retention using AB1DJL7PH4 Filters

Filter Serial Number	Forward Flow* (mL/min)	Sterile Effluent	Titer Reduction
IB9497002	18.5	Yes	> 9.4×10^{10}
IB9524054	19.0	Yes	> 3.3×10^{10}
IB9497040	19.5	Yes	> 1.3×10^{11}
IB9497007	20.5	Yes	> 1.1×10^{11}
IB9497016	20.6	Yes	> 1.5×10^{11}
IB9497021	20.6	Yes	> 1.4×10^{10}
IB9497037	21.5	Yes	> 1.1×10^{11}
IB9524078	21.8	Yes	> 3.8×10^{10}
IB9524001	21.9	Yes	> 3.3×10^{10}
IB9497027	22.2	Yes	> 2.2×10^{10}
IB9524086	22.4	Yes	> 6.8×10^{10}
IB9497023	22.5	Yes	> 4.5×10^{10}
IB9524013	22.6	Yes	> 7.5×10^{10}
PB454095	22.6	Yes	> 1.8×10^{11}
IB9524038	22.8	Yes	> 1.8×10^{11}
IB9524050	23.0	Yes	> 1.4×10^{10}
IB9524018	23.2	Yes	> 8.7×10^{10}
IB9497005	23.4	Yes	> 1.1×10^{11}
IB9524029	23.5	Yes	> 1.9×10^{10}
IB9497033	23.6	Yes	> 1.6×10^{11}
IB9497055	23.9	Yes	> 8.8×10^{10}
IB9524079	24.3	Yes	> 1.2×10^{10}
IB9524031	24.5	Yes	> 4.7×10^{10}
IB9524066	24.8	Yes	> 1.2×10^{11}
IB9497066	24.8	Yes	> 1.8×10^{11}
PB454094	25.9	Yes	> 1.8×10^{11}
PB454122	27.3	Yes	> 1.5×10^{11}
PB454138	30.1	Yes	> 1.6×10^{11}
PB454133	34.1	Yes	> 1.5×10^{11}
PB454105	34.8	Yes	> 1.7×10^{11}
PB454013	37.1	Yes	> 1.4×10^{11}

* Forward Flow values measured at 4475 mbar (65 psi) air test pressure, filters wet with water

1.4 Conclusions

Based on the results of the validation study, the Forward Flow test was shown to be a suitable non-destructive integrity test for **Fluorodyne** II DJL filters. Forward Flow test parameters for **Fluorodyne** II DJL filters (part number AB1DJL7PH4) were set as follows:

Test pressure	4475 mbar (65 psi)
Wetting liquid	Water
Temperature	20°C (68°F) ± 5°C
Test gas	Air
Maximum allowable Forward Flow limit	29 mL/min

These test parameters include a safety margin based on the results in Table II-1. The validity of Forward Flow values are continually reviewed and monitored during routine production tests. Test values are issued and controlled by Pall Scientific and Laboratory Services.

Please contact Pall for further details.

1.5 Microbial Validation of Other Filter Styles

During the development of other styles of **Fluorodyne** II DJL filters, production samples were subjected to bacterial challenge tests and Forward Flow integrity tests as described earlier in section 1.2.

The bacterial challenge and Forward Flow results are shown in Table II-2. The higher of the two Forward Flow values are presented.

Table II-2 Bacterial Challenge and Forward Flow Results obtained using Other Filter Styles

Filter Part Number	Filter Serial Number	Forward Flow (mL/min)	Sterile Effluent	Titer Reduction
AB05DJL2PH4	IC2132099	10.2	Yes	> 6.9×10^{10}
	IC2132112	11.4	Yes	> 5.0×10^{10}
	IC2132030	11.8	Yes	> 4.1×10^{10}
	IC2132032	11.9	Yes	> 7.2×10^{10}
	IC2132110	11.9	Yes	> 8.1×10^{10}
MCY4440DJLPH4	IC2353038	5.9	Yes	> 2.3×10^{10}
	IC2353004	6.1	Yes	> 3.9×10^{10}
	IC2353013	6.3	Yes	> 2.6×10^{10}
	IC2353029	6.3	Yes	> 2.8×10^{10}
	IC2834084	6.3	Yes	> 4.6×10^{10}
	IC2834077	6.4	Yes	> 2.6×10^{10}
	IC2353034	6.5	Yes	> 3.6×10^{10}
	IC2353019	6.7	Yes	> 2.6×10^{10}
	IC2834030	7.0	Yes	> 1.1×10^{11}
	IC2353007	7.2	Yes	> 3.6×10^{10}
	IC2834068	7.4	Yes	> 7.1×10^{10}
	IC2834007	9.2	Yes	> 2.1×10^{10}
	IC2834034	9.6	Yes	> 3.2×10^{10}

* Forward Flow values measured at 4475 mbar (65 psi) air test pressure, filters wet with water

Based on the core validation of 25 cm (10") **Fluorodyne** II DJL filters (Table II-1), and subsequent testing of other filter styles, Forward Flow test parameters were set as follows for the other filter styles:

Filter Part Number	Wetting Liquid*	Air Test Pressure	Forward Flow Limit
AB05DJL2PH4	Water	4475 mbar (65 psi)	14.5 mL/min
MCY4440DJLPH4	Water	4475 mbar (65 psi)	8.2 mL/min

* Temperature 20°C (68°F) ± 5°C

2. Microbial Challenge Tests using *Acholeplasma laidlawii*

2.1 Introduction

There is no standard organism for validating 0.1 µm filters, however mycoplasma species have often been used because of their small size and deformable nature. Mycoplasma lack a rigid cell wall and are bounded only by a plasma membrane, the cells are pleomorphic and are known to penetrate 0.2 µm-rated filters.

The aim of this stage of the validation was to determine the typical removal efficiency of **Fluorodyne** II DJL filters using the mycoplasma, *A. laidlawii.*

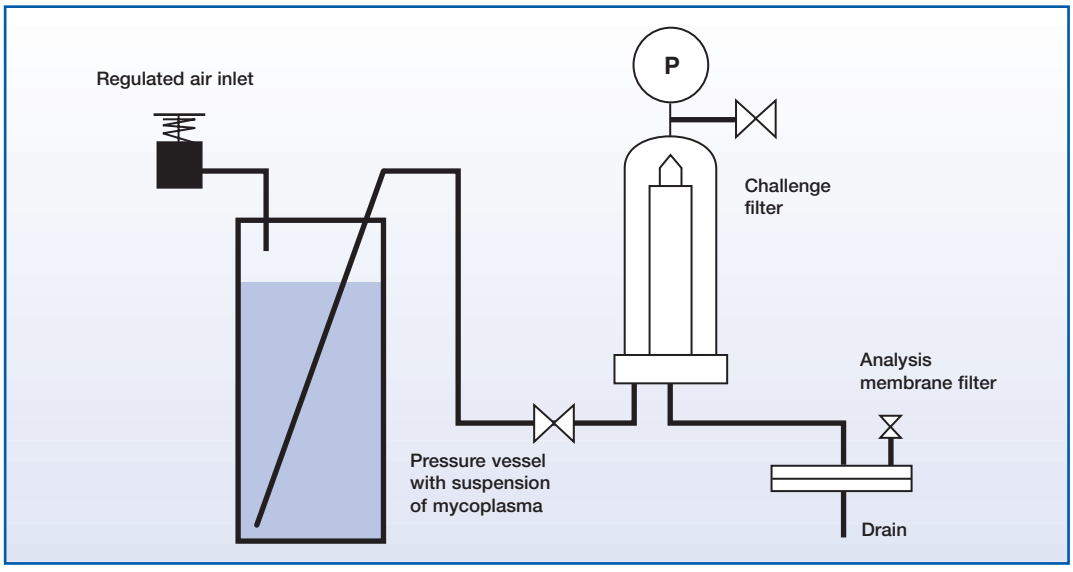
2.2 Summary of Methods

Fluorodyne II DJL filters (part number AB1DJL7PH4) with a range of Forward Flow values were selected from standard manufacturing lots and subjected to challenge tests using an aqueous suspension of *A. laidlawii* (ATCC 23206).

Each filter sample was installed in a housing and tested for integrity using the Forward Flow method, prior to being autoclaved at 121°C (250°F) for 60 minutes. The filter assembly was aseptically connected to the pre-sterilized challenge apparatus, as shown in Figure II-3.

An aqueous suspension of *A. laidlawii* was passed through the filter at a challenge level of > 1 x 10^7 CFU per cm^2 of effective filtration area. On completion of the challenge a second Forward Flow test was performed.

Figure II-3 Mycoplasma Challenge Apparatus



During the challenge test the entire filter effluent was passed through a 0.1 µm-rated analysis disc on the downstream side of the test filter assembly. The filter disc was incubated on agar and, following incubation, the disc was examined to determine if any mycoplasma had passed through the test filter during the challenge. The titer reduction (TR) for each filter was determined as follows:

$$T_R = \frac{\text{Total number of organisms influent to the filter}}{\text{Number of colonies recorded on the downstream analysis disc}}$$

Please contact Pall if a more detailed description of the test methods is required.

2.3 Results

The mycoplasma challenge and Forward Flow test results are shown in Table II-3. The higher of the two Forward Flow values are presented.

Table II-3 Results of *A. laidlawii* Challenge Tests using AB1DJL7PH4 filters

Filter Serial Number	Forward Flow* (mL/min)	Titer Reduction
IB9497009	22.5	6.1×10^{8}
IB9524028	22.5	1.8×10^{9}
IB9497054	24.0	5.4×10^{8}
IB9524060	24.5	2.2×10^{8}
PB4540149	25.0	1.0×10^{9}
IB9497057	25.0	6.7×10^{7}
IB9524022	26.0	3.5×10^{9}
PB4540081	26.1	2.9×10^{8}

* Forward Flow values measured at 4475 mbar (65 psi) air test pressure, filters wet with water

2.4 Conclusions

Users of **Fluorodyne** II DJL filters can expect to obtain high titer reductions of mycoplasma, typically in the region of 10^{8} as verified using *A. laidlawii*.

Part III. Validation of Physical Characteristics

1. Resistance to Steam Sterilization

1.1 Introduction

The aim of these validation tests was to determine the resistance of **Fluorodyne** II DJL filters to steam in place sterilization.

1.2 Summary of Methods

Typical **Fluorodyne** II DJL filters (part number AB1DJL7PH4) were subjected to repeated steam in place cycles at 125°C (257°F) and 142°C (288°F). During the tests, filters were installed in stainless steel filter housings and exposed in line to saturated condensate-free steam. Steam pressure and flow were held constant during the sterilization period and after each steam cycle, the filters were cooled by passing dry compressed air through them. The differential pressure during the tests was controlled and maintained at < 300 mbar (4.35 psi).

Following exposure to steam, the integrity of the filters was measured using the Forward Flow test. In some cases, a bacterial challenge test using *B. diminuta* was also performed (according to the method described earlier in Part II).

A summary of the test conditions is shown in Table III-1.

Table III-1 Steam Sterilization Test Conditions

Steam Temperature	Number of One Hour Steam Cycles	Measurement of Filter Integrity after Exposure to Steam
125°C (257°F)	30	Forward Flow and bacterial challenge using *B. diminuta*
125°C (257°F)	60	Forward Flow
142°C (288°F)	5	Forward Flow and bacterial challenge using *B. diminuta*
142°C (288°F)	10	Forward Flow

Please contact Pall if a more detailed description of the test methods is required.

1.3 Results

1.3.1 Steam Sterilization Tests at 125°C (257°F)

The Forward Flow and bacterial challenge test results obtained after steaming at 125°C (257°F) are shown in Tables III-2 and III-3.

Table III-2 Forward Flow and Bacterial Challenge Results after exposure to 30 one hour cycles at 125°C (257°F)

Filter Serial Number	Forward Flow (mL/min)*		Sterile Effluent when challenged with *B.diminuta***
	Initial Values	After exposure at 30 x 1 hour cycles	
IB9524005	17.3	22.0	Yes
IB9524017	19.5	21.8	Yes
IB9524056	18.1	21.4	Yes
IB9524057	18.4	20.4	Yes
IB9524070	18.1	21.3	Yes

* Forward Flow air test pressure 4475 mbar (65 psi), filters wet with water, maximum allowable limit 29 mL/min

** After 30 hours accumulated exposure to steam, challenge level ≥1 x 10^7 CFU/cm^2

Table III-3 Forward Flow before and after 60 one hour cycles at 125°C (257°F)

Filter Serial Number	Forward Flow (mL/min)*	
	Initial Values	After exposure to 60 x 1 hour cycles
IB9497039	22.1	22.8
IB9497058	18.5	22.7
IB9497062	18.6	22.2
IB9497067	21.3	23.9
IB9497068	18.8	27.4

* Forward Flow air test pressure 4475 mbar (65 psi), filters wet with water, maximum allowable limit 29 mL/min

1.3.2 Steam Sterilization Tests at 142°C (288°F)

The Forward Flow and bacterial challenge test results obtained after steaming at 142°C (288°F) are shown in Tables III-4 and III-5.

Table III-4 Forward Flow and Bacterial Challenge Results after exposure to 5 one hour cycles at 142°C (288°F)

Filter Serial Number	Forward Flow (mL/min)*		Sterile Effluent when challenged with *B.diminuta***
	Initial Values	After exposure at 5 x 1 hour cycles	
IB9524008	21.0	20.0	Yes
IB9524012	21.5	19.0	Yes
IB9524021	19.5	20.5	Yes
IB9524046	20.0	19.5	Yes
IB9524062	19.5	20.0	Yes

* Forward Flow air test pressure 4475 mbar (65 psi), filters wet with water, maximum allowable limit 29 mL/min

** After 2 hours accumulated exposure to steam, challenge level ≥ 1 x 10^7 CFU/cm^2

Table III-5 Forward Flow before and after 10 one hour cycles at 142°C (288°F)

	Forward Flow (mL/min)*	
Filter Serial Number	Initial Values	After exposure to 10 x 1 hour cycles
IB9497049	21.0	20.0
IB9497059	18.5	21.0
IB9497060	23.0	16.0

* Forward Flow air test pressure 4475 mbar (65 psi), filters wet with water, maximum allowable limit 29 mL/min

1.4 Conclusions

Fluorodyne II DJL filters retained integrity after repeated steam in place cycles in the forward direction at 125°C (257°F) and 142°C (288°F). These data demonstrate that **Fluorodyne** II DJL filters are resistant to cumulative steam exposure typical of process conditions.

2. Validation of Temperature and Pressure Limits

2.1 Introduction

The aim of these tests was to determine the resistance of **Fluorodyne** II DJL filters to temperature and pressure.

2.2 Summary of Methods

Tests were performed on typical production filters. The filters were installed in a stainless steel housing and purposefully blocked with an aqueous suspension of standard test dust in order that set differential pressures could be maintained across the filter elements. Upstream water pressures were applied and held constant for 30 minutes. The temperature was controlled by immersing the whole assembly in a heated water bath. Forward Flow tests were performed before and after each test.

The test conditions used were as follows:

Flow Direction	Differential Pressure at Temperature
Forward	5.3 bar (77 psi) at 50°C (122°F) 3.4 bar (49 psi) at 80°C (176°F)
Reverse	1 bar at (15 psi) 40°C (104°F)

Please contact Pall if a more detailed description of the test methods is required.

2.3 Results

Forward Flow results of filters exposed to differential pressures in the forward and reverse flow directions are shown in Tables III-6 to III-8.

Table III-6 Forward Flow after exposure to 5.3 bar (77 psi) Differential Pressure in the forward direction at 50°C (122°F)

Filter Serial Number	Initial Forward Flow* (mL/min)	Forward Flow* (mL/min) after exposure to Differential Pressure
IB9524042	23.0	22.0
IB9524059	22.0	21.0
IB9524072	23.0	25.0

* Forward Flow air test pressure 4475 mbar (65 psi), filters wet with water, maximum allowable limit 29 mL/min

Table III-7 Forward Flow after exposure to 3.4 bar (49 psi) Differential Pressure in the forward direction at 80°C (176°F)

Filter Serial Number	Initial Forward Flow* (mL/min)	Forward Flow* (mL/min) after exposure to Differential Pressure
IB9497017	22.5	20.0
IB9497069	21.0	27.0
IB9497073	16.0	13.0

* Forward Flow air test pressure 4475 mbar (65 psi), filters wet with water, maximum allowable limit 29 mL/min

Table III-8 Forward Flow after exposure to 1 bar (15 psi) Differential Pressure in the reverse direction at 40°C (104°F)

Filter Serial Number	Initial Forward Flow* (mL/min)	Forward Flow* (mL/min) after exposure to Differential Pressure
PILF4844159	18.0	20.0
IB9497076	26.0	22.0
IB9497078	24.0	20.0

* Forward Flow air test pressure 4475 mbar (65 psi), filters wet with water, maximum allowable limit 29 mL/min

2.4 Conclusions

These data indicate that **Fluorodyne** II DJL filters can withstand high differential pressures in both the forward and reverse directions.

Part IV. Extractables, Biological Safety and Protein Transmission

1. Extractables Testing of Fluorodyne II DJL Filters

1.1 Introduction

The aim of this series of tests was to quantify and characterize the material which can be extracted from **Fluorodyne** II DJL filters by water and ethanol.

1.2 Summary of Methods

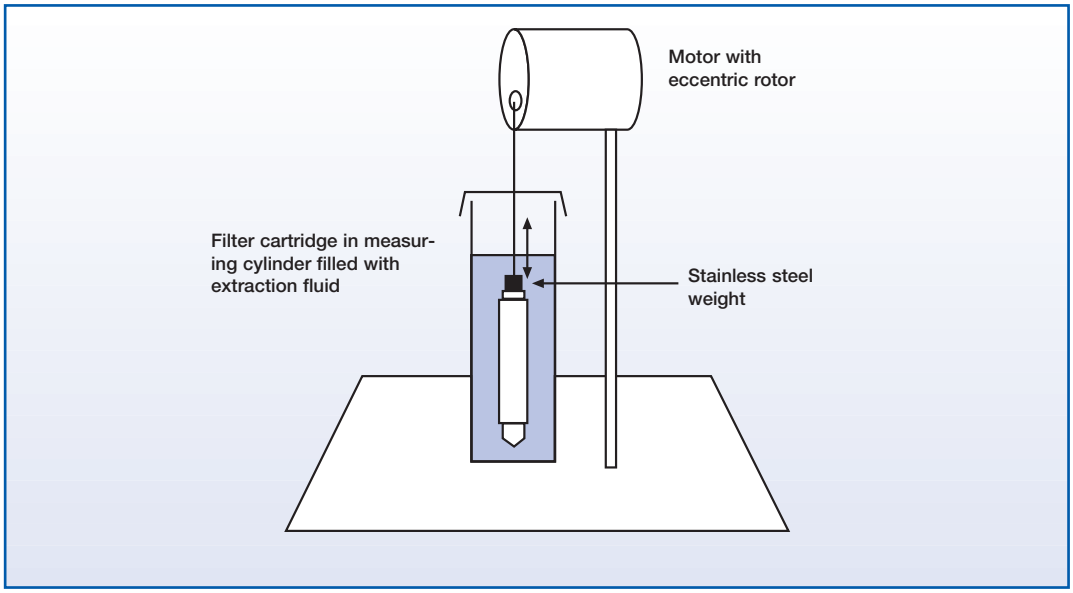
Preparation of Filter Samples

Extractables tests were performed on typical production filter cartridges that had been autoclaved in order to maximize the quantity of any extractable material present. The filters were wrapped in aluminium foil and autoclaved for one hour at 121°C (250°F), using a slow exhaust cycle. Visible droplets of water remaining on the filter elements were allowed to evaporate at room temperature before the extraction was performed.

Extraction Procedure

Dynamic extraction tests were performed in water and ethanol. The test filters were immersed in 1500 mL of extraction fluid in a clean measuring cylinder, as shown in Figure IV-1. For four hours the filter was gently moved up and down. This movement created flow through the filter membrane as a result of the pressure head that was created each time the element was partially lifted out of the liquid.

Figure IV-1 Filter Extraction Apparatus



Analysis of Material Extracted

After the extraction, 1000 mL of the extraction liquid was evaporated to dryness and the non-volatile extractables were determined gravimetrically. The ethanol extractables were also analysed by Fourier Transform Infra Red Spectroscopy (FTIR).

Please contact Pall if a more detailed description of the test methods is required.

1.3 Results

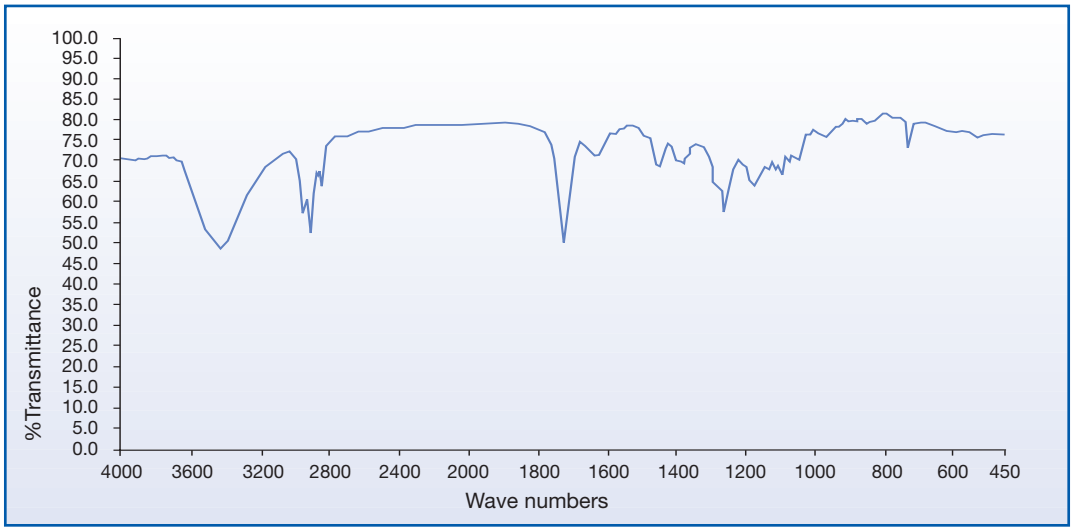
Table IV-1 shows the levels of aqueous and ethanol extractables obtained using typical production **Fluorodyne** II filters (part number AB1DJL7PH4).

Table IV-1 Non-volatile Aqueous and Ethanol extractables obtained using 25 cm (10") filters

Extraction Fluid	Filter Serial Number	Residue
Water at 20°C (68°F)	PB4540092	2.1 mg
	PB4540144	2.0 mg
	PB4540161	1.2 mg
Ethanol at 20°C (68°F)	IB9524025	160 mg
	IB9524058	131 mg
	IB9524073	166 mg

Infra red spectra (Figure IV-2) of ethanol extracts from **Fluorodyne** II DJL filters indicate the presence of extractables typical of polypropylene resins (stearate salts or esters, antioxidant and small amounts of polypropylene oligomers) and of the acrylate copolymer used to render the membrane hydrophilic.

Figure IV-2. Infra Red Spectrum of Ethanol Extract from Fluorodyne II DJL filters



1.4 Conclusions

The levels of aqueous extractables determined for **Fluorodyne** II DJL filters are extremely low, and the results reported are typical for production elements.

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

2. Biological Safety Tests on Components of Fluorodyne II DJL Filter Cartridges

2.1 Introduction

The purpose of this study was to evaluate the biological suitability of the materials of construction of **Fluorodyne** II DJL filters. The materials of construction are as follows:

Filter membrane:	Double layered Pall hydrophilic PVDF
Support/drainage layers:	Natural unpigmented polypropylene homopolymer
Core, endcaps and adapter:	Natural unpigmented polypropylene homopolymer
Cage:	Polypropylene homopolymer with titanium dioxide filler

2.2 Summary of Methods

The tests were performed in accordance with the Biological Reactivity Tests *in vivo* for Class VI Plastics (121°C (250°F)) as described in the current United States Pharmacopeia.

The tests were conducted by Gibraltar Laboratories Inc., Fairfield, New Jersey.

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 (122°F) for 72 hours, 70°C (158°F) for 24 hours, or 121°C (250°F) for 1 hour. The most stringent condition not resulting in physical changes in the plastic is recommended, therefore the filters were extracted at 121°C (250°F).

Acute Systemic Injection Tests

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

Intracutaneous Tests

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

Implantation Tests

Implantation tests were also performed, in order to subject the materials of construction to the most stringent conditions included in the USP. Each of the components of the **Fluorodyne** II DJL membrane filter cartridges were implanted separately.

2.3 Results

The **Fluorodyne** II DJL membrane filter cartridges passed all of the tests specified. Please see Appendix 1 for copies of the test reports.

2.4 Conclusions

Fluorodyne II DJL membrane filter cartridges meet the requirements of the USP for Class VI (121°C) Plastics *(in vivo)*.

3. Protein Transmission Characteristics

3.1 Introduction

The aim of these tests was to determine the typical protein transmission characteristics through **Fluorodyne** II DJL filters using a dilute solution of immunoglobulin IgG.

3.2 Summary of Methods

A solution of ^{125}I-labelled IgG was prepared in phosphate buffered saline (pH 7). The total protein concentration was 100 µg/mL. The solution was filtered through discs of **Fluorodyne** II DJL filter membrane (13 mm (0.5") diameter) at a flow rate of 0.5 mL/min, until a total of 250 µg of protein had passed through each sample. Excess solution was carefully blotted from the surface of the discs and the amount of ^{125}I-labelled protein retained by each disc was determined using a gamma counter. Protein transmission was calculated by subtracting the amount of protein on the filter disc from the total that had been passed through the filter.

Please contact Pall if a more detailed description of the test methods is required.

3.3 Results

The transmission of IgG through **Fluorodyne** II DJL filter membrane is shown in Table IV-2. In all cases the measured protein transmission was in excess of 98%.

Table IV-2 Transmission of IgG through Fluorodyne II DJL Filter Membrane

Filter Sample	Protein Transmission
Membrane 1	98.1 %
Membrane 2	98.4 %
Membrane 3	98.5 %
Membrane 4	98.4 %

3.4 Conclusions

Fluorodyne II DJL filters exhibit very high transmission characteristics with dilute solutions of IgG, and can therefore be used to filter dilute protein solutions to maximize product recoveries.

Appendix 1

Independent Report on Biological Reactivity of **Fluorodyne** II DJL Filter Cartridges

GIBRALTAR LABORATORIES, INC.

Quality research & regulatory testing services since 1970

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ACCREDITED

REPORT No.
G-96206
04/01/97
1 of 3

LABORATORY REPORT

FINAL REPORT

Sponsor: (662)
Pall Corporation Technical Center
25 Harbor Park Drive
Port Washington,NY,11050-4630
Attn: Janet Mathus
Purchase Order #: 409757

GBL Ref.: 1156-196-5800
GBL Sample No.:87333/1.30
and 87333/1-3.127
Lot #1:IA9816 036 DJL
Date Received: 03/17/97
Date Tested: 03/19/97
Date Completed: 03/27/97

USP 23 CLASS VI on AB1DJL7PH4 Filter
Interim Report Activity:

Description:
One cartridge filter identified as: "AB1DJL7PH4 Filter".
Note: Perform testing on each of the following components of the filter after cutting it apart:

Item #	Description
/1 -	Hydrophilic Polyvinylidene Fluoride (PVDF) Filter Medium
/2 -	Polypropylene support layer
/3 -	Polypropylene endcaps, core or adaptor
/4 -	Polypropylene cage containing 1% white pigment

1 Purpose
To determine the reaction of living, normal animals to the presence of extracts or portions of the test material.

2 Test System
2.1 New Zealand albino rabbits, either sex, 2.00 to 3.09 kg. Eight for intracutaneous injection, eight for implantation.

2.2 Swiss Webster albino mice, either sex 19.6 to 23.8 grams. Five test and control (intravenous and intraperitoneal injection).

Respectfully Submitted,
GIBRALTAR LABORATORIES, INC.

Date
Written: 04/01/97
Analyst: 41, 117, 123

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

AUDIT 4-4-97 GBL

LABORATORY REPORT

3 Method

Test Material Preparation and Extraction

3.1 Solvents

3.1.1 USP Sodium Chloride for Injection (Saline)
3.1.2 5% Ethanol in Sodium Chloride (ETOH/Saline)
3.1.3 Cottonseed Oil (CSO)
3.1.4 Polyethylene glycol 400 (PEG)

3.2 One unit of the test material was cut into sections using a heated scalpel blade.

3.3 For the saline, ethanol and saline and cottonseed oil extracts, the following ratios of material to solvents were used: 30 cm^2 each of Hydrophilic Polyvinylidene Fluoride (PVDF) Filter Medium and Polypropylene support layer and between 0.96 and 1.03 grams each of Polypropylene endcaps, core or adaptor and Polypropylene cage containing 1% white pigment. The above materials were placed in 20 mL of each solvent.

3.4 For the PEG 400 extract, the following ratio of material to solvent was used: 15 cm^2 each of Hydrophilic Polyvinylidene Fluoride (PVDF) Filter Medium and Polypropylene support layer and between 0.51 and 0.53 grams each of Polypropylene endcaps, core or adaptor and Polypropylene cage containing 1% white pigment. The above materials were placed in 10 mL of PEG 400.

3.5 Extraction Conditions: 121C for one hour

3.6 Dosing Procedures

3.6.1 IC Injection - three day observation period
3.6.2 Systemic Injection (IV and IP) - three day observation period
3.6.3 Intramuscular Implantation - seven day observation period

4 Results: See Tables 1 to 3.

Table 1: Intracutaneous Irritation

Extract	Test Score	Control Score	Difference
Saline	(0.00)	(0.00)	(0.00)
ETOH/Saline	(0.00)	(0.00)	(0.00)
CSO	(0.00)	(0.00)	(0.00)
PEG 400	(0.00)	(0.00)	(0.00)

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Table 2: Systemic Toxicity

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

Table 3: Intramuscular Implantation

*GBL Lab#	Test Score	Average Control Score	Difference
/1	(0.50)	(0.00)	(0.50)
/2	(0.38)	(0.00)	(0.38)
/3	(0.25)	(0.00)	(0.25)
/4	(0.25)	(0.00)	(0.25)

*See Description on page 1.

5 Conclusion

The test material conformed to the requirements of this test.

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03.2004