BioPharmaceuticals

Validation Guide

USTR 2099

Validation Guide for Pall 'Kleenpak' Nova Filter Capsules

*Filtration. Separation. Solution.*SM

Contents

1. Validation Overview

1.1 Introduction

This report contains data applicable to 'Pall' 'Kleenpak' Nova filter capsules. These filter capsules have integrally moulded inlet and outlet connections and precision-moulded vent and drain fittings. The purpose of this report is to document the testing that has been performed to demonstrate the strength and suitability of the capsules for use in pharmaceutical filtration applications.

Kleenpak Nova filter capsules incorporate standard 25cm (10") **Pall** filter cartridges and are available with a number of different membrane types. Information about the validation of the different **Pall** filter membranes is available in separate validation guides.

Kleenpak Nova filter capsules with a 'G' designation in the part number are provided non-sterile. Depending on the filter membrane incorporated in the filter capsules, some versions are suitable for gamma-irradiation by the user. Further details about the tolerance of different **Pall** membranes to gamma-irradiation are available from your local Pall Sales company or distributor.

1.2 Summary of conclusions

Burst testing

Forty-nine **Kleenpak** Nova capsules were burst pressure tested and in all cases the burst pressures were ≥ 16.6 bar (240 psi), thereby fulfilling the required 5:1 safety ratio between burst pressure and the maximum recommended operating pressure of 3 bar (43.5 psi). This testing included untreated samples, samples that had been exposed to repeated autoclave cycles at 125°C and samples that had been exposed to gamma-irradiation at doses of approximately 35 and 50 kGy.

Creep rupture testing

Kleenpak Nova filter capsules have been designed to be capable of operating at up to 3 bar (43.5 psi) for 168 hours (1 week) in continuous use. In order to allow for integrity testing the capsules have also been designed to be capable of operating at up to 5.9 bar (85 psi) for a maximum of 10 hours. The creep-rupture data presented using typical **Kleenpak** Nova capsules demonstrates the very large safety margins that have been incorporated into these pressure claims.

Pressure fatigue testing

Kleenpak Nova filter capsules can withstand repeated pressurisation cycles, as demonstrated by fatigue testing where typical capsules were exposed to 100,000 pressure cycles from 0 to 3 barg (43.5 psi) and back to 0 barg again.

Environmental testing

The data presented provide assurance that **Kleenpak** Nova filter capsules will not be damaged by extremes in temperature that they could potentially be subjected to during storage or transit.

Filter/capsule seal testing

The integrity of the filter/housing seal of **Kleenpak** Nova filter capsules has been demonstrated by performing Forward Flow integrity tests and liquid bacterial challenge tests on filter samples that have been exposed to either autoclave sterilisation or gamma-irradiation at doses of approximately 35 or 50 kGy.

Water flow characteristics

The water flow / pressure drop data presented for empty **Kleenpak** Nova filter capsules can be used in conjunction with the pressure drop characteristics of standard 25cm (10") **Pall** filter cartridges to form the basis for sizing filter systems employing **Kleenpak** Nova filter capsules.

Extractables testing

The level of aqueous and ethanol extractables for empty **Kleenpak** Nova filter capsules, irradiated with doses of up to 53.0 kGy were found to be extremely low. Out of 12 extraction tests performed the non-volatile residue extracted was ≤ 1 mg when water was used as the extraction fluid and ≤ 6 mg when 96% ethanol was used as the extraction fluid.

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

Shelf life studies

Burst pressure tests performed on irradiated **Kleenpak** Nova filter capsules that had been stored for up to six months demonstrated that a high safety factor was maintained between the burst strength and the maximum recommended operating pressure.

Microbial challenge tests demonstrated that irradiated filter cartridges incorporating 'Ultipor' 'N66' NFZ-grade membrane and 'Fluorodyne' II DFL-grade gave sterile effluent following four months storage at room temperature (20 ± 5°C). Longer terms tests are in progress at time of writing.

Biological reactivity tests

Kleenpak Nova filter capsules were found to meet the requirements of the current United States Pharmacopoeia for Class VI-121°C Plastics.

2. Burst testing

2.1 Introduction

The purpose of these tests was to demonstrate that **Kleenpak** Nova capsules withstand the maximum pressure rating of 3 bar (43.5 psi) with an appropriate safety margin. Burst tests were performed on empty untreated **Kleenpak** Nova capsules and also on samples that had been autoclaved and gamma-irradiated at doses of approximately 35 and 50 kGy.

2.2 Summary of methods

Standard empty **Kleenpak** Nova capsules from production were used for these tests. Prior to the burst testing samples of the capsules were pre-treated with one of the conditions shown below:

- **Six one-hour autoclave cycles at 125°C**
- **Gamma irradiated at a dose of approximately 35 kGy**
- **Gamma irradiated at a dose of approximately 50 kGy**

Before the burst tests were performed the vent and drain valves were removed and replaced with blanking plugs. The whole assembly was placed in a water bath at 40°C. The empty capsules were filled with water and the outlet was blanked off. Prior to starting the burst tests the capsules were held within the water for a minimum of 15 minutes to ensure that they had equilibrated with the water bath temperature. The inlet of the water-filled capsule was then connected to a pressure source and the upstream pressure was gradually increased until the filter capsule burst.

2.3 Results

The results of the burst pressure tests are shown in Tables 2-1 to 2-4. The average burst pressure of the non-treated **Kleenpak** Nova capsules was found to be 22.3 bar (324 psi). After exposure to six one-hour autoclave cycles at 125°C the average burst pressure fell slightly to 20.8 bar (301 psi). Exposure to gamma-irradiation was also found to have an effect on the burst pressures of the **Kleenpak** Nova filter capsules. The average burst pressure of samples exposed to approximately 35 kGy was 18.5 bar (269 psi) and the average burst pressure after exposure to approximately 50 kGy was 18.8 bar (272 psi).

In all cases the measured burst pressures far exceeded the 5:1 ratio between burst pressure and the 3 bar (43.5 psi) maximum recommended operating pressure that is the design requirement for the **Kleenpak** Nova capsule.

Table 2-1. Burst pressures of non-sterilised Kleenpak Nova capsules

Pall Kleenpak Nova capsule serial number	Burst pressure
PB550001	22.1 bar / 320 psi
PB550002	22.1 bar / 320 psi
PB550003	23.4 bar / 340 psi
PB550004	23.4 bar / 340 psi
PB554001	22.8 bar / 330 psi
PB554002	22.8 bar / 330 psi
PB554003	22.1 bar / 320 psi
PB554004	24.1 bar / 350 psi
PB554005	23.4 bar / 340 psi
PB555001	22.1 bar / 320 psi
PB555002	22.1 bar / 320 psi
PB555003	21.4 bar / 310 psi
PB555004	24.1 bar / 350 psi
PB555005	24.1 bar / 350 psi
PB556001	22.8 bar / 330 psi
PB556002	19.3 bar / 280 psi
PB556003	21.4 bar / 310 psi
PB556004	23.4 bar / 340 psi
PB556005	20.7 bar / 300 psi
PB557001	22.1 bar / 320 psi
PB557002	22.8 bar / 330 psi
PB557003	24.1 bar / 350 psi
PB557004	22.8 bar / 330 psi
PB557005	16.6 bar / 240 psi
Average burst pressure	**22.3 bar / 324 psi**

Table 2-2. Burst pressures of Kleenpak Nova capsules autoclaved for 6 one-hour cycles at 125°C

Pall Kleenpak Nova capsule serial number	Burst pressure
PB550010	20.3 bar / 295 psi
PB550015	20.0 bar / 290 psi
PB550018	21.0 bar / 305 psi
PB550027	22.1 bar / 320 psi
PB555045	20.7 bar / 300 psi
PB551001	21.4 bar / 310 psi
PB551011	21.0 bar / 305 psi
PB551010	20.7 bar / 300 psi
PB551009	20.7 bar / 300 psi
PB551002	20.7 bar / 300 psi
PB552006	21.4 bar / 310 psi
PB552002	20.7 bar / 300 psi
PB552007	20.7 bar / 300 psi
PB552005	20.0 bar / 290 psi
PB552008	20.0 bar / 290 psi
Average burst pressure	**20.8 bar / 301 psi**

Table 2-3. Burst pressures of Kleenpak Nova capsules gamma irradiated with a dose of 35.6 – 37.2 kGy

Pall Kleenpak Nova capsule serial number	Burst pressure
PB554016	19.3 bar / 280 psi
PB554021	18.3 bar / 265 psi
PB554008	17.2 bar / 250 psi
PB554009	19.3 bar / 280 psi
PB554010	18.6 bar / 270 psi
Average burst pressure	**18.5 bar / 269 psi**

Table 2-4. Burst pressures of Kleenpak Nova capsules gamma irradiated with a dose of 50.1 – 53.0 kGy

Pall Kleenpak Nova capsule serial number	Burst pressure
PB554026	19.0 bar / 275 psi
PB554025	18.6 bar / 270 psi
PB554023	18.6 bar / 270 psi
PB554024	19.0 bar / 275 psi
PB554037	18.6 bar / 270 psi
Average burst pressure	**18.8 bar / 272 psi**

2.4 Conclusions

Forty-nine **Kleenpak** Nova capsules were burst pressure tested and in all cases the burst pressures were ≥ 16.6 bar (240 psi), thereby fulfilling the required 5:1 safety ratio between burst pressure and the maximum recommended operating pressure of 3 bar (43.5 psi). This testing included untreated samples, samples that had been exposed to repeated autoclave cycles at 125°C and samples that had been exposed to gamma-irradiation at doses of approximately 35 and 50 kGy.

3. Creep-rupture testing

3.1 Introduction

Creep testing was performed in order to demonstrate the strength and stability of **Kleenpak** Nova capsule bodies over extended periods of time whilst under pressure.

3.2 Summary of methods

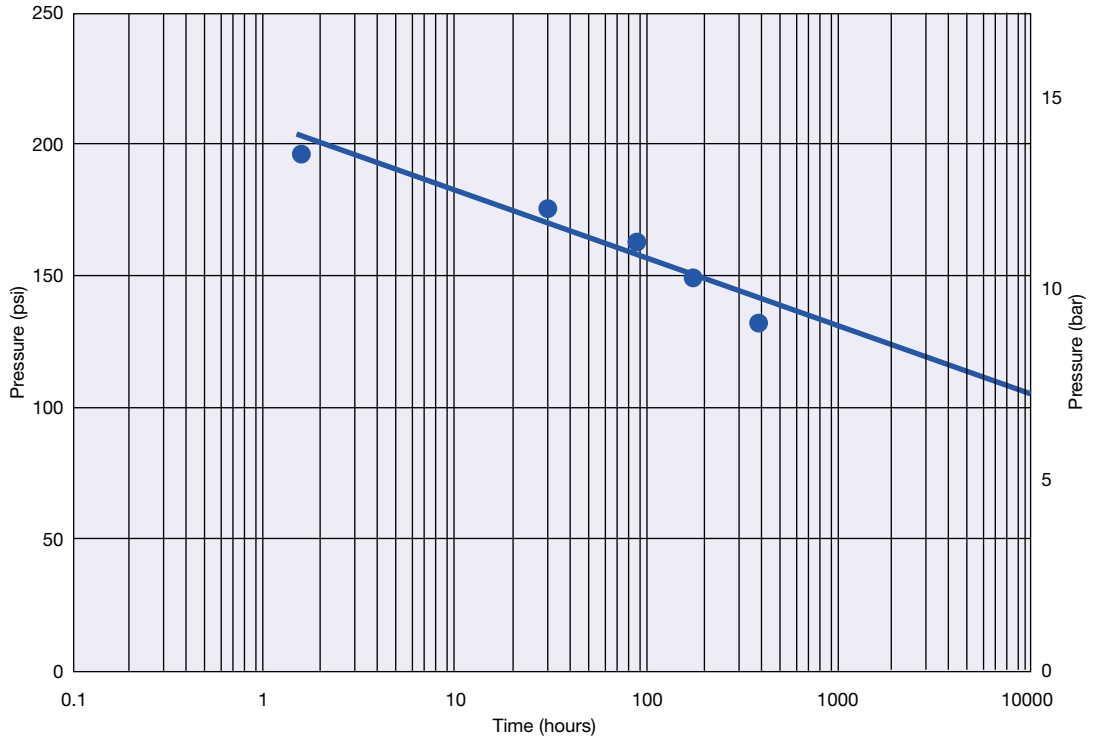
Typical **Kleenpak** Nova empty filter capsules were used for the tests. Prior to the creep-rupture testing the capsules were autoclaved for six one-hour cycles at 125°C.

For the creep-rupture tests the capsules were immersed in a water bath held at 40°C. The capsules were filled with the warm water and connected to a creep-rupture rig designed to maintain set pressures within the inside of the capsule until failure of the capsule occurred. At this end point, the failure time and mode was noted.

3.3 Results

The results are shown in Figure 3-1. The points on the graph represent the average values obtained from testing a minimum of two empty **Kleenpak** Nova filter capsules at each pressure. The data has been extrapolated to predict the creep-rupture of samples maintained at constant pressure for up to 10,000 hours (416 days). Based on this extrapolation, it can be expected that **Kleenpak** Nova filter capsules will withstand approximately 1,000 hours (41 days) at 8.6 bar (125 psi) and 10,000 hours (416 days) at 6.9 bar (100 psi).

Figure 3-1. Mean creep rupture pressures for empty Kleenpak Nova filter capsules



3.4 Conclusions

Kleenpak Nova filter capsules have been designed to be capable of operating at up to 3 bar (43.5 psi) for 168 hours (1 week) in continuous use. In order to allow for integrity testing the capsules have also been designed to be capable of operating at up to 5.9 bar (85 psi) for a maximum of 10 hours. The creep-rupture data presented using typical **Kleenpak** Nova capsules demonstrates the very large safety margins that have been incorporated into these pressure claims.

4. Pressure fatigue testing

4.1 Introduction

The purpose of these tests was to demonstrate that **Kleenpak** Nova filter capsules can withstand repeated cycles of pressurisation up to the maximum recommended operating pressure of 3 bar (43.5 psi).

4.2 Summary of methods

Typical **Kleenpak** Nova empty capsules were used for the tests. Six samples with triclover inlet and outlet connections were used (P1-option) and six samples with hosetail inlet and outlet connections were used (P6-option).

The tests were performed using a hydraulic test stand specially designed to produce controlled and repeated pressure pulses. The vent and drain valves of the capsules were closed and the capsule outlets blanked off. Prior to fatigue testing the capsules were filled with mineral oil and connected to the hydraulic test stand. The pressure of the oil within the capsules was cycled from 0 barg to 3 barg (43.5 psi) and back to 0 barg in 4 seconds. The pressure cycles were repeated 100,000 times on each **Kleenpak** Nova capsule tested.

On completion of the fatigue testing the **Kleenpak** Nova capsules were carefully examined to determine if there was any visible evidence of leaks or damage.

4.3 Results

The fatigue test results are shown in Table 4-1. Twelve **Kleenpak** Nova capsules were tested and none of them showed any visible signs of leak paths or damage after 100,000 pressure cycles of 0 to 3 barg (43.5 psi).

Table 4-1. Results of fatigue testing for Kleenpak Nova capsules

Inlet/outlet connection option	Pall Kleenpak Nova capsule serial number	Signs of leak paths/damage following exposure to 100,000 pressure cycles from 0 to 3 barg (43.5 psi)
Triclover inlet/outlet connections (P1-option)	PB557011	None
	PB557017	None
	PB551006	None
	PB551007	None
	PB557018	None
	PB551005	None
Hosetail inlet/outlet connections (P6-option)	PB580002	None
	PB586032	None
	PB586033	None
	PB586034	None
	PB586023	None
	PB586035	None

4.4 Conclusions

Kleenpak Nova filter capsules can withstand repeated pressurisation cycles, as demonstrated by fatigue testing where typical capsules were exposed to 100,000 pressure cycles from 0 to 3 barg (43.5psi) and back to 0 barg again.

5. Environmental testing

5.1 Introduction

The purpose of these tests was to demonstrate that **Kleenpak** Nova filter capsules could withstand extremes in temperature that they could potentially be exposed to during storage or transit.

5.2 Summary of methods

Typical **Kleenpak** Nova empty capsules were used for the tests. Three samples with triclover inlet and outlet connections were used (P1-option) and three samples with hosetail inlet and outlet connections were used (P6-option).

All of the samples were carefully examined for visual defects prior to leak testing. The leak tests were performed by immersing the assemblies in water and pressurising the internal capsules with 3 bar (45 psi) air pressure for one minute. The capsules were then dried and placed in an environmental chamber where they were exposed to 25 cycles of the following conditions:

- **Heated from ambient to 60°C**
- **Temperature held at 60°C for one hour**
- **Cooled from 60°C to – 40°C**
- **Temperature held at – 40°C for one hour**
- **Heated from – 40°C to 30°C**

On completion of the 25 temperature cycles, the filter capsules were inspected, diameter measurements were made and then they were leak tested again using compressed air at 3 bar (45 psi).

5.3 Results

All of the capsules passed the leak testing before and after exposure to the thermal cycling (Table 5-1). The capsules were carefully inspected and no signs of deformation, degradation or stress cracking were observed.

Table 5-1. Results of environmental chamber testing

Inlet/outlet connection option	Pall Kleenpak Nova capsule serial number	Signs of leak paths/damage following exposure to 25 cycles of thermal cycling
Triclover inlet/outlet connections (P1-option)	PB557010	None
	PB557016	None
	PB557012	None
Hosetail inlet/outlet connections (P6-option)	PB586042	None
	PB586009	None
	PB586010	None

5.4 Conclusions

The data presented provide assurance that **Kleenpak** Nova filter capsules will not be damaged by extremes in temperature that they could potentially be subjected to during storage or transit.

6. Filter/capsule seal testing

6.1 Introduction

The purpose of this series of tests was to confirm that the seal between the filter and the outer capsule of **Kleenpak** Nova filters is integral. The testing of the seal was achieved by performing integrity and bacterial challenge tests on typical **Kleenpak** Nova filter capsules. The testing was performed on untreated samples and also on samples that had been exposed to gamma-irradiation at doses of approximately 35 and 50 kGy.

6.2 Summary of methods

Standard **Kleenpak** Nova filter capsules incorporating 0.2m-rated **Ulitpor N66** 'Posidyne' filter membrane were used for the tests (part number NP6NFZP1G). Prior to challenge testing, six samples were each subjected to one of the following conditions:

- **One hour autoclave cycle at 125°C**
- **Gamma irradiation at a dose of approximately 35 kGy**
- **Gamma irradiation at a dose of approximately 50 kGy**

The filter capsules were aseptically connected to the challenge apparatus shown schematically in Figure 6-1 and subjected to a liquid bacterial challenge test using *Brevundimonas diminuta* (ATCC 19146) at a minimum challenge level of $1x10^7$ colony forming units per cm^2 of effective filtration area.

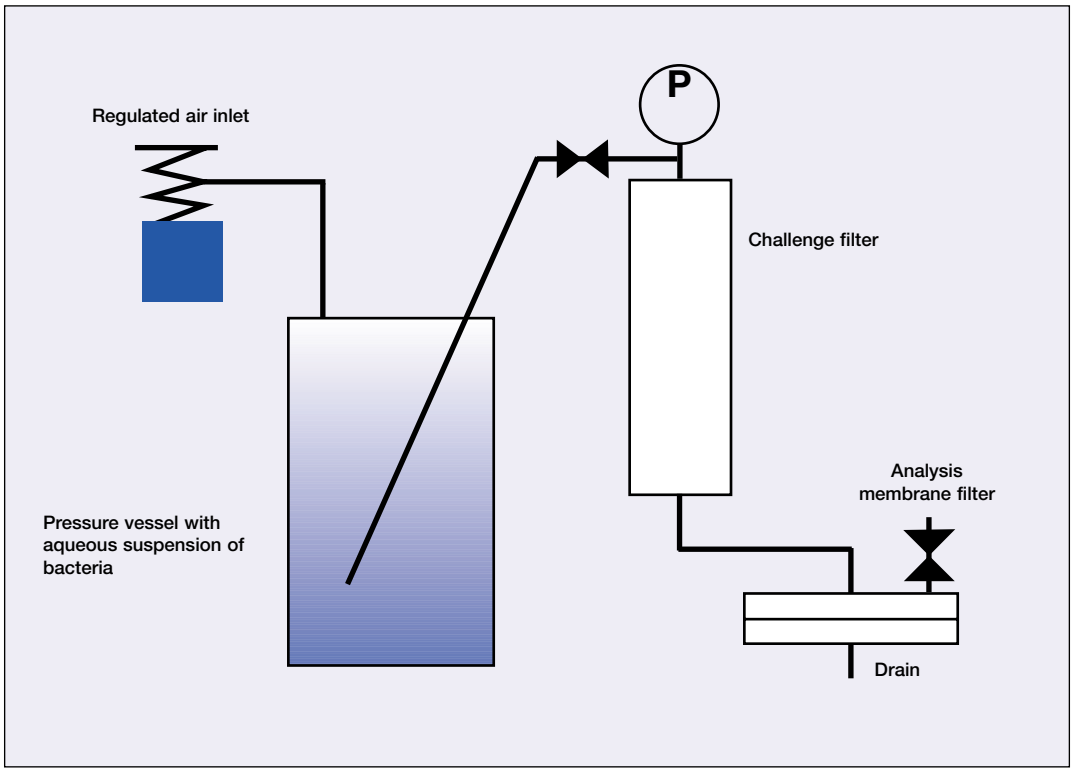


Figure 6-1. Bacterial challenge apparatus

During the challenge test, the entire filter effluent was passed through a 0.2µm-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 bacteria had passed through the test filter during the challenge. The titre 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 titre reduction was expressed as:

$$> \text{Total number of organisms influent to the filter (e.g. } > 1 \times 10^{11})$$

On completion of the challenge test the filter capsules were Forward Flow integrity tested.

6.3 Results

The results are shown in Tables 6-1 to 6-3. Each of the filters tested passed the Forward Flow integrity test and gave sterile effluent when challenge tested with an aqueous suspension of *B. diminuta.*

Table 6-1. Forward Flow and bacterial challenge results for autoclaved Kleenpak Nova filter capsules (part number NP6NFZP1G)

Pall capsule serial number	Forward Flow* (ml/min)	Sterile effluent	Titre reduction
PB58301	2.7	Yes	$> 2.2 \times 10^{11}$
PB583016	2.6	Yes	$> 2.4 \times 10^{11}$
PB583017	3.4	Yes	$> 2.0 \times 10^{11}$
PB583018	2.4	Yes	$> 2.3 \times 10^{11}$
PB583021	1.8	Yes	$> 2.6 \times 10^{11}$
PB583022	3.0	Yes	$> 2.6 \times 10^{11}$

** Tested water wet, air test pressure 2760 mbar (40 psi), maximum allowable limit 12 ml/min*

Table 6-2. Forward Flow and bacterial challenge results for Kleenpak Nova filter capsules gamma-irradiated at 37.0 – 38.9 kGy (part number NP6NFZP1G)

Pall capsule serial number	Forward Flow* (ml/min)	Sterile effluent	Titre reduction
PB583001	2.3	Yes	$> 2.8 \times 10^{11}$
PB583004	2.1	Yes	$> 3.6 \times 10^{11}$
PB583007	1.5	Yes	$> 3.7 \times 10^{11}$
PB583008	2.0	Yes	$> 3.3 \times 10^{11}$
PB583014	2.1	Yes	$> 3.3 \times 10^{11}$
PB583025	1.8	Yes	$> 4.0 \times 10^{11}$

** Tested water wet, air test pressure 2760 mbar (40 psi), maximum allowable limit 12 ml/min*

Table 6-3. Forward Flow and bacterial challenge results for Kleenpak Nova filter capsules gamma-irradiated at 52.7 – 53.5 kGy (part number NP6NFZP1G)

Pall capsule serial number	Forward Flow* (ml/min)	Sterile effluent	Titre reduction
PB583005	2.8	Yes	$> 1.7 \times 10^{11}$
PB583006	3.8	Yes	$> 2.2 \times 10^{11}$
PB583009	2.4	Yes	$> 2.4 \times 10^{11}$
PB583010	2.5	Yes	$> 2.2 \times 10^{11}$
PB583011	3.2	Yes	$> 2.5 \times 10^{11}$
PB583027	2.0	Yes	$> 2.7 \times 10^{11}$

** Tested water wet, air test pressure 2760 mbar (40 psi), maximum allowable limit 12 ml/min*

6.4 Conclusions

The integrity of the filter/housing seal of **Kleenpak** Nova filter capsules has been demonstrated by performing Forward Flow integrity tests and liquid bacterial challenge tests on filter samples that have been exposed to either autoclave sterilisation or gamma-irradiation at doses of approximately 35 or 50 kGy.

7. Determination of water flow characteristics

7.1 Introduction

The purpose of these tests was to determine the pressure differential characteristics of **Kleenpak** Nova filter capsules when subjected to different inlet water flow rates.

7.2 Summary of methods

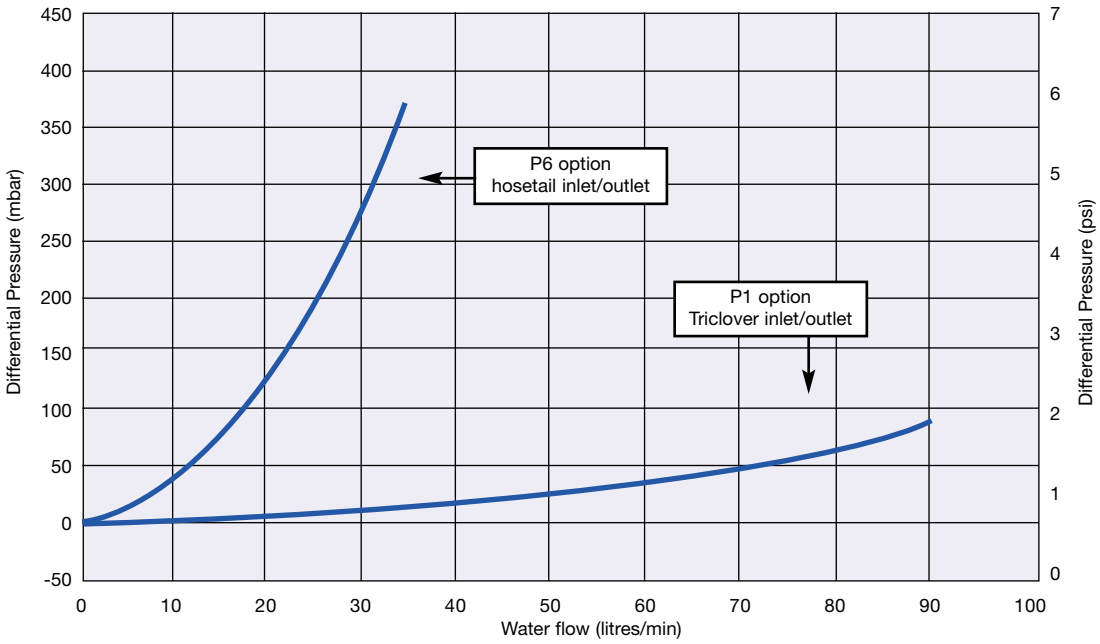
The tests were performed on standard empty **Kleenpak** Nova filter capsules. Deionised water was pumped through the filter capsules in the normal flow direction. Pressure transducers on the upstream and downstream side of the assembly were monitored in order to calculate the differential pressure at different water flow rates.

All of the data were corrected to a standard temperature of 20°C.

7.3 Results

The water flow/differential pressure characteristics of empty **Kleenpak** Nova filter capsules are shown in Figure 7-1. **Kleenpak** Nova filter capsules are available with a range of different membrane types, incorporating standard Pall 25cm (10") filter cartridges. The water flow/differential pressure data presented here can be used to provide guidance on filter sizing.

Figure 7-1. Water flow/differential pressure characteristics for empty Kleenpak Nova filter capsules



7.4 Conclusions

The water flow / pressure drop data presented for empty **Kleenpak** Nova filter capsules can be used in conjunction with the pressure drop characteristics of standard 25cm (10") Pall filter cartridges to form the basis for sizing filter systems employing **Kleenpak** Nova filter capsules.

8. Extractables testing

8.1 Introduction

The purpose of these tests was to determine the amount of material that can be extracted from irradiated typical **Kleenpak** Nova filter capsules by water and ethanol at ambient temperature.

8.2 Summary of methods

Typical **Kleenpak** Nova empty filter capsules were used for the tests. Prior to the extraction tests the capsule samples were gamma-irradiated at doses of either approximately 35 or 50 kGy.

The extraction tests were performed by recirculating the extraction fluid (deionised water or 96% ethanol) through the filter capsule for four hours. In order to minimise any background residues a diaphragm pump with PTFE liquid contact parts and tubing was used. The non-volatile residue (NVR) was determined by evaporating the extraction fluid to dryness. The material extracted from the **Kleenpak** Nova filter capsules was compared with control samples of water and ethanol that had been recirculated through the same extraction system but without the filter capsule installed.

8.3 Results

The results (Table 8-1) show the typical quantities of non-volatile residue extracted per **Kleenpak** Nova capsule using deionised water and 96% ethanol at ambient temperature. The results reported are typical for standard production capsules.

Table 8-1. Aqueous and ethanol extractables for Kleenpak Nova filter capsules

Extraction fluid	Gamma irradiation (kGy)	Pall Kleenpak Nova capsule serial number	Non-volatile residue (mg)
Water	35.6 - 37.2	PB554006	0
		PB554007	1
		PB554022	1
	50.1 - 53.0	PB554028	0
		PB554036	0
		PB554029	1
96% ethanol	35.6 - 37.2	PB554012	0
		PB554013	0
		PB554019	0
	50.1 - 53.0	PB554026	6
		PB554034	5
		PB554038	6

8.4 Conclusions

The level of aqueous and ethanol extractables for **Kleenpak** Nova filter capsules, irradiated with doses of up to 53.0 kGy are extremely low. Out of 12 extraction tests performed the non-volatile residue extracted was ≤ 1 mg when water was used as the extraction fluid and ≤ 6 mg when 96% ethanol was used as the extraction fluid.

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

9. Shelf life studies

9.1 Introduction

There were two aims for this series of tests:

- **To demonstrate that an adequate safety margin is maintained for the burst pressures of gamma-irradiated Kleenpak Nova filter capsules following storage at room temperature for up to six months**

- **To demonstrate that Pall N66 Posidyne and Fluorodyne II sterilising-grade membranes provide sterile filtrate following storage for four months after exposure to gamma irradiation**

9.2 Summary of methods

Burst pressure testing

Samples of typical **Kleenpak** Nova empty filter capsules were irradiated at a dose of approximately 35 or 50 kGy and then stored at ambient temperature for up to six months. On completion of the storage interval, the burst pressures of the filter capsules were determined according to the method described previously in Section 2.

Bacterial challenge testing

Typical AB1-style (25 cm) filter cartridges incorporating **N66 Posidyne** NFZ-grade membrane and **Fluorodyne** II DFL-grade membrane (part numbers AB1NFZ7PH4 and AB1DFL7PH4) were irradiated with doses of approximately 35 or 50 kGy. The filter cartridges were then stored in their original packaging for four months at room temperature and then subjected to microbial challenge testing according to the method described previously in Section 6.

9.3 Results

The results of the burst pressure tests performed on **Kleenpak** Nova filter capsules stored for up to 6 months following irradiation with doses of approximately 35 and 50 kGy are shown in Tables 9-1 and 9-2. In all cases the burst pressure measurements were ≥ 15.2 bar / 220 psi, thereby fulfilling the required 5:1 safety ratio between burst pressure and the maximum recommended operating pressure of 3 bar (43.5 psi).

The results of the bacterial challenge tests performed on irradiated AB1-style filter elements in corporating **N66 Posidyne** NFZ-grade membrane and **Fluorodyne** II DFL-grade that had been stored for four months are shown in Tables 9-3 and 9-4. All of the filters tested produced sterile effluent when challenged.

Table 9-1. Burst pressures of Kleenpak Nova capsules irradiated at 35.6 – 37.2 kGy and then stored for up to 6 months

Storage time	Pall Kleenpak Nova capsule serial number	Burst pressure
3 months	PB556014	20.0 bar / 290 psi
	PB556018	17.2 bar / 250 psi
	PB556022	18.6 bar / 270 psi
	PB556020	19.3 bar / 280 psi
	PB556015	19.3 bar / 280 psi
	PB556019	16.6 bar / 240 psi
	Average burst pressure	**18.5 bar / 268 psi**
6 months	PB556010	17.2 bar / 250 psi
	PB556017	19.0 bar / 275 psi
	PB556006	19.3 bar / 280 psi
	PB556012	20.7 bar / 300 psi
	PB556016	20.7 bar / 300 psi
	Average burst pressure	**19.4 bar / 281 psi**

Table 9-2. Burst pressures of Kleenpak Nova capsules irradiated at 50.1 – 53.0 kGy then stored for up to 6 months

Storage time	Pall Kleenpak Nova capsule serial number	Burst pressure
3 months	PB556030	17.9 bar / 260 psi
	PB556031	16.6 bar / 240 psi
	PB556034	15.9 bar / 230 psi
	PB556025	18.6 bar / 270 psi
	PB556032	18.6 bar / 270 psi
	PB556023	17.2 bar / 250 psi
	Average burst pressure	**17.4 bar / 253 psi**
6 months	PB555032	19.3 bar / 280 psi
	PB555023	18.6 bar / 270 psi
	PB555026	19.3 bar / 280 psi
	PB555027	19.3 bar / 280 psi
	PB555029	15.2 bar / 220 psi
	Average burst pressure	**18.3 bar / 266 psi**

Table 9-3. Bacterial challenge results for N66 Posidyne grade NFZ filters (part number AB1NFZ7PH4) stored for four months following irradiation

Irradiation dose	Pall filter serial number	Foward Flow* (ml/min)	Sterile effluent	Titre reduction
34.2 - 36.7 kG	ID0619124	1.9	Yes	> 1.9×10^{11}
	ID0619650	3.0	Yes	> 2.1×10^{11}
	ID0619131	3.4	Yes	> 1.6×10^{11}
45.4 - 49.4 kG	ID0619565	2.6	Yes	> 1.6×10^{11}
	ID0619663	3.5	Yes	> 1.8×10^{11}
	ID0619618	2.2	Yes	> 2.2×10^{11}

** Tested water wet, air test pressure 2760 mbar (40 psi), maximum allowable limit 12 ml/min*

Table 9-4. Bacterial challenge results for Fluorodyne II grade DFL filters (part number AB1DFL7PH4) stored for four months following irradiation

Irradiation dose	Pall filter serial number	Foward Flow* (ml/min)	Sterile effluent	Titre reduction
34.2 - 36.7 kG	ID0859185	6.9	Yes	> 1.5×10^{11}
	ID1090244	5.9	Yes	> 2.2×10^{11}
	ID1090409	6.0	Yes	> 2.0×10^{11}
45.4 - 49.4 kG	ID1090238	5.8	Yes	> 1.9×10^{11}
	ID1090240	6.1	Yes	> 2.5×10^{11}
	ID1090250	6.2	Yes	> 2.1×10^{11}

** Tested water wet, air test pressure 2760 mbar (40 psi), maximum allowable limit 12 ml/min*

9.4 Conclusions

Burst pressure tests performed on irradiated **Kleenpak** Nova filter capsules that had been stored for up to six months demonstrated that a high safety factor was maintained between the burst strength and the maximum recommended operating pressure.

Microbial challenge tests demonstrated that irradiated filter cartridges incorporating **N66 Posidyne** NFZ-grade membrane and **Fluorodyne** II DFL-grade gave sterile effluent following four months storage at room temperature (20 ± 5°C).

10. Biological safety tests

10.1 Introduction

The purpose of these tests was to evaluate the biological suitability of the materials of construction of the **Kleenpak** Nova filter capsules. The materials of construction are as follows :

Capsule components:	Polypropylene
Valve and filter element seals:	Silicone elastomer

10.2 Summary of methods

The tests were performed in accordance with the Biological Reactivity Tests *in vivo* for Class VI Plastics (121°C) as described in the current United States Pharmacopeia. The tests were conducted by Gilbraltar Laboratories, Inc., New Jersey, USA.

The testing procedures described in the USP include:

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

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

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

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

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.

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 **Kleenpak** Nova filter capsule was implanted separately.

10.3 Results

The components of **Kleenpak** Nova filter capsules passed all of the tests specified. Appendix 1 shows a copy of the test certificates.

10.4 Conclusions

Components of **Kleenpak** Nova capsules meet the requirements of the USP for Class VI -121°C Plastics.

Appendix 1

Copy of Biological Safety test report

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13504-C SOUTHPOINT BLVD., CHARLOTTE, NC 28273-6763 • PHONE: [704] 588-7288 • FAX: [704] 583-6156
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REPORT No. G-29577
05/10/00
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FINAL REPORT

Sponsor:(0850)
Pall Corporation
25 Harbor Park Drive
Port Washington,NY,11050
Attn: Janet Mathus
Purchase Order #:P434807

GBL Ref.: 1434- 196- 7435
GBL Sample No.:18536/1-4.339
Lot #1:N/A
Lot #2: None
Lot #3: None
Date Received: 04/12/00
Date Tested: 04/28/00
Date Completed: 05/09/00

USP 24 CLASS VI on Kleenpak Nova Capsule Components
Interim Report Activity:

Description:
Structural components for Kleenpak Nova Capsule.
/1 = 3" x 4" whitened polypropylene capsule head
/2 = 4" x 11" natural polypropylene capsule bowl
/3 = 3" x 3" polypropylene endcap
/4 = 13" extruded polypropylene-based composite weld strand

1 Purpose
To determine the reaction of normal animal tissue and living animals to the presence of extracts and/or portions of the test material.

2 Test System
2.1 New Zealand albino rabbits, either sex, 1.85 to 2.92 kg.
Two per extract and two per implant.
2.2 Swiss Webster albino mice, male, 16.5 to 23.0 grams.
Five test and control (intravenous and intraperitoneal injection).

3. Method: Test Material Preparation and Extraction
A composite of the following materials was extracted in 20 mL of each of the below solvents. Each component was tested separately for implantation.
/1 1 gram - Hydrophilic polyvinylidene fluoride filter membrane
/2 1 gram - Polypropylene support layer
/3 1 gram - Polypropylene core
/4 1 gram - Polypropylene capsule body or endcaps

3.1 Solvents
(X) USP Sodium Chloride for Injection (Saline)
(X) 5% Ethanol in Sodium Chloride (ETOH/Saline)
(X) Cottonseed Oil (CSO)
(X) Polyethylene glycol 400 (PEG)

Conclusion: The material conforms to the requirements of this test.
Respectfully Submitted,
GIBRALTAR LABORATORIES, INC.

Date
Written: 05/10/00 Approved By: J. Chris Knutsen, Ph.D. or Daniel L. Prince, Ph.D.
Analyst: 41
Protocol #: None

AUDIT HCP 5/10/00 GBL

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LABORATORY REPORT

3.2 Extraction Conditions
(X) 121C for one hour

3.3 Dosing Procedures
(X) IC Injection - three day observation period
(X) Systemic Injection (IV and IP) - three day observation period
(X) Intramuscular Implantation - seven day observation period

4. Results: See Tables 1 to 6.

Table 1: Intracutaneous Irritation

Extract	Average Test Score	Average Control Score	Difference
Saline	(0.00)	(0.00)	(0.00)
ETOH/Saline	(0.00)	(0.00)	(0.00)
CSO	(1.08)	(1.67)	(0.59)
PEG 400	(0.00)	(0.00)	(0.00)

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 - Whitened Polypropylene Capsule Head

Sample Size	Average Test Score	Average Control Score	Difference
1 x 10 mm	(0.13)	(0.25)	(0.12)

Table 4: Intramuscular Implantation - Natural Polypropylene Capsule Head

Sample Size	Average Test Score	Average Control Score	Difference
1 x 10 mm	(0.13)	(0.00)	(0.13)

Table 5: Intramuscular Implantation - Polypropylene Endcap

Sample Size	Average Test Score	Average Control Score	Difference
1 x 10	(0.00)	(0.00)	(0.00)

Table 6: Intramuscular Implantation - Extruded Polypropylene-Based Composite Weld Strand

Sample Size	Average Test Score	Average Control Score	Difference
1 x 10	(0.00)	(0.00)	(0.00)

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FINAL REPORT

Sponsor:(0850)
Pall Corporation
25 Harbor Park Drive
Port Washington,NY,11050
Attn: Janet Mathus
Purchase Order #:P434807

GBL Ref.: 1435- 196- 7449
GBL Sample No.:18672/ 1.339
Lot #1:N/A
Lot #2: None
Lot #3: None
Date Received: 04/14/00
Date Tested: 05/01/00
Date Completed: 05/09/00

USP 24 CLASS VI on White Silicone Gasket
Interim Report Activity:

Description:
Ten products of 3.25" White Silicone Gasket" placed in an 8" x 5" zip-lock bag.

1. Purpose
To determine the reaction of normal animal tissues and living animals to the presence of extracts and/or portions of the test material.

2. Test System
2.1 New Zealand albino rabbits, either sex, 1.97 to 3.03 kg.
Two for intracutaneous injection, two for implantation.

2.2 Swiss Webster albino mice, either sex 17.2 to 21.8 grams.
Five test and control (intravenous and intraperitoneal injection).

3. Method: Test Material Preparation and Extraction
3.1 4.0 grams of material per 20.0 mL of solvent were extracted in:
(X) USP Sodium Chloride for Injection (Saline)
(X) 5% Ethanol in Sodium Chloride (ETOH/Saline)
(X) Cottonseed Oil (CSO)
(X) Polyethylene glycol 400 (PEG)

3.2 Extraction Conditions
( ) 50C for 72 hours
( ) 70C for 24 hours
(X) 121C for one hour

3.3 Dosing Procedures
(X) IC Injection - three day observation period
(X) Systemic Injection (IV and IP) - three day observation period
(X) Intramuscular Implantation - seven day observation period

Respectfully Submitted,
GIBRALTAR LABORATORIES, INC.

Date
Written: 05/10/00
Analyst: 41
Protocol #: None

Approved By: J. Chris Knutsen, Ph.D. or Daniel L. Prince, Ph.D.

AUDIT
REP 5/10/00
GBL

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4. Results: See Tables 1 to 3.

Table 1: Intracutaneous Irritation

Extract	Average Test Score	Average 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)

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

Sample Size	Average Test Score	Average Control Score	Difference
1 x 10 mm	(0.00)	(0.00)	(0.00)

5. Conclusion: The material conformed to the requirements of this test.

PALL BioPharmaceuticals

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