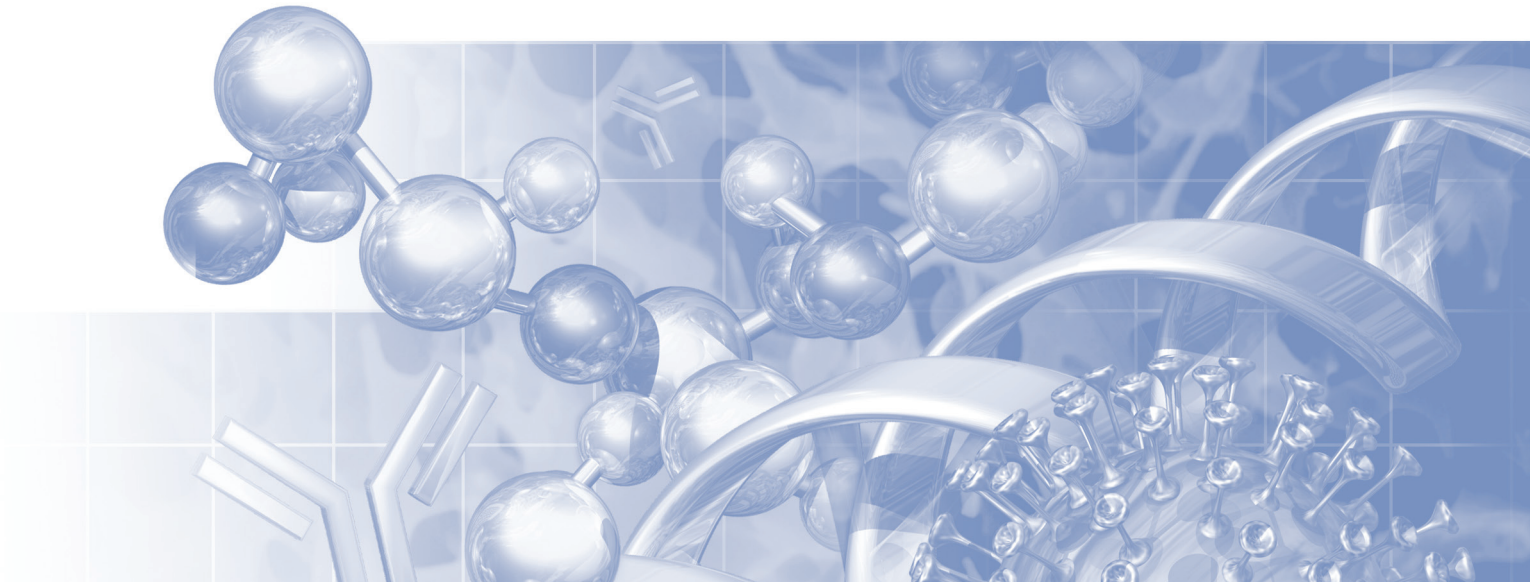
PALL Biotech

Validation Guide

USTR3130b

Kleenpak® Presto Sterile Connector



Contents

1. Validation Overview

1.1 Introduction

The purpose of this report is to document testing that has been performed to demonstrate the suitability of the Kleenpak Presto sterile connector for use in biopharmaceutical applications. The Kleenpak Presto sterile connector is designed to enable the dry sterile connection of two (2) fluid paths in biopharmaceutical applications. It can be sterilized either by gamma irradiation or by autoclaving. The Kleenpak Presto sterile connector device provides an easy to use, single-use method for the connection of two sterile fluid path assemblies even in an uncontrolled environment while maintaining the sterility of the fluid pathway.

The Kleenpak Presto sterile connector is a genderless connector made of polyethersulfone (PES), which has excellent chemical compatibility with aqueous solutions over a wide pH range. Each connector half is covered by a peel strip that maintains the sterility of the closed fluid pathway prior to use. The seal in contact with fluid is made of low extractable platinum cured silicone.

Both parts of Kleenpak Presto sterile connector are provided to the user in the closed position, in which the fluid cannot be transferred through the device. When the operator needs to connect the two sterile fluid pathways via the Kleenpak Presto sterile connector, the operator will connect the two parts, remove the peel strips, and actuate the device (open position). In the open position, fluid can be transferred from one end to the other while maintaining sterility and containment of the fluid path.

The validation testing program includes a demonstration of the mechanical and functional performance of the Kleenpak Presto sterile connector. It also includes data demonstrating the level of extractables, particulate testing, endotoxin and biological safety tests, and shelf life studies.

The Kleenpak Presto sterile connector range includes a ¼ in., ⅜ in., ⅝ in., ½ in. , and ¾ in. internal diameter hose barb fitting and ½ in. sanitary fitting each with a universal face for step up or step down connections. The Kleenpak Presto sterile connector part numbers are shown in Table 1 below.

Table 1

Kleenpak Presto sterile connector part numbers

Kleenpak Presto Sterile Connector	
Part Number	**Size**
PSC1G07	¼ in. hose barb
PSC1G10	⅜ in. hose barb
PSC1G11	⅝ in. hose barb
PSC1G06	½ in. hose barb
PSC1G05	¾ in. hose barb
PSC1G08	½ in. sanitary connection

1.2 Summary of Conclusions

1.2.1 Mechanical Tests

Two mechanical tests were carried out on the Kleenpak Presto sterile connector: the leak test and the burst test.

The purpose of the leak test is to demonstrate the strength and suitability of the seal to maintain the integrity of Kleenpak Presto sterile connectors after conditioning via autoclaving, gamma irradiation, and -80 °C freezing to support the pressure rating of the device.

The purpose of the burst test is to demonstrate the strength and suitability of the seal to maintain the integrity of Kleenpak Presto sterile connector after conditioning via autoclaving, gamma irradiation or freezing when subjected to spikes in pressure after the connection has been performed.

All Kleenpak Presto sterile connectors passed the acceptance criteria.

1.2.2 Functional Tests

Determination of Water Flow Characteristics Test
Water flow vs. pressure drop data have been determined for the Kleenpak Presto sterile connector. This data can be used in conjunction with the pressure drop characteristics of other system components (e.g. filter, tubing) as a basis to define sizing for systems using Kleenpak Presto sterile connectors.

Extended Duration Operation Test
The purpose of this test was to demonstrate that sterilized Kleenpak Presto sterile connector devices can be used for continuous transfer of fluid with a working pressure of 3 barg (43.5 psig) from 2 ± 2 °C to 60 ± 2 °C for 90 days. They can also be exposed at 4 barg (58 psig) for 48 hours from 2 ± 2 °C to 60 ± 2 °C when used in in high pressure operation applications.

All Kleenpak Presto sterile connections passed the extended duration operation test.

Bacterial Soiling Testing
The purpose of the bacterial soiling test is to evaluate the ability of a Kleenpak Presto sterile connector to maintain a sterile fluid path during connection and fluid transfer after the device is intentionally soiled by exposure to *Brevundimonas diminuta* suspended in an aqueous solution at a minimum level of 10^6 CFU/device

The soiling test data presented in this Validation Guide provides assurance that the Kleenpak Presto sterile connector is able to maintain a sterile fluid path before connection and during fluid transfer in an uncontrolled environment.

All Kleenpak Presto sterile connectors passed the bacterial soiling test.

Compatibility Testing
The purpose of this testing is to demonstrate the strength and suitability of Kleenpak Presto sterile connectors with a wide variety of solvents and solutions that are used in biopharmaceutical applications.

All Kleenpak Presto sterile connectors passed the acceptance criteria.

1.2.3 Extractables

The purpose of this test is to quantify and characterize the extractables from the device in various solvents, based on recommendations of the BioPhorum Operations Group (BPOG) Extractables Working group and are representative of most biopharmaceutical fluids.

The study was carried out on Kleenpak Presto sterile connectors that had been irradiated at 50 ± 5 kGy or autoclaved (1 cycle, 75 minutes, 130 ± 2 °C).

Extracts were analyzed using analytical techniques aiming to detect various compounds that could be extracted from the connectors.

The results indicate that the extractable level in the test contact fluid was extremely low. Bis-phenol A was not detected in any samples.

1.2.4 Biological Safety, Cleanliness and Physicochemical Tests

Biological Reactivity Tests, *in vitro*, USP <87>

The purpose of this test is to determine the biological reactivity of mammalian cells (mouse fibroblasts) following contact with the material exposed to the fluid in Kleenpak Presto sterile connector as per USP <87> (MEM cytotoxicity). These tests were conducted using devices which had been gamma irradiated at 50 ± 5 kGy.

The Kleenpak Presto sterile connector meets the specifications of the USP <87>.

Biological Reactivity Tests, *in vivo*, USP < 88>

The purpose of this test is to determine the biological reactivity of mammalian cells (mouse fibroblasts) following contact with the material exposed to the fluid in Kleenpak Presto sterile connector as per USP <88> for Class VI plastics. These tests were conducted using devices which had been gamma irradiated at 50 ± 5 kGy.

The Kleenpak Presto sterile connector meets the specifications of the USP <88>.

Physicochemical Test as per USP < 661>

The purpose of this test was to evaluate the physicochemical suitability on each part of the Kleenpak Presto sterile connector for contact with parenterals. These tests were conducted using devices which had been gamma irradiated at 50 ± 5 kGy.

The Kleenpak Presto sterile connector meets specifications of the USP <661>.

Particle Release Test, USP < 788> Particulate Matter in Injections

The purpose of this test is to determine the particulate level present in the Kleenpak Presto sterile connector. A total of 10 non-connected parts from one batch were tested.

Rinse solution from the Kleenpak Presto sterile connector meets the requirement of particulate testing performed as per the specifications of the USP <788>.

Endotoxin Test, USP < 85>

The purpose of this test is to quantify the bacterial endotoxin level that may be present in the Kleenpak Presto sterile connector. A total of 9 non-connected parts from 3 batches were tested.

Rinse solution from the Kleenpak Presto sterile connector meets the requirement of USP <85> endotoxin content.

1.2.5 Shelf Life Studies

Shelf life studies were conducted on Kleenpak Presto sterile connectors in order to establish up to a 3 year claim for devices gamma irradiated prior to storage.

Samples of Kleenpak Presto sterile connectors were subjected to gamma irradiation and accelerated storage conditions to simulate 3 years of storage. Connectors then underwent leak testing, burst testing and bacterial liquid soiling tests. Extractable levels were also determined on irradiated and aged connectors and compared with new connectors that had not been irradiated.

The results indicated that the functionality of the Kleenpak Presto sterile connector remained intact after 3 years of storage.

Shelf life studies for a 5 year claim for devices not sterilized prior to storage are currently in progress. Results will be available once complete.

2. Mechanical Tests

2.1 Introduction

The following tests were carried out on the Kleenpak Presto sterile connectors to check their ability to withstand mechanical stress after autoclave sterilization, for 2 cycles at 130 ± 2 °C for 75 minutes/cycle and post exposure to gamma irradiation at 50 ± 5 kGy with or without freezing (-80 °C)/thawing treatment:

- Leak test
- Burst test

2.2 Leak test

2.2.1 Introduction

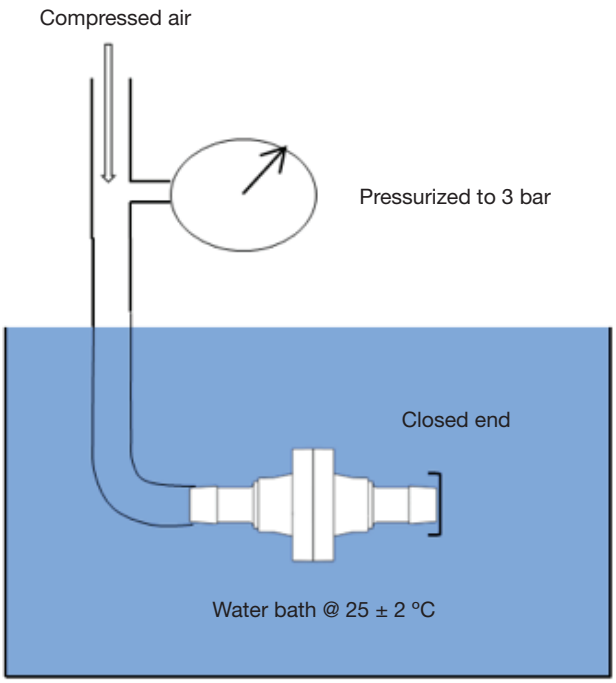
The purpose of this test was to demonstrate the ability of the seal to maintain integrity of the Kleenpak Presto sterile connector after exposure to worst-case conditioning steps (sterilization, freezing/thawing).

2.2.2 Summary of the Test Method

The leak test consists of placing the connected assembly in a bath of water at 25 °C ± 2 °C and pressurizing the Kleenpak Presto sterile connectors with 3 barg air whilst underwater for 5 minutes. The acceptance criteria was the absence of bubbles at the end of the five minutes.

Figure 1

Schematic of the Kleenpak Presto sterile connector in a water bath for the leak test



2.2.3 Results

Table 2

Results of leak testing for Kleenpak Presto sterile connector

Batch Number	Number of Pairs Tested	Conditioning Device 1	Conditioning Device 2	Conditioning Device 1 and 2	Leak Test Result (Pass/Fail)
DV0001	10	G	G	N/A	Pass (10/10)
DV0001	10	AC	AC	N/A	Pass (10/10)
DV0001	10	AC	G	N/A	Pass (10/10)
DV0001	10	G	G	F	Pass (10/10)
DV0001	10	AC	AC	F	Pass (10/10)
DV0001	10	AC	G	F	Pass (10/10)
DV0001	10	G	G	N/A	Pass (10/10)
DV0001	10	AC	AC	N/A	Pass (10/10)
DV0001	10	AC	G	N/A	Pass (10/10)
DV0001	10	G	G	F	Pass (10/10)
DV0001	10	AC	AC	F	Pass (10/10)
DV0001	10	AC	G	F	Pass (10/10)

Where:

G refers to the device was gamma irradiated at 50 ± 5 kGy.

AC refers to the device was autoclaved at 130 ± 2 °C for two cycles of 75 minutes.

F refers to the device being frozen for seven days and then thawed before the test

Device 1 refers to one half of the pair tested, while Device 2 refers to the other half of the pair.

2.2.4 Conclusion

All the Kleenpak Presto sterile connectors passed the leak test criteria of holding 3 barg of pressure for 5 minutes with no bubbles seen for connected parts. Sterilization and freezing/thawing post-sterilization do not affect the integrity of the sealing of the Kleenpak Presto sterile connectors.

2.3 Burst Test

2.3.1 Introduction

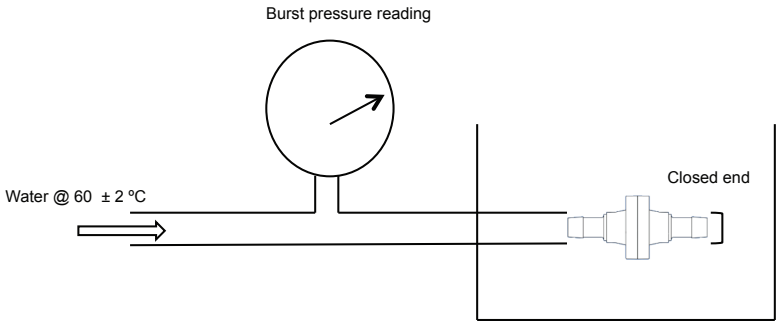
The purpose of this test is to demonstrate that the gamma irradiated and autoclaved Kleenpak Presto sterile connector can withstand the maximum pressure rating of 3 barg (43.5 psig) in the actuated position.

2.3.2 Summary of the Test Method

The gamma irradiated and autoclaved parts were stored in a 60 °C tank for a minimum of 30 minutes before being taken out for testing.

Figure 2

Burst Pressure test set-up for Kleenpak Presto sterile connector



Before the burst test, the two connectors were connected together and actuated. One end of the connected device was connected, using suitable fittings, to the burst test system. The connected device was filled with water at 60 ± 2 °C and the other end of the connected device was blanked off with a suitable connection able to withstand the burst test pressures. The water pressure was increased until the device ruptured. The maximum pressure was recorded.

2.3.3 Results

Table 3

Summary of burst test results for Kleenpak Presto sterile connector

Batch Number	Number of Pairs Tested	Conditioning Device 1	Conditioning Device 2	Conditioning Device 1 and 2	Average Burst Pressure (barg/psig)
DV0001	10	G	G	N/A	29.8/432
DV0001	10	AC	AC	N/A	30.8/447
DV0001	10	AC	G	N/A	31.8/462
DV0001	10	G	G	F	28.1/407
DV0001	10	AC	AC	F	25.6/371
DV0001	10	AC	G	F	23.8/345
DV0001	10	G	G	N/A	26.9/390
DV0001	10	AC	AC	N/A	24.9/361
DV0001	10	AC	G	N/A	24.9/362
DV0001	10	G	G	F	31.4/455
DV0001	10	AC	AC	F	26.9/391
DV0001	10	AC	G	F	26.4/383

Where:

G refers to the device was gamma irradiated at 50 ± 5 kGy.

AC refers to the device was autoclaved at 130 ± 2 °C for two cycles of 75 minutes.

F refers to the device being frozen for seven days and then thawed before the test

Device 1 refers to one half of the pair tested, while Device 2 refers to the other half of the pair.

2.3.4 Conclusion

All Kleenpak Presto sterile connector after connection had burst pressures higher than the maximum recommended operating pressure of 3 barg (43.5 psig), and the maximum short term operating pressure of 4 barg (58 psig), with adequate safety margin.

3. Functional Tests

3.1 Introduction

The following tests were carried out on the Kleenpak Presto sterile connector to provide information on its functional performances:

- Determination of water flow characteristics
- Extended duration operation test
- Bacterial soiling test

3.2 Determination of Water Flow Characteristics Test

3.2.1 Introduction

The purpose of these tests is to determine the differential pressures of an assembled Kleenpak Presto sterile connector when subjected to different water flow rates.

3.2.2 Summary of the Test Method

Total of 18 assembled Kleenpak Presto sterile connectors were tested, of which 3 were from each of the fitting types available. Deionized water was pumped through the assembled Kleenpak Presto sterile connectors at various flow rates and the differential pressures were recorded via pressure transducers on the upstream and downstream sides of the connector.

3.2.3 Results

The water flow versus differential pressure characteristics of Kleenpak Presto sterile connector are shown in Figure 3 and Figure 4.

Figure 3

Pressure drop graph for Kleenpak Presto sterile connectors

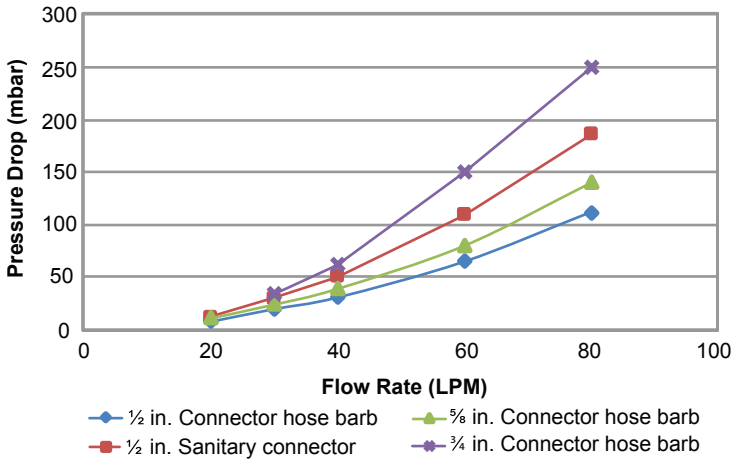
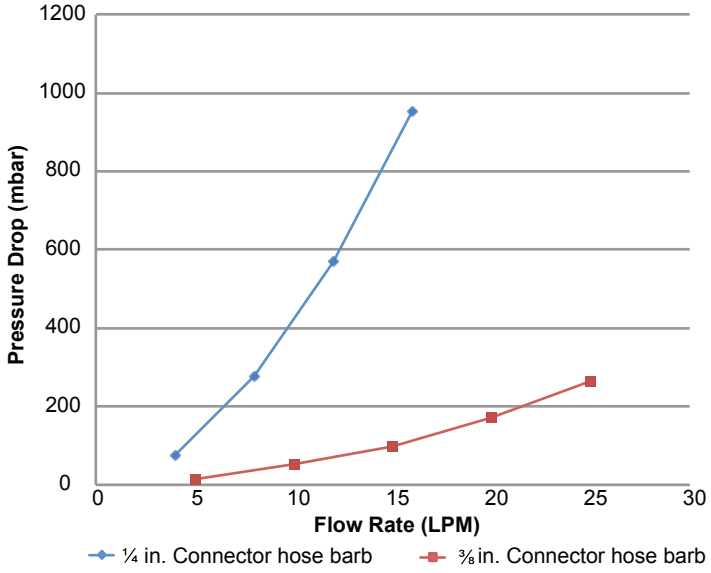


Figure 4

Pressure drop graph for Kleenpak Presto sterile connectors



3.2.4 Conclusions

The water flow versus differential pressure data presented for assembled Kleenpak Presto sterile connectors can be used in conjunction with the pressure drop characteristics of other system components (e.g. tubing, filter capsules and biocontainers) to form the basis for sizing disposable systems using the Kleenpak Presto sterile connector device.

3.3 Extended Duration Operation Test

3.3.1 Introduction

The purpose of these tests is to demonstrate that pre-sterilized Kleenpak Presto sterile connector devices can be used for continuous transfer of fluid with a working pressure of 3 barg (43.5 psig) from 2 ± 2 °C to 60 ± 2 °C, or with short term rating of 4 barg (58 psig) for 48 hours (for example for use in TFF applications).

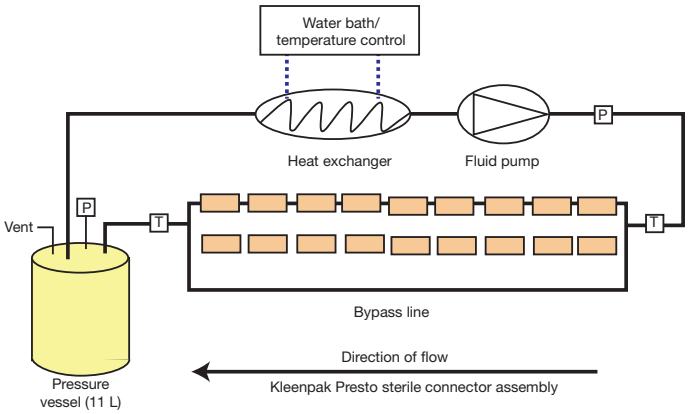
3.3.2 Summary of the Test Method

A total of 84 pre-sterilized Kleenpak Presto sterile connectors were tested. 60 devices were pre-sterilized using gamma irradiation at 50 ± 5 kGy, 12 were pre-sterilized using two (2) 75 minutes autoclave cycles at 130 ± 2 °C and the remaining 12 were pre-sterilized using a combination of the methods (autoclave and gamma irradiation). 36 devices from each of the two sterilization method were used for testing at 2 ± 2 °C and 60 ± 2 °C at 3 barg (43.5 psig) pressure. The remaining 48 gamma irradiated (50 ± 5 kGy) devices were used for testing at 4 barg pressure.

The tests were performed by circulating an aqueous fluid under 3 barg (43.5 psig) pressure through a Kleenpak Presto sterile connector assembly. Schematic of the experimental setup is shown in figure 5. The tests were performed at 2 ± 2 °C and 60 ± 2 °C at 3 barg for 90 days and at 4 barg for 48 hours.

Figure 5

Test setup for extended operation tests for Kleenpak Presto sterile connector



The pressure in the Kleenpak Presto sterile connector assembly was maintained using an 11 liter pressure vessel. A pump was used to circulate the fluid through the Kleenpak Presto sterile connector assembly, pressure vessel, and in-line heat exchanger at a fixed flow rate of 5 L/minute. The desired temperature of circulating fluid was achieved by adjusting the temperature of the water bath.

First, the system was assembled as shown above. The pressure vessel was filled with 8 liters of diluted red dye solution. The pump was used to bleed out the air and fill the Kleenpak Presto sterile connector assembly with the dye solution. The pump speed was adjusted to circulate the dye solution through the Kleenpak Presto sterile connector assembly at ~5 L/minute.

The pressure vessel was pressurized to 3 barg (43.5 psig) or 4 barg (58 psig) pressure while continuing to recirculate the fluid. The water bath/heat exchanger was set to a pre-determined value to achieve the desired temperature in the Kleenpak Presto sterile connector assembly. The system pressure and temperatures were monitored at regular intervals for the duration of the experiment after the fluid in the assembly reached the desired temperature. The Kleenpak Presto sterile connector samples were visually inspected twice daily for fluid leakage for the course of the study.

3.3.3 Results

Table 4

Results of extended operation for the Kleenpak Presto sterile connector

Batch Number	Temperature	Test Duration	Pressure	Conditioning Device 1	Sterilization Method Device 2	Number of Devices Tested	Results
DV0001	2 ± 2 ºC	90 days	3 barg (43.5 psig)	AC	AC	6	Pass
DV0001	2 ± 2 ºC	90 days	3 barg (43.5 psig)	G	G	6	Pass
DV0001	2 ± 2 ºC	90 days	3 barg (43.5 psig)	AC	G	6	Pass
DV0001	60 ± 2 ºC	90 days	3 barg (43.5 psig)	AC	AC	6	Pass
DV0001	60 ± 2 ºC	90 days	3 barg (43.5 psig)	G	G	6	Pass
DV0001	60 ± 2 ºC	90 days	3 barg (43.5 psig)	AC	G	6	Pass
DV0001	2 ± 2 ºC	2 days	4 barg (58 psig)	G	G	6	Pass
DV0001	60 ± 2 ºC	2 days	4 barg (58 psig)	G	G	6	Pass
DV0004 DV0005	2 ± 2 ºC	2 days	4 barg (58 psig)	G	G	18	Pass
DV0004 DV0005	60 ± 2 ºC	2 days	4 barg (58 psig)	G	G	18	Pass

Where:

G refers to the device was gamma irradiated at 50 ± 5 kGy.

AC refers to the device was autoclaved at 130 ± 2 ºC for two cycles of 75 minutes.

Device 1 refers to one half of the pair tested, while Device 2 refers to the other half of the pair.

3.3.4 Conclusions

The results from this test show that the Kleenpak Presto sterile connector can be used continuously for 90 days at a maximum pressure of 3 barg and in a temperature range of 2 – 60 °C. It is also suitable for high pressure applications at 4 barg and in a temperature range of 2 – 60 °C for up to 2 days.

3.4 Bacterial Liquid Soiling Test

3.4.1 Introduction

The purpose of the bacterial liquid soiling test to evaluate the ability of a Kleenpak Presto sterile connector to maintain a sterile fluid path during connection and fluid transfer after the device is intentionally soiled by exposure to a 4.0% CMC solution inoculated with *Brevundimonas diminuta* bacteria (ATCC 19146).

3.4.2 Summary of the Test Method

A total of 180 unconnected Kleenpak Presto sterile connectors (90 pairs) from three manufacturing batches, were used for the test work. 60 devices were pre-sterilized via gamma-irradiation using 50 ± 5 kGy, 60 were pre-sterilized via autoclave using two (2) 75 minutes autoclave cycles at 130 ± 2 ºC, and the remaining 60 were pre-sterilized using a combination of irradiation and autoclave.

A summary of the bacterial liquid soiling test is explained below. For each set of Kleenpak Presto sterile connectors 2 glass bottles of 125 mL capacity was used. One glass bottle was filled with 100 mL of Tryptic Soy Broth and the second was left empty. The open ends were wrapped in autoclave tape. The glass bottles were then autoclaved. After autoclaving tubing and a pre sterilized Kleenpak Presto sterile connector were attached. A closed clamp was installed between the glass bottle and the Kleenpak Presto sterile connector.

Once each system was assembled the tamper resistance caps were removed from each connector. The peel strip was then immersed in 1.0 x 10^6 CFU/mL of *B. diminuta*. This was done for both Kleenpak Presto sterile connectors. The soiled devices were then dried and then connected as per the standard assembly procedure. The clamp between the glass bottle was opened. The tryptic soy broth (TSB) was then transferred through the Kleenpak Presto sterile connector assembly under gravity. The TSB material was flushed back and forth several times to ensure that the entire interior of the assembly was exposed. After flushing back and forth the TSB was collected in one bottle. The glass bottle containing the TSB was then clamped off and placed in an incubator at 30 ºC for 2 days.

After the 2 days incubation the samples were evaluated for bacterial growth by examining for turbidity. For each manufacturing batch one positive and one negative control was used.

As a positive control the protective peel strip was removed from the connector before soiling of the device with the *B. diminuta*.

A negative control was handled in an identical manner to the test plan describe above but was not exposed to *B. diminuta*.

Figure 6
Soiling of both ends of Kleenpak Presto sterile connector assemblies prior to connection.

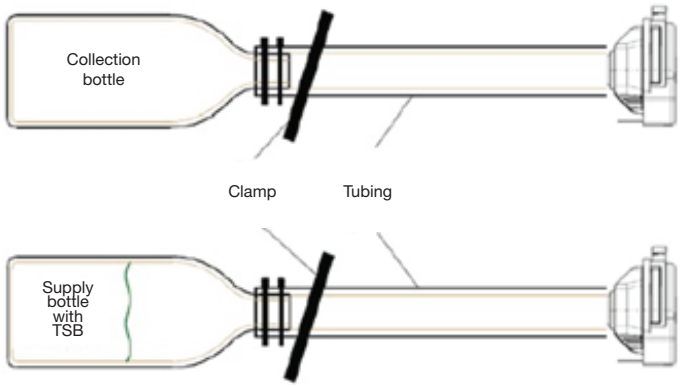
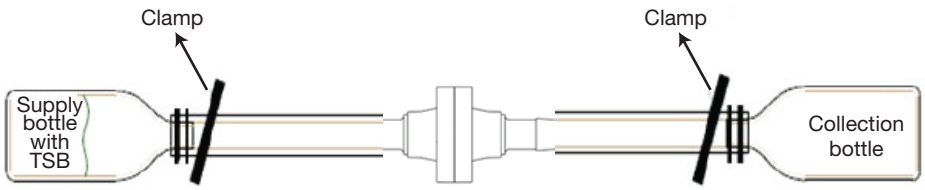


Figure 7
Assembly connected by Kleenpak Presto sterile connector for transfer of TSB



3.4.3 Results

Table 5

Liquid soiling test results of connected Kleenpak Presto sterile connector (gamma irradiated at 50 ± 5 kGy). Contamination level: 1.0 x 10^6 CFU/mL of B. diminuta

	Batch Number	Quantity Tested (Pairs)	Conditioning Device 1	Conditioning Device 2	Growth Observed
Batch 1	DV0001	6	G	G	None
Batch 2	DV0004	6	G	G	None
Batch 3	DV0005	6	G	G	None
Negative control	DV0001 DV0004 DV0005	1 1 1	G	G	None
Positive control	DV0001 DV0004 DV0005	1 1 1	G	G	Turbid growth

Table 6

Liquid soiling test results of connected Kleenpak Presto sterile connector (autoclaved at 130 °C ± 2 °C for 2 x 75 minutes cycles). Contamination level: 1.0 x 10^6 CFU/mL of B. diminuta

	Batch Number	Quantity Tested (Pairs)	Conditioning Device 1	Conditioning Device 2	Growth Observed
Batch 1	DV0001	6	AC	AC	None
Batch 2	DV0004	6	AC	AC	None
Batch 3	DV0005	6	AC	AC	None
Negative control	DV0001 DV0004 DV0005	1 1 1	AC	AC	None
Positive control	DV0001 DV0004 DV0005	1 1 1	AC	AC	Turbid growth

Table 7

Liquid soiling test results of connected Kleenpak Presto sterile connector (one part autoclaved at 130 °C ± 2 °C for 2 x 75 minutes cycles and another part gamma irradiated at 50 ± 5 kGy). Contamination level: 1.0 x 10^6 CFU/mL of B. diminuta

	Batch Number	Quantity Tested (Pairs)	Conditioning Device 1	Conditioning Device 2	Growth Observed
Batch 1	DV0001	6	AC	G	None
Batch 2	DV0004	6	AC	G	None
Batch 3	DV0005	6	AC	G	None
Negative control	DV0001 DV0004 DV0005	1 1 1	AC	G	None
Positive control	DV0001 DV0004 DV0005	1 1 1	AC	G	Turbid growth

3.4.4 Conclusion

A total of 180 Kleenpak Presto sterile connectors from three (3) different manufacturing batches were used for the test work. No bacterial growth was detected in the samples collected after the transfer through the Kleenpak Presto sterile connector units previously contaminated by a minimum challenge level of 10^6 CFU/mL of *B. diminuta* spores. The input verification tests indicated that the inoculation step described in the liquid soiling test procedure will ensure a sufficient amount of spores adhere to each Kleenpak Presto sterile connector to pass the acceptance criteria of $\geq 1.00 \times 10^6$ CFU per connector.

Negative controls did not show any growth, the positive controls demonstrated a confluent growth as expected. The soiling test demonstrated that the Kleenpak Presto sterile connectors can maintain a sterile pathway even in worst case bacterial challenging conditions.

The Kleenpak Presto sterile connector assemblies met the test requirements of the bacterial soiling challenge.

3.5 Chemical Compatibility

3.5.1 Introduction

The purpose of the chemical compatibility test is to evaluate the ability of the Kleenpak Presto sterile connector to remain intact after the transfer of aggressive fluids with a minimum working pressure of 15 barg.

3.5.2 Summary of the Test Method

A total of 80 pre-sterilized Kleenpak Presto sterile connectors were tested. 40 mini sanitary flange (MTC) devices were pre-sterilized using gamma irradiation at 50 ± 5 kGy, 40 (MTC) were pre-sterilized using two (2) 75 minutes autoclave cycles at 130 ± 2 °C. The devices were exposed to 8 aggressive fluids, referenced in Table 9. All tests were run at 2 L/min where the leak test (Section 2.2.2) was done at 3 barg while the burst test had a minimum of 15 barg.

3.5.3 Results

Table 9

Chemical compatibility burst test results of connected Kleenpak Presto sterile connector

	Temperature (°C)	Duration	Quantity Tested	Autoclaved Burst Pressure (barg/psig)	Gamma Irradiated Burst Pressure (barg/psig)
2 M NaOH	25	21 Days	10	23.4/339.5	25.1/364.0
HCL, pH 2	25	21 Days	10	23.7/343.8	21.1/306.4
20% DMA	40	24 Hrs	10	24.4/353.4	25.6/371.4
25% DMSO	25	24 Hrs	10	24.2/351.6	25.8/374.2
30% PEG	25	24 Hrs	10	19.8/286.6	25.8/373.6
1% Tween 80	25	24 Hrs	10	22.6/328.0	27.2/394.4
Ultrapure water	40	24 Hrs	10	25.8/374.8	24.8/359.2
100% Ethanol	40	24 Hrs	10	23.2/337.0	31.2/452.8

3.5.4 Conclusion

All Kleenpak Presto sterile connectors achieved the test requirement of holding 3 barg of pressure for 5 minutes with no bubbles after chemical exposure. All achieved the burst test requirement of > 15 barg.

4. Extractables

4.1 Introduction

The purpose of this test is to quantify and characterize the extractables from the Kleenpak Presto Sterile connector in various test fluids, which are representative of most biopharmaceutical fluids. These are based on recommendations of the BPOG (BioPhorum Operations Group) Extractables Working Group.

4.2 Summary of the Test Method

Extractables tests were performed on 144 connected Kleenpak Presto sterile connectors subjected to an appropriate pre-treatment: a total of 72 connected connector parts, from two separate manufacturing batches, were irradiated at 50 ± 5 kGy; a total of 72 connected connector parts from two separate manufacturing batches, were submitted to 1 autoclave cycle for 75 minutes at 130 ± 2 °C.

Table 10

Solvents and incubation conditions

Solvents					
Water	50% Ethanol	0.5 N NaOH	0.1 M Phosphoric acid	1% PS80	5 M NaCl
Incubation Conditions					
30 minutes at 25 °C	24 hours at 40 °C	7 days at 40 °C			

Table 11

Detail of test units quantity and batch number

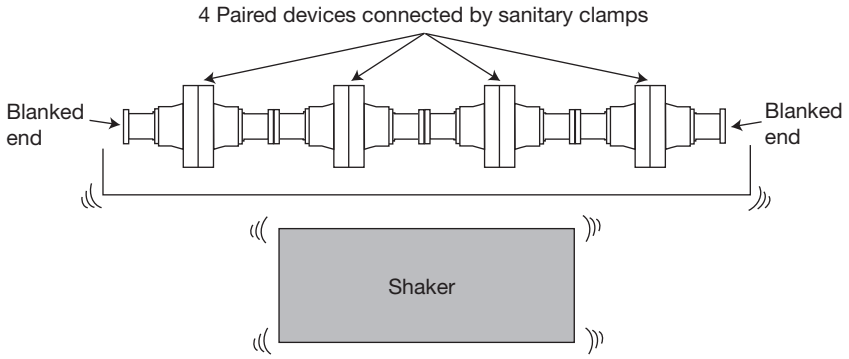
Batch Number	Quantity
DV0001	144
DV0002	144

Four pairs of connectors were connected in series for each of the six solvents, referenced in Table 10. The extraction was performed at ambient temperature (25 °C) for time 0 (≤30 mins) and at 40 °C for 24 hours and 7 days. A negative control was used while using the same test system conditions as above without incorporating the Kleenpak Presto sterile connector in the assembly.

Test systems were assembled for each solvent to accommodate the four assembled Kleenpak Presto sterile connectors as shown in Figure 8.

Figure 8

Extractables test schematic of four Kleenpak Presto sterile connectors in series



4.3 Results

4.3.1 Kleenpak Presto Sterile Connector Extractables Summary

Results of the test performed to quantify and identify the extractables from Kleenpak Presto sterile connectors having been submitted to 1 x 75 minutes autoclave cycle at 130 ± 2 ºC or to gamma irradiation at 50 ± 5 kGy; are given below.

Table 12

Summary of organic extractables from Kleenpak Presto sterile connector

			Worst-Case Amount, µg/cm²								
			Autoclaved			Gamma Irradiated					
Solvent	Compound[a]	CAS	½ hr	24 hrs	7 days	½ hr	24 hrs	7 days	Worst-Case Amount (µg/cm²)	Worst-Case Total Daily Intake (µg/day)[b]	PDE or Risk Index (µg/day)
Water	None detected	N/A	–	–	–	–	–	–	–	–	–
5 M NaCl	None detected	N/A	–	–	–	–	–	–	–	–	–
0.1 M H_3PO_4	None detected	N/A	–	–	–	–	–	–	–	–	–
0.5 N NaOH	Unknown (mass ions 477.2, 183.2)	N/A	–	–	0.418	–	–	–	0.418	0.0693	–
	Acetone	67-64-1	–	–	–	–	0.396	0.407	0.407	0.0674	21,000[c]
50% Ethanol	Hexamethylene diacrylate	1304[illegible]-33-4	–	6.80	14.9	–	–	–	14.9	2.47	5320[d]
	2,2-Dimethoxy-2-phenylaceto-phenone	2465[illegible]42-8	–	1.86	5.22	–	0.320	0.982	5.22	0.865	672[d]
	Benzil	134-81-6	–	2.71	4.69	–	–	0.424	4.69	0.777	1750[d]
	5-Hexenyl propionate	N/A	–	1.14	2.82	–	–	–	2.82	0.467	525[d]
	Unknown (mass ions 267.2, 245.5)	N/A	–	–	–	–	–	0.714	0.714	0.118	–
	1,1-Diethoxyethane	105-57-7	–	–	–	–	–	0.529	0.529	0.0876	3,500[c]
	Diphenyl sulfone	127-63-9	–	–	0.506	–	–	–	0.506	0.0838	22,200[c]
	Aliphatic alcohols (n = 10 - 20)	N/A	–	–	–	–	–	0.446	0.446	0.0739	14,000[c,e]
	Unsaturated fatty acid related compound	N/A	–	–	0.405	–	–	–	0.405	0.0671	–
	Unknown (mass ions 283.2, 278.3)	N/A	–	–	–	–	–	0.349	0.349	0.0578	–
1% PS80	None detected	N/A	–	–	N/A	–	–	N/A	–	–	–

Notes:

a. Only compounds at ≥ 0.25 µg/cm² are reported. For compounds detected at < 0.25 µg/cm² and details, please contact Pall Corporation.

b. Total Daily Intake (TDI) was calculated based on worst-case process conditions (5 L process volume using 2 assembled connectors and a 10 mL drug product daily dosage).

Example calculation:

TDI (Hexamethylene diacrylate) = 14.9 µg/cm² x 41.42 cm²/connector x 2 connectors x 1 / 5000 mL x 10 mL/day = 2.47 µg/day

c. Risk Index from Jenke et. al, PDA Pharm J Sci and Tech 2014, 68, Pages 407 – 455.

d. PDE values from Pall Internal Toxicological Report. For more details, please contact Pall Corporation.

e. Based on 1-Octadecanol (C-18 alipathic alcohol, CAS No. 112-92-5).

Table 13

Summary of elemental impurities from Kleenpak Presto sterile connector

Solvent	Elements[a]	ICH Q3D Class	Worst-Case Amount, µg/cm² Autoclaved	Gamma Irradiated
Water	Na	N/A	0.005	–
	Ca	N/A	0.006	0.007
5 M NaCl	Mg	N/A	0.018	0.014
	Ca	N/A	0.014	0.012
	Fe	N/A	0.007	–
	Zn	N/A	–	0.014
	K	N/A	0.207	–
0.1 M H_3PO_4	Na	N/A	–	0.013
	Al	N/A	–	0.006
	Ca	N/A	0.020	0.014
	Mn	N/A	–	0.013
	Fe	N/A	0.133	–
	Zn	N/A	0.006	–
	K	N/A	–	0.006
0.5 N NaOH	None detected	N/A	N/A	N/A
50% Ethanol	B	N/A	–	0.014
	Ti	N/A	–	0.014
	Fe	N/A	0.009	–
1% PS80	None detected	N/A	N/A	N/A

Note: No ICH Q3D elements were detected at ≥ 0.005 µg/cm² (20 ppb).

4.4 Conclusion

Water, 50% Ethanol, 0.5 N NaOH, 0.1 M Phosphoric Acid, 1% PS80, and 5 M NaCl were selected as generic model solvents to evaluate the extractables level that could be generated by the Kleenpak Presto sterile connector.

The study was conducted on 4 sets of Kleenpak Presto sterile connectors connected in series which were previously gamma irradiated (50 ± 5 kGy) or autoclaved (1 cycle, 75 min, 130 °C. The extracts were analyzed using a variety of analytical techniques aiming to detect various compounds that might be present. No Bis-phenol A was detected with limit of detection of 0.05 ppm.

5. Biological Safety, Cleanliness and Physicochemical Tests

5.1 Introduction

The purpose of these tests is to evaluate the biological and physicochemical suitability of the material of construction of the fluid path of the Kleenpak Presto sterile connector, which are as follows:

Table 14

Materials of construction of the fluid path in Kleenpak Presto sterile connector

Connector Body	Polyethersulfone (PES)
Seal	Platinum cured silicone

5.2 Biological Reactivity Tests, *in vitro*, USP <87>

5.2.1. Introduction

The purpose of this test is to determine the biological reactivity of mammalian cells (mouse fibroblasts) following contact with the material exposed to the fluid in Kleenpak Presto sterile connector as per USP <87> (MEM cytotoxicity).

5.2.2 Summary of the Test Method

Tests were performed as per USP <87>, MEM Elution Method. The samples to be tested, gamma-irradiated to 50 ± 5 kGy, and then totally immersed in the cell culture for 24 hours at 37 ºC in a 5% CO_2 incubator.

The extracts are then examined to determine any biological reactivity.

5.2.3 Results

Kleenpak Presto sterile connector passed the USP tests as described above. Copies of the test reports are available by contacting Pall Corporation.

5.2.4 Conclusion

The Kleenpak Presto sterile connector meets the USP <87> specifications.

5.3 Biological Reactivity Tests, *in vivo*, USP <88>

5.3.1 Introduction

The purpose of this test is to determine the biological response of animals to direct and indirect contact with the materials of construction of Kleenpak Presto sterile connector or injection of extract from the materials of construction of the Kleenpak Presto sterile connector, as per USP <88> for Class VI plastics.

5.3.2 Summary of the Test Method

Tests include USP Biological Reactivity Tests, *in vivo* for Class VI Plastics (121 ºC) as described in the United States Pharmacopoeia Chapter <88>. These tests were conducted using devices previously gamma irradiated at 50 ± 5 kGy and on the fluid contact material.

The Biological Reactivity Tests *in vivo* for Class VI-121 ºC Plastics as described in the United States Pharmacopoeia Chapter <88> 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)

USP <88> states that extracts may be prepared at one of three standard conditions: 50 ºC for 72 hours, 70 ºC for 24 hours, or 121 ºC for 1 hour. The most stringent condition not resulting in physical changes in the plastic is recommended; therefore the fluid contact material was extracted at 121 °C for 1 hour. The extracts were then used in the following tests to determine the biological effects they have:

- **Systemic Injection Tests**
  A systemic injection test is performed to evaluate systemic biological responses of animals to plastics, polymers and biomaterials by a single dose injection. The Sodium Chloride Injection and Alcohol Sodium Chloride Injection extracts are injected intravenously. The Polyethylene Glycol 400 and Vegetable Oil extracts are injected intraperitoneally.
- **Intracutaneous Tests**
  An intracutaneous test is performed to evaluate the local biological response to a single injection of an extract to produce tissue irritation. All four of the extracts listed above will be used for these tests.
- **Implantation Tests**
  Implantation test is also performed, in order to evaluate animal response in case of direct contact with Kleenpak Presto sterile connector construction materials.

5.3.3 Results

The Kleenpak Presto sterile connector passed the USP <88> tests as described above. Copies of the test reports are available by contacting Pall Corporation.

5.3.4 Conclusion

The Kleenpak Presto sterile connector meets the USP <88> specifications.

5.4 Physicochemical Test as per USP <661>

5.4.1 Introduction

The purpose of these tests is to evaluate the physicochemical suitability of Kleenpak Presto sterile connector for contact with parenterals.

5.4.2 Summary of the Test Method

Plastic devices that are intended for packaging products for parenteral use must meet the requirements of Physicochemical Testing-Plastics found in the current USP. These tests are designed to measure the properties of impurities extracted from plastics when leached with extraction medium over a specified period and temperature. Irradiated samples at a dose of 50 ± 5 kGy from Kleenpak Presto sterile connector were extracted at 70 ºC for 24 hours in purified water. Samples of the liquids are then tested for the following under USP <661> guidelines:

- **Buffering Capacity** - measures the alkalinity and acidity of the extracts.
- **Non-Volatile Residue (NVR)** - measures organic/inorganic residues soluble in extraction media.
- **Residue on Ignition** - performed when the NVR is greater than 5 milligrams.
- **Heavy Metals** - detects the presence of metals such as lead, tin, and zinc. These tests were performed on Kleenpak Presto sterile connector giving a surface area sample equivalent to 120 cm^2 /20 mL as per USP and previously gamma irradiated at 50 ± 5 kGy.

5.4.3 Results

The Kleenpak Presto sterile connector meets acceptance criteria for all three (3) tests.

Residue on ignition was not performed as NVR residue was lower than 5 mg.

Copies of the test reports are available by contacting Pall Corporation.

5.4.4 Conclusion

The Kleenpak Presto sterile connector meets the specification of the USP <661> and therefore is appropriate for parenteral use.

5.5 Particles Release Test, USP <788> Particulate Matter in Injections

5.5.1 Introduction

The purpose of this test is to determine the particulate level present in Kleenpak Presto sterile connector.

5.5.2 Summary of the Test Method

Ten (10) Kleenpak Presto sterile connector devices (gamma sterilized at 50 ± 5 kGy) were tested individually for particle level, as per United States Pharmacopoeia (USP <788>).

Each unit was rinsed with 100 mL water for injection and the fluid was tested for the presence of particles with sizes equal to or greater than 10 µm and 25 µm.

5.5.3 Results

Table 15

Kleenpak Presto sterile connector (sanitary flange) particle release results

Batch Details	Single Devices or Pairs	Quantity	Pass/Fail
DV0009	Single devices	10	Pass

5.5.4 Conclusion

The Kleenpak Presto sterile connectors meet the requirements of particulate testing performed as per the specifications of USP <788>.

5.6 Endotoxin Test, USP <85>

5.6.1 Introduction

The purpose of this test was to quantify the bacterial endotoxin level that may be present in Kleenpak Presto sterile connector.

5.6.2 Summary of the Test Method

Nine (9) assembled Kleenpak Presto sterile connector devices from each of three (3) batches were tested individually for endotoxin level as per USP <85>. All the samples were gamma sterilized at 50 ± 5 kGy.

5.6.3 Results

Table 16

Kleenpak Presto sterile connector (sanitary flange) endotoxin level

Kleenpak Presto Sterile Connector			
Sample Batch and Size	**Quantity Tested**	**Single Devices or Pairs**	**Endotoxin Level per Device (EU/device)**
Batch 1			
DV0003	3	Single devices	<6.66
Batch 2			
DV0004	3	Single devices	<6.66
Batch 3			
DV0005	3	Single devices	<6.66

All results as measured are below the endotoxin limit of 20.0 EU/device.

5.6.4 Conclusion

Rinse solutions from Kleenpak Presto sterile connectors meet acceptance criteria for endotoxin content when tested in accordance with USP <85>.

6. Shelf Life Studies

6.1 Introduction

Shelf life studies were conducted on Kleenpak Presto sterile connectors in order to establish up to a 3 year claim for devices gamma irradiated prior to storage. Shelf life studies for a 5 year claim for devices not sterilized prior to storage are also in progress.

6.2 Summary of the Test Method

Testing was undertaken using 44 Kleenpak Presto sterile connectors that had been gamma irradiated (50 ± 5 kGy) and stored at 50 ºC with 75% relative humidity for 138 days to simulate 3 years of storage. A sample of connectors (20) was subjected to leak testing (Section 2.2) and burst testing (Section 2.3). Another sample of connectors (20) was subjected to bacterial liquid soil testing (Section 3.4). For the soiling test two connectors were also used as positive controls and two connectors were used as negative controls.

Four pairs of gamma sterilized and aged Kleenpak Presto sterile connectors were also connected together, incubated in water for 24 hours at 40 ºC and Total Organic Carbon (TOC) measured to demonstrate the general extractables quantity. This was compared with TOC results from 4 new pairs of Kleenpak Presto sterile connectors subjected to the same incubation conditions.

Table 17

Summary of shelf life studies

Accelerated or Real-Time Aging Test Duration	Leak Test	Burst Test	Bacterial Liquid Soiling Test	Extractables
0 days	√	√	√	√
3 years accelerated[1]	√	√	√	√
3 years real-time[1]	√	√	√	x
5 years real-time[2]	√	√	√	x

1 A set of samples were gamma irradiated at 50 ± 5 kGy prior to storage
2 Sample sets were not sterilized prior to storage

6.3 Results

6.3.1 Leak Test

All ten (10) Kleenpak Presto sterile connector pairs passed leak testing as a constant stream of bubbles were not detected over a 5-minute period.

Table 17

Leak test results of Kleenpak Presto sterile connector (gamma irradiated at 50 ± 5 kGy prior to storage)

Kleenpak Presto Sterile Connector Batch Number	Number of Pairs Tested	Result
IZ4831	10	Pass

6.3.2 Burst Test

All connected Kleenpak Presto sterile connectors had a burst pressure higher than the maximum recommended operating pressure of 3 barg (43.5 psig) and the maximum short term operating pressure of 4 barg (59 psig), with adequate safety margin.

Table 18

Burst pressure of connected Kleenpak Presto sterile connector (gamma irradiated at 50 ± 5 kGy prior to storage)

Kleenpak Presto Sterile Connector Batch Number	Number of Pairs Tested	Minimum / Maximum Burst Pressure	Average Burst Pressure
IZ4831	10	21.7 / 36 barg (315 / 522 psig)	28.1 barg (408 psig)

6.3.3 Bacterial Liquid Soiling Test

All Kleenpak Presto sterile connector samples passed the soiling test with no bacterial growth. The positive control showed bacterial growth and the negative control did not show any bacterial growth.

Table 19

Liquid soiling test results of connected Kleenpak Presto sterile connectors (gamma irradiated at 50 ± 5 kGy prior to storage)

	Batch Number	Number of Connected Devices Tested	*Brevundimonas diminuta* Recovered in TSB Samples
Kleenpak Presto sterile connector	IZ4831	10	None
Negative control	IZ4831	1	None
Positive control	IZ4831	1	Growth

Concentration of Brevundimonas diminuta suspension: 1 x 10^6 cfu/mL

6.3.4 Extractables

The Kleenpak Presto sterile connector extract from 4 pairs of gamma sterilized and aged connectors incubated for 24-hour incubation in water at 40 ºC, had a TOC of <6 ppm compared to the water extract from new connectors that also had a TOC of <6 ppm under the same conditions. This indicated that the extractable levels remain low after 3 years of storage.

6.4 Conclusion

Following gamma irradiation and accelerated storage conditions to simulate 3 years of storage, Kleenpak Presto sterile connectors passed leak testing, burst testing and bacterial liquid soiling tests. Extractable levels were also shown to remain low after 3 years of storage.

The results indicated that the functionality of the Kleenpak Presto sterile connector remained intact after 3 years of storage.

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