Life Sciences

Validation Guide

USTR 2734a

Kleenpak™ Sterile Disconnector

*Filtration. Separation. Solution.*SM

Content

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 sterile disconnector for use in biopharmaceutical applications. The disposable Kleenpak sterile disconnector device provides an easy to use, single-use method for the separation of sterile fluid path assemblies even in an uncontrolled environment while maintaining the sterility and containment of the separated system fluid paths. It also provides optical visibility of the fluid path.

The disposable Kleenpak sterile disconnector consists of a male and a female connector made in polysulfone, which has excellent chemical resistance to aqueous solutions over a wide pH range.

The Kleenpak sterile disconnector is provided to the user in the open position, in which the fluid can be transferred through the device. When the operator wishes to disconnect the assemblies connected via the Kleenpak sterile disconnector, the operator will actuate the device and separate the Kleenpak sterile disconnector into its two halves (closed position). In the closed position, flow is blocked while the sterility and containment of the assembly fluid paths is maintained.

The testing program includes demonstration of the mechanical and functional performances of the Kleenpak sterile disconnector. It also includes the data demonstrating the level of extractables, the internal fluid path cleanliness in terms of particles and endotoxins, and the biological safety.

The Kleenpak sterile disconnectors presented in this report are referenced KPD01HB6, KPD01HB7 and KPD01HB10 and can be used respectively in conjunction with ½ in., ¼ in., and ⅜ in. ID tubing.

1.2 Summary of Conclusions

1.2.1 Mechanical Tests

Leak Test, Assembled Device

The air pressure leak test is conducted on connected Kleenpak sterile disconnectors with sealed fitting ports. It challenges the Kleenpak sterile disconnector to not leak in the connected position.

Kleenpak sterile disconnectors of part numbers KPD01HB6 (½ in.), KPD01HB7 (¼ in.) and KPD01HB10 (⅜ in.) were leak tested after being subjected to gamma irradiation. All tested Kleenpak sterile disconnectors passed this test which indicates that they can withstand gamma irradiation in the connected position at the maximum allowed dose of 50 +/-5 kGy without leaking.

Kleenpak sterile disconnectors KPD01HB6 (½ in.), KPD01HB7 (¼ in.) and KPD01HB10 (⅜ in.) were leak tested after being subjected to an autoclave cycle. All tested Kleenpak sterile disconnectors passed this test which indicates that they can withstand autoclave in the connected position up to the maximum allowed cycle of 75 min at 130 +/- 2 °C without leaking.

Burst Test

The purpose of this test was to demonstrate that the Kleenpak sterile disconnector in the connected position can withstand the maximum pressure rating of 3 barg (43.5 psi) with an appropriate safety margin.

Tests units previously gamma irradiated at 50+/-5 kGy or autoclaved at 130+/-2 °C for 2 cycles of 75 min, were then burst pressure tested in the connected position.

All Kleenpak sterile disconnectors had burst pressures higher than 9.03 barg (131 psig), giving an acceptable safety factor over the maximum recommended operating pressure in connected position of 3 barg (43.5 psig).

Male Half Burst Test

The purpose of this test was to demonstrate that the Kleenpak sterile disconnector male half in the disconnected position can withstand the maximum pressure rating of 3 barg (43.5 psi) with an appropriate safety margin.

Tests units previously gamma irradiated at 50+/-5 kGy or autoclaved at 130+/-2 °C for 2 cycles of 75 min and were then burst pressure tested in the disconnected position.

All Kleenpak sterile disconnectors male halves have burst pressures higher than 16.55 barg (240 psig), giving an acceptable safety factor over the maximum recommended operating pressure in connected position of 3 barg (43.5 psig).

Creep Rupture Test

The objective of a creep rupture test is to demonstrate the gradual deformation of a material under constant stress. Kleenpak sterile disconnector has been designed to be capable of operating at up to 3 barg (43.5 psig) for 4 weeks in continuous use and creep rupture test was then performed to validate those claims.

Kleenpak sterile disconnectors KPD01HB6, KPD01HB7 and KPD01HB10 were subjected to a creep rupture test in the connected position after gamma irradiation at 50+/-5 kGy or 2 autoclave cycles of 75 min at 130 +/-2 °C. The data demonstrate absence of measurable creep in under design conditions, and the large safety margins that have been incorporated into these claims.

Pressure Hold Test, Disconnected Device

The air pressure hold test is conducted on disconnected Kleenpak sterile disconnectors. The objective is to confirm seal integrity of male and female Kleenpak sterile disconnector assemblies after the disconnection operation.

Test units were subjected to a pressure hold test after an appropriate pre-treatment: gamma irradiation at 50 +/- 5 kGy or 2 autoclave cycles of 75 min at 130+/-2 °C.

The pressure hold test confirmed that each part of the Kleenpak sterile disconnector can be maintained under pressure (350 mbar) for 30 days without leakage.

1.2.2 Resistance to Freezing Storage

Freezing Storage, Assembled Device

Test units (assembled device) were subjected to a storage period of a minimum 30 days at -80 °C after having been submitted to gamma irradiation at 50+/-5 kGy. Assemblies were then submitted to burst test and leak test.

The storage hold test confirmed that the Kleenpak sterile disconnector can withstand a freezing / defrost cycle with storage held at -80 °C for a minimum of 30 days and that the functional properties remain unchanged.

Freezing Storage, Disconnected Device

Test units (disconnected device) were submitted to an appropriate pre-treatment: gamma irradiation at 50+/-5 kGy, or autoclaved twice at 130+/-2 °C for 75 min per cycle. Water was then passed through the units and those were then subjected to a storage period of 30 days at -80 °C. After the storage period, units were thawed and checked for leakage.

The storage test confirmed that each part of the Kleenpak sterile disconnector can be maintained for 30 days at -80 °C without any leakage. Instructions for Use (USD2727) detail recommended practices for storage.

1.2.3 Functional Tests

Water Flow Characteristics

The water flow vs. pressure drop data are presented for the Kleenpak sterile disconnector. They can be used in conjunction with the pressure drop characteristics of other system components (e.g. filter, tubing) as a basis to define a sizing for systems using Kleenpak sterile disconnectors.

Soiling (Microbial Ingress) Test

Kleenpak sterile disconnectors were submitted to a soiling (Microbial Ingress) test that is comprised of an intentional contamination by a suspension of *Brevundimonas diminuta* bacteria during a growth medium transfer and the disconnection operation.

The presented soiling test provides assurance that the Kleenpak sterile disconnector is able to maintain a sterile fluid path during fluid transfer, and upon disconnection.

Autoclave Sterilization Testing With Biological Indicators

Kleenpak sterile disconnector assemblies were subjected to 1 autoclave cycle of 30 min at 122+/-1 °C, with a spore strip of *B. stearothermophilus* ATCC 7953 placed inside each assembly.

This test demonstrated that Kleenpak sterile disconnector can be rendered sterile by an autoclave cycle of 30 min at 122+/-1 °C.

Determination of Residual Volume

The residual volume that has been recorded on Kleenpak sterile disconnector from part number KPD01HB6, KPD01HB7 and KPD01HB10 is 29 µL max with an average value below 18 µL.

To ensure absence of spillage, units should be fully drained prior to the disconnection operation as described in Instructions for Use (USD2727).

1.2.4 Extractables

Water and ethanol were selected as generic model solvents to evaluate the level of extractables that could be generated by the Kleenpak sterile disconnector. Water is a polar solvent representative of most aqueous process fluid solutions, while Ethanol is a semi-polar solvent that represents a “worst than worst case” for typical bioprocess formulations, including presence of surfactants.

Study was conducted on a set comprising 4 Kleenpak sterile disconnectors previously gamma-irradiated or autoclaved. Extraction was conducted with water at 60-65 °C and ethanol at 20-25 °C for 24 h. Extracts were analyzed using analytical techniques aiming to detect various compounds that could be present.

The results indicate that the extractables level in tested contact fluids was extremely low (below 1 ppm for most of the detected compounds).

Data are applicable to all variants, using the scaling factor.

1.2.5 Biological Safety, Particulates and Physico-Chemical Tests

Data are applicable to all variants.

Biological Tests, *in vitro*

The purpose of this test is to determine the biological reactivity of mammalian cell cultures following contact with the material to which the fluid is exposed when passing through the Kleenpak sterile disconnector as per USP <87> (MEM Elution Cytotoxicity).

The Kleenpak sterile disconnector meets the specifications of the USP <87> (MEM Elution Cytotoxicity).

Biological Tests, *in vivo*

The purpose of this test is to determine the biological reactivity following contact with the material to which the fluid is exposed when passing through the Kleenpak sterile disconnector as per USP <88>.

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

Particle Release

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

Rinse solution from the Kleenpak sterile disconnector meets the requirements of particulate testing performed as per the specifications of USP <788> (for volumes below 100 mL).

Endotoxins Test

The purpose of this test is to quantify the bacterial endotoxins level that may be present in the Kleenpak sterile disconnector.

The endotoxin level tested as per <USP85> of rinse solution from the Kleenpak sterile disconnector was below 0.25 EU/mL (specification of Purified Water).

Physicochemical Test

The purpose of this test was to evaluate the physicochemical suitability on each part of the Kleenpak sterile disconnector for contact with parenterals. The physicochemical tests are designed to measure the properties of impurities (buffering capacity, non-volatile residue, residue on ignition, heavy metal content) extracted from plastics when leached with extraction medium over a specified period and temperature.

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

1.2.6 Shelf Life Studies

Shelf life studies are conducted in order to establish a 3-year shelf life for Kleenpak sterile disconnector after gamma irradiation and a 5-year shelf life for non sterile products.

Results for a 3 years shelf life (accelerated storage conditions) are available at the time this guide is written. Results for longer period will be available as developed.

Samples of Kleenpak sterile disconnectors were subjected to a leak test, a burst test, a pressure hold test, a soiling test, and a NVR evaluation on samples exposed to 3 year shelf life in accelerated conditions.

The tests indicate that the functionality of the Kleenpak sterile disconnectors remained intact.

2. Mechanical Tests

2.1 Introduction

The following tests were carried out on Kleenpak sterile disconnectors to check their ability to withstand mechanical stress after gamma irradiation or autoclave pre-treatment:

- Leak Test
- Burst Test
- Male Burst Test
- Creep Rupture Test
- Pressure Hold Test

2.2 Leak Test

Introduction

The purpose of this test was to demonstrate that Kleenpak sterile disconnector can withstand the autoclave sterilization cycle or the gamma irradiation in the connected position with an appropriate safety margin.

Summary of the Test Method

Kleenpak sterile disconnectors (assembled devices) previously gamma irradiated at 50 +/-5 kGy or submitted to 2 autoclave cycles of 75 min at 130 +/-2 °C were subjected after this pre-treatment to the in-process leak test. Tested quantities and batch number per part number are detailed below:

Table 1

Detail of test units quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number
KPD01HB6 (½ in.)	180	T09/00071H T09/00072L T09/00073N T10/00027H T10/00028N T10/00029L
KPD01HB7 (¼ in.)	60	T11/00067H T11/00067N T11/00067/L
KPD01HB10 (⅜ in.)	60	T11/00066H T11/00066N T11/00066/L

This test has been designed to verify the absence of leak of manufactured devices during the process. Each unit is pressurized with air at 3.1 bar and pressure decay is recorded. A max allowable leak rate has been defined, for which units do not demonstrate any visible leak when placed in a water bath. They also met the acceptance criteria of the soiling (microbial ingress) test as described in Section 4.3.

Results

Kleenpak sterile disconnectors were leak tested in the connected position at a pressure of 3.1 bar (air). All units passed this test (leak-free).

Conclusion

Kleenpak sterile disconnectors from part number KPD01HB6 (½ in.), KPD01HB7 (¼ in.) and KPD01HB10 (⅜ in.) were leak tested in the connected position after being subjected to gamma irradiation. All connected Kleenpak sterile disconnectors passed this test which indicates that the connected Kleenpak sterile disconnector can withstand gamma irradiation at the maximum allowed dose (50 +/-5 kGy) without leaking.

Kleenpak sterile disconnectors from part number KPD01HB6 (½ in.), KPD01HB7 (¼ in.) and KPD01HB10 (⅜ in.) were leak tested in the connected position after being subjected to 2 autoclave cycles. All connected Kleenpak sterile disconnectors passed this test which indicates that connected Kleenpak sterile disconnector can withstand autoclave up to the maximum allowed cycle of 75 min at 130 +/-2 °C without leaking.

2.3 Burst Test

Introduction

The purpose of this test was to demonstrate that connected Kleenpak sterile disconnectors can withstand the maximum pressure rating of 3 barg (43.5 psi) with an appropriate safety margin.

Summary of the Test Method

The burst test was performed on assembled Kleenpak sterile disconnectors subjected to an appropriate pre-treatment: gamma irradiation at 50 +/-5 kGy or 2 autoclave cycles of 75 min at 130 +/-2 °C. Tested quantities and batch number per part number are detailed below :

Table 2

Detail of test units quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number
KPD01HB6 (½ in.)	120	T09/00071H T09/00072L T09/00073N
KPD01HB10 (⅜ in.)	60	T11/00066H T11/00066N T11/00066/L
KPD01HB7 (¼ in.)	60	T11/00067H T11/00067N T11/00067/L

Before the burst tests were performed, Kleenpak sterile disconnectors were connected to suitable fittings and a calibrated pressure gauge placed upstream. The Kleenpak sterile disconnector assemblies were filled with water, the air purged and one end blanked off. Prior to starting tests, units were maintained at 40 °C for a minimum of 15 min to ensure temperature was equilibrated.

The other end of the assembly was connected to a pressure source and the pressure increased until failure of the Kleenpak sterile disconnector occurred. The types of failure such as shut off O-ring extrusion, shut off clip broken, plunger forced out of the connector were recorded.

Results

Table 3

Burst pressure of Kleenpak sterile disconnector (Gamma irradiated at 50 +/-5 kGy)

Part Number (Connector Size)	Batch Number	Burst Pressure Minimum/Maximum (barg / psig)	Burst Pressure Average (barg / psig)
KPD01HB6 (½ in.)	T09/00071H	12.13 - 14.48 / 176 -210	13.42 / 194.6
	T09/00072L	12.07 - 13.79 / 175-200	13.09 / 189.9
	T09/00073N	12.40 -14.34 / 180 -208	13.34 / 193.6
KPD01HB10 (⅜ in.)	T11/00066 H	12.48 - 13.86 / 181 -201	13.13 / 190.4
	T11/00066 N	13.10 - 14.62 / 190-212	13.80 / 200.2
	T11/00066 L	13.24 -14.76 / 192 -214	13.86 / 201.0
KPD01HB7 (¼ in.)	T11/00067 H	13.31 - 14.13 / 193 -205	13.60 / 197.2
	T11/00067 N	12.48 - 14.62 / 181-212	13.71 / 198.9
	T11/00067 L	12.41 -14.62 / 180 -212	13.66 / 198.1

Table 4

Burst pressure of Kleenpak sterile disconnector (Autoclaved 2 x 75 min at 130 +/- 2 °C)

Part Number (Connector Size)	Batch Number	Burst Pressure Minimum/Maximum (barg / psig)	Burst Pressure Average (barg / psig)
KPD01HB6 (½ in.)	T09/00071H	9.03 - 13.44 / 131 - 195	11.35 / 164.55
	T09/00072L	10.41 - 14.34 / 151 - 208	11.74 / 170.25
	T09/00073N	9.79 - 14.41 / 142 - 209	13.07 / 189.6
KPD01HB10 (⅜ in.)	T11/00066 H	9.72 - 12.76 / 141 - 185	11.47 / 166.3
	T11/00066 N	10.00 - 11.24 / 145 - 163	10.62 / 154.1
	T11/00066 L	9.79 - 11.99 / 142 - 174	10.77 / 156.2
KPD01HB7 (¼ in.)	T11/00067 H	10.62 - 11.99 / 154 - 174	11.33 / 164.3
	T11/00067 N	10.27 - 13.03 / 149 - 189	10.93 / 158.5
	T11/00067 L	9.65 - 12.55 / 140 - 182	11.21 / 162.6

Conclusion

Kleenpak sterile disconnectors from part number KPD01HB6 (½ in.), KPD01HB7 (¼ in.) and KPD01HB10 (⅜ in.) were burst pressure tested after being submitted to a gamma irradiation cycle (50+/-5 kGy) or an autoclave cycle (2 x 75 min at 130+/-2 °C). All Kleenpak sterile disconnectors had burst pressures higher than 9.03 barg (131 psig) giving an acceptable safety factor over the maximum recommended operating pressure of 3 barg (43.5 psig).

2.4 Burst Test of Male Half

Introduction

The purpose of this test was to demonstrate that the disconnected male half of Kleenpak sterile disconnector can withstand the maximum pressure rating of 3 barg (43.5 psig) with an appropriate safety margin.

Summary of the Test Method

The burst test was performed on disconnected male half of Kleenpak sterile disconnectors subjected to an appropriate pre-treatment: gamma irradiation at 50 +/-5 kGy and/or 2 autoclave cycles of 75 min at 130 +/-2 °C.

Tested quantities and batch number per part number are detailed below:

Table 5

Detail of test units quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number
KPD01HB6 (½ in.)	30	T10/00030H T10/00031N T10/00032L
KPD01HB10 (⅜ in.)	60	T11/00066H T11/00066N T11/00066/L
KPD01HB7 (¼ in.)	60	T11/00067H T11/00067N T11/00067/L

Before the burst tests were performed, the male half Kleenpak sterile disconnector is connected to suitable fittings and a calibrated pressure gauge placed upstream. The Kleenpak sterile disconnector male halves were filled with water, the air purged and one end blanked off. Prior to starting test, units were maintained at 40 °C for a minimum of 15 min to ensure temperature was equilibrated.

The other end of the assembly was connected to a pressure source and the pressure increased until failure of the Kleenpak sterile disconnector occurred. The major type of failure was a breakage of the 4 connecting ribs after a stretching of the plunger part.

Results

Table 6

Burst pressure of Kleenpak Sterile Disconnector Male Half (gamma irradiated at 50 +/-5 kGy)

Part Number (Connector Size)	Batch Number	Burst Pressure Minimum/Maximum (barg / psig)
KPD01HB6 (½ in.)	T10/00030H	38.44-45.89 / 558-666
	T10/00031N	36.52-50.85 / 530-738
	T10/00032L	38.17- 49.26 / 554-715
KPD01HB10 (⅜ in.)	T11/00066 H	>16.55 / >240*
	T11/00066 N	>16.55 / >240*
	T11/00066 L	>16.55 / >240*
KPD01HB7 (¼ in.)	T11/00067 H	>16.55 / >240*
	T11/00067 N	>16.55 / >240*
	T11/00067 L	>16.55 / >240*

**240 psi was the maximum obtainable pressure for the test rig. No failures were observed from the tests units. The minimum pressure represents a high safety margin comparing to the max. recommended operating pressure and was then recorded.*

Table 7

Burst pressure of Kleenpak Sterile Disconnector Male Half (Autoclaved 2 x 75 min at 130 +/- 2 °C)

Part Number (Connector Size)	Batch Number	Burst Pressure Minimum/Maximum (barg / psig)
KPD01HB10 (⅜ in.)	T11/00066 H	>16.55 / >240*
	T11/00066 N	>16.55 / >240*
	T11/00066 L	>16.55 / >240*
KPD01HB7 (¼ in.)	T11/00067 H	>16.55 / >240*
	T11/00067 N	>16.55 / >240*
	T11/00067 L	**>16.55 / >240***

**240-260 psi which was the maximum obtainable pressure for the test rig at which no failure was observed. The minimum pressure represents a high safety margin comparing to the max. recommended operating pressure and was then recorded.*

Conclusion

Kleenpak sterile disconnector male units for all 3 variants KPD01HB6 (½ in.), KPD01HB7 (¼ in.) and KPD01HB10 (⅜ in.) were burst pressure tested after an appropriate pre-treatment: gamma irradiation at 50 +/-5 kGy and/or 2 autoclave cycles of 75 min at 130 +/-2 °C.

Burst pressures recorded were higher than 16.55 barg (240 psig) giving an acceptable safety factor over the maximum recommended operating pressure of 3 barg (43.5 psig).

2.5 Creep Rupture Test

Introduction

The objective of a creep rupture test is to demonstrate the gradual deformation of a material under constant stress. A creep rupture test was then performed in order to check the strength and the stability of Kleenpak sterile disconnectors over extended period of time whilst under pressure.

Summary of the Test Method

Creep rupture testing was performed on assembled Kleenpak sterile disconnector subjected to an appropriate pre-treatment: gamma irradiated at 50 +/-5 kGy or 2 autoclave cycles of 75 min at 130 +/-2 °C. Tested quantities and batch number per part number are detailed below:

Table 8

Detail of test units quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number
KPD01HB6 (½ in.)	22	T09/00052H T09/00053N T09/00054L T09/00071H T09/00072L T09/00073N
KPD01HB10 (⅜ in.)	2*	T11/00066H T11/00066N T11/00066/L
KPD01HB7 (¼ in.)	5*	T11/00067H T11/00067N T11/00067/L

** Taken at random from listed batches*

Before the creep rupture testing was performed, the Kleenpak sterile disconnector assemblies were filled with water, the air purged and one end blanked off. Prior to starting tests, units were maintained at 40 °C for a minimum of 15 min to ensure temperature was equilibrated.

The other end of the assembly was connected to a pressure source and the pressure increased to a fixed pressure. Kleenpak sterile disconnectors were left under pressure until failure is noticed. The time at which the failure occurred was recorded.

Results

Kleenpak sterile disconnectors from part number KPD01HB6, KPD01HB7 and KPD01HB10 were held at different pressures between 5.2 barg (75 psig) and 10.3 barg (150 psig) for time periods up to 1000 h. Data generated confirmed that the Kleenpak sterile disconnectors can be used at a maximum pressure of 3 bar up to 4 weeks (672 h) with safety margin.

Conclusion

The creep-rupture data generated using typical Kleenpak sterile disconnectors (previously gamma irradiated at 50+/-5 kGy or autoclaved at 130 +/-2 °C for two 75 min cycles) demonstrate that Kleenpak sterile disconnector whatever the connection size is; can be used up to 4 weeks (672 h) at the maximum pressure of 3 bar. This claim incorporates a large safety margin.

2.6 Pressure Hold Test

Introduction

The pressure hold test was performed in order to confirm the seal integrity of the Kleenpak sterile disconnector after having been disconnected.

Summary of the Test Method

Pressure hold tests were performed on disconnected Kleenpak sterile disconnectors subjected to an appropriate pre-treatment: gamma irradiation 50 +/-5 kGy or 2 autoclave cycles of 75 min at 130 +/-2 °C. Tested quantities and batch number per part number are detailed below:

Table 9

Detail of test units quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number
KPD01HB6 (½ in.)	120	T09/00052H T09/00053N T09/00054L
KPD01HB10 (⅜ in.)	60	T11/00066H T11/00066N T11/00066/L
KPD01HB7 (¼ in.)	60	T11/00067H T11/00067N T11/00067/L

Before the pressure hold testing was performed, each device was disconnected in two halves. Male and female parts were connected to appropriate fittings through the hose barb adaptors. A pressure gauge was placed upstream. The pressure was increased up to 350 mbar (5 psi) applied for 3 minutes. After that time, pressure was cut off and units were left under pressure for 15 min. Any air leak was recorded and units visually inspected for air bubbles under water.

10 female halves and 10 male halves for each configuration were then taken randomly and connected again to manifold. The pressure was increased up to 350 mbar (5 psi) and units were left under pressure for 30 days. Any air leak was recorded and units visually inspected for air bubbles under water at the end of this test period.

Results

No leak was detected in any samples for the 15 min test and no leak was detected after 30 days under pressure.

Conclusion

Kleenpak sterile disconnectors from part number KPD01HB6 (½ in.), KPD01HB7 (¼ in.) and KPD01HB10 (⅜ in.) were submitted to a pressure hold test after being submitted to a gamma irradiation cycle (50+/-5 kGy) or an autoclave cycle (2 x 75 min at 130+/-2 °C).

The pressure hold test confirmed that each part (after having been disconnected) of the Kleenpak sterile disconnector can be maintained under pressure (350 mbar) for 30 days.

3. Resistance to Freezing Storage

3.1 Introduction

The following tests were carried out on Kleenpak sterile disconnectors to check their ability to withstand freezing conditions and to operate after being stored in freezing conditions.

3.2 Freezing Storage, Assembled Device

Introduction

The purpose of this test was to demonstrate that the connected Kleenpak sterile disconnector is capable of operating after being stored at -80 °C.

Summary of the Method

Kleenpak sterile disconnector KPD01HB7 (¼ in.) and KPD01HB10 (⅜ in.) were irradiated at 50 +/- 5 kGy and then stored at -80°C for 30 days. These results apply also to KPD01HB6 as the functional part remains unchanged between the 3 variants.

After the end of the storage period, assemblies were left at ambient temperature for 24 hours, and then submitted to leak test and burst test as described in this document section 2.2 and 2.3.

Tested quantities and batch number per part number are detailed below:

Table 10

Detail of test units quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number
KPD01HB10 (⅜ in.)	12	T11/00066H T11/00066N T11/00066/L
KPD01HB7 (¼ in.)	12	T11/00067H T11/00067N T11/00067/L

Results

Table 11

Burst pressure of Kleenpak sterile disconnector (Gamma irradiated at 50 +/- 5 kGy) after minimum 30 days of storage at -80 °C

Part Number (Connector Size)	Batch Number	Leak Test	Burst Pressure (average of the 4 samples)
KPD01HB10 (⅜ in.)	T11/00066 H	pass	13.10 barg (190 psig)
	T11/00066 N	pass	13.76 barg (199.5 psig)
	T11/00066 L	pass	13.70 barg (198.75 psig)
KPD01HB7 (¼ in.)	T11/00067 H	pass	13.27 barg (192.5 psig)
	T11/00067 N	pass	13.41 barg (194.5 psig)
	T11/00067 L	pass	13.60 barg (197.25 psig)

Conclusion

Kleenpak sterile disconnectors from part number KPD01HB7 (¼ in.) and KPD01HB10 (⅜ in.) were irradiated at 50 +/-5 kGy and then stored for 30 days at -80 °C. These results apply also to KPD01HB6 as the functional part remains unchanged between the 3 variants.

The storage hold test confirmed that connected Kleenpak sterile disconnector can be stored at -80 °C for 30 days and that the functional properties remained unchanged.

3.3 Freezing Storage, Disconnected Device

Introduction

The purpose of this test was to demonstrate that disconnected Kleenpak sterile disconnectors are capable of storing fluid at -80 °C.

Summary of the Method

Freezing storage tests were performed on initially connected Kleenpak sterile disconnectors KPD01HB7 (¼ in.) and KPD01HB10 (⅜ in.) subjected to an appropriate pre-treatment: gamma irradiation at 50 +/-5 kGy or 2 autoclave cycles of 75 min at 130 +/-2 °C. Results obtained with KPD01HB7 and KPD01HB10 samples apply also to KPD01HB6 as the functional part remains unchanged between the 3 variants.

Tested quantities and batch number per part number are detailed below:

Table 12

Detail of test units, quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number
KPD01HB10 (⅜ in.)	12	T11/00066H T11/00066N T11/00066/L
KPD01HB7 (¼ in.)	12	T11/00067H T11/00067N T11/00067/L

Tubing was attached to each side of the Kleenpak sterile disconnector and water was passed through and drained. A clamp was then placed on the tubing and the device was disconnected into its male and female parts. Each assembly was then stored at -80 °C for 30 days.

After the end of the storage period, Kleenpak sterile disconnector units were left at ambient temperature for 24 hours, and then checked for water leakage.

Results

No leak was observed on any Kleenpak sterile disconnector halves that have been submitted to storage of 30 days at -80 °C.

Conclusion

Kleenpak sterile disconnectors from part number KPD01HB7 (¼ in.) and KPD01HB10 (⅜ in.) were irradiated at 50 +/-5 kGy or 2 autoclave cycles of 75 min at 130 +/-2 °C and then stored for 30 days at -80 °C after water flushing and disconnection. They were then checked for leakage.

The storage test confirmed that each part of the Kleenpak sterile disconnector can be maintained for 30 days at -80 °C without any leakage. These results apply also to KPD01HB6 as the functional part remains unchanged between the 3 variants.

Please refer to Instructions For Use (USD2727) for storage instructions: units should be fully drained prior to storage.

4. Functional Tests

4.1 Introduction

The objective of the functional tests was to provide informations on the functional performances of the Kleenpak sterile disconnector. This conducted were:

- Determining the water flow characteristics
- Checking ability for the Kleenpak sterile disconnector to be used in contaminated environment (Soiling test)
- Checking the ability to be rendered sterile by autoclaving
- Determining if any fluid is spilled during disconnection

4.2 Determination of Water Flow Characteristics

Introduction

The purpose of these tests was to determine the water flow rates of connected Kleenpak sterile disconnectors when subjected to differential pressures.

Summary of the Method

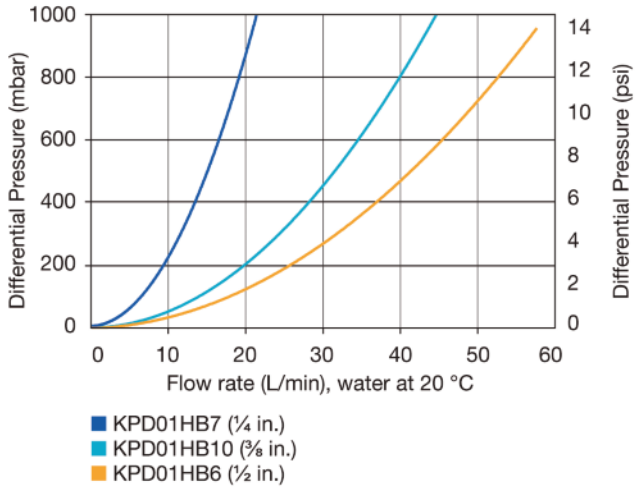
Three Kleenpak sterile disconnectors of each variant were tested. Deionized water was pumped through the assembled Kleenpak sterile disconnectors at various flow rates and the differential pressure was recorded via pressure transducer on the upstream and downstream side of the connection. All data were corrected to a standard temperature of 20 °C.

Results

The water flow vs. differential pressure characteristics of Kleenpak sterile disconnector are shown on Figure 1.

Figure 1

Water flow vs. differential pressure characteristics for Kleenpak sterile disconnector



Conclusion

The water flow/pressure drop data presented in Figure 1 for connected Kleenpak sterile disconnectors 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 the disposable system employing the Kleenpak sterile disconnector.

4.3 Soiling (Microbial Ingress) Test

Introduction

The purpose of the soiling test was to evaluate the ability of a Kleenpak sterile disconnector device to maintain a sterile fluid path during fluid transfer, and upon disconnection.

Summary of Test Method

In order to demonstrate that an external microbial soiling of the device does not impact the ability of the Kleenpak sterile disconnector to provide a sterile pathway before disconnection and to maintain internal sterility during the disconnection, the Kleenpak sterile disconnector devices were exposed to a suspension of *Brevundimonas diminuta* ATCC 19146 containing a minimum of 10^7 CFU per mL:

- For the verification of the lack of microbial ingress in assembled device, each Kleenpak sterile disconnector assembly was dipped in a bacterial suspension of *B. diminuta* for about 30 seconds before performing a Tryptic Soya Broth (TSB) transfer.
- For the verification of the lack of microbial ingress during the disconnection, the Kleenpak sterile disconnector assembly was dipped in the *B. diminuta* suspension for about 30 seconds before the operation of disconnection.

Tested quantities and batch number per part number are detailed below:

Table 13

Detail of test units quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number
KPD01HB6 (½ in.)	47	T10/00027 T10/00028 T10/00029
KPD01HB10 (⅜ in.)	36	T11/00066H T11/00066N T11/00066/L

Prior to test, Kleenpak sterile disconnectors were submitted to 2 autoclave cycles (130+/-2°C for 75 min) or to a gamma irradiation cycle (50 +/- 5 kGy) which was followed by an autoclave cycle for test purpose.

The following procedure was followed for each of the tests:

A presterilized glass transfer vessel was connected to one side of the Kleenpak sterile disconnector (via short presterilized tubing length) and then filled with sterile tryptic soy broth (TSB). A presterilized glass collection vessel was connected to the opposite side of the Kleenpak sterile disconnector. The presterilized Kleenpak sterile disconnector devices were dipped for 30 s in the *B. diminuta* solution. TSB was transferred through the Kleenpak sterile disconnector to the glass collection vessel using a low-pressure air source.

The devices were then again dipped into the *B.diminuta* suspension. Each Kleenpak sterile disconnector device was then actuated and separated in its male and female parts (Figure 3). A volume of presterilized TSB was used to rinse the pathway of each of the 2 disconnected parts and aseptically collected.

Figure 2
Assembly to transfer TSB through the Kleenpak sterile disconnector

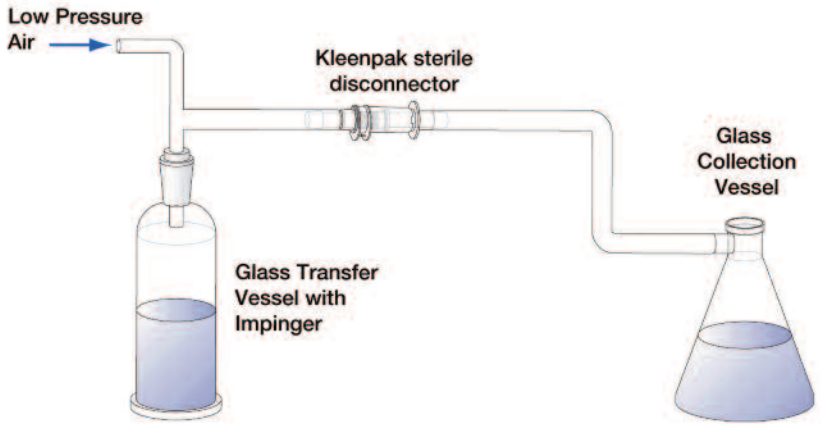
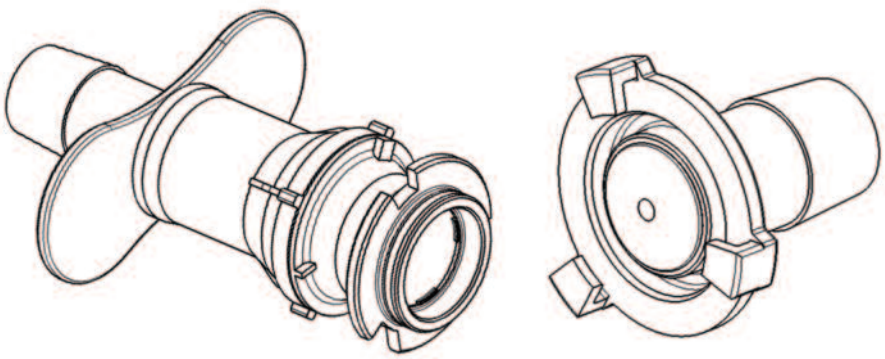


Figure 3
Kleenpak sterile disconnector parts after disconnection



Each collected TSB sample is incubated at 30 °C for 7 days.

Positive and negative controls were performed as part of the soiling tests. The negative control was not exposed to the bacterial solution. The positive control was not actuated before being separated in order to leave the pathway exposed to the *B.diminuta* suspension.

Absence of colonies of *B. diminuta* on the recovery membrane or absence of turbidity would demonstrate that the sterility of the pathway was maintained.

Results

Table 14

Soiling test results of Kleenpak sterile disconnector (Gamma irradiated at 50 +/- 5 kGy + autoclave cycle)

		Number of CFU of *B.diminuta* Recovered in TSB Samples	
Part Number	**Sample Number**	**Male Part Post Disconnection**	**Female Part Post Disconnection**
KPD01HB6	T10/00027	0	0
KPD01HB6	T10/00028	0	0
KPD01HB6	T10/00029	0	0
KPD01HB6	Negative control	0	0
KPD01HB6	Positive control	Confluent growth	Confluent growth

Concentration of the *B. diminuta* suspension: 1.9 x 10^7 cfu/mL

Table 15

Soiling test results of Kleenpak sterile disconnector (Autoclaved twice at 130 +/-2°C)

		Number of CFU of *B.diminuta* Recovered in TSB Samples	
Part Number	**Sample Number**	**Male Part Post Disconnection**	**Female Part Post Disconnection**
KPD01HB6	T10/00027	0	0
KPD01HB6	T10/00028	0	0
KPD01HB6	T10/00029	0	0
KPD01HB6	Negative control	0	0
KPD01HB6	Positive control	Confluent growth	Confluent growth

Concentration of the *B. diminuta* suspension: 1.9 x 10^7 cfu/mL

Table 16

Soiling test results of Kleenpak sterile disconnector (Gamma irradiated at 50 +/- 5 kGy + autoclave cycle)

		Turbidity in TSB Samples	
Part Number	**Sample Number**	**Male Part Post Disconnection**	**Female Part Post Disconnection**
KPD01HB10	T11/00066H	no	no
KPD01HB10	T11/00066N	no	no
KPD01HB10	T11/00066/L	no	no
KPD01HB10	Negative control	no	no
KPD01HB10	Positive control	yes	yes

Concentration of the *B. diminuta* suspension: 2.7 x 10^7 cfu/mL

Table 17

Soiling test results of Kleenpak sterile disconnector (Autoclaved twice at 130 +/-2°C)

		Turbidity in TSB Samples	
Part Number	Sample Number	Male Part Post Disconnection	Female Part Post Disconnection
KPD01HB10	T11/00066H	no	no
KPD01HB10	T11/00066N	no	no
KPD01HB10	T11/00066/L	no	no
KPD01HB10	Negative control	no	no
KPD01HB10	Positive control	yes	yes

Concentration of the *B. diminuta* suspension: 4.0 x 10^7 cfu/mL

Conclusion

No bacterial growth was detected in the TSB samples collected during the transfer through the Kleenpak sterile disconnector units and the disconnection in 2 halves, previously contaminated by a *B. diminuta* suspension.

Negative control did not show any growth, the positive controls demonstrated a presence of growth as expected.

The soiling test demonstrated that the Kleenpak sterile disconnector can maintain a sterile pathway even in highly bacterial contaminated conditions. Results of this test also apply to KPD01H7 as the functional part remains unchanged between the 3 variants.

4.4 Autoclave Sterilization Testing with Biological Indicators

Introduction

The purpose of this test is to determine the ability of the Kleenpak sterile disconnector to be rendered sterile by autoclaving.

Summary of the Test Method

A spore strip of *Geobacillus* (formely *Bacillus*) *stearothermophilus* ATCC 7953 was placed inside each assembly in the fluid path. Kleenpak sterile disconnector assemblies were submitted to 1 autoclave cycle of 30 min at 122 °C +/-1 °C.

After autoclave cycle, spore strips were removed and transferred into a trypticase soya broth (TSB). Incubation was performed during 24 h at 55-60 °C. After incubation, growth evidence was checked for each strip in parallel with negative and positive controls.

Tested quantities and batch number per part number are detailed below:

Table 18

Detail of test units quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number
KPD01HB6 (½ in.)	12	T09/00071, T09/00072 T09/00073
KPD01HB10 (⅜ in.)	6	T11/00066H T11/00066N T11/00066/L
KPD01HB7 (¼ in.)	6	T11/00067H T11/00067N T11/00067/L

Results

Table 19

Autoclave testing Kleenpak sterile disconnector (1 x 30 min autoclave cycle at 122+/-1 °C)

Part Number (Connector Size)	Lot number Batch Number	Sterility of TSB
KPD01HB6 (½ in.)	T09/00071	No growth
	T09/00072	No growth
	T09/00073	No growth
	Negative control	No growth
	Positive control	Growth
KPD01HB10 (⅜ in.)	T11/00066 L	No growth
	T11/00066 N	No growth
	T11/00066 H	No growth
	Negative control	No growth
	Positive control	Growth
KPD01HB7 (¼ in.)	T11/00067 L	No growth
	T11/00067 N	No growth
	T11/00067 H	No growth
	Negative control	No growth
	Positive control	Growth

Conclusion

This test demonstrated that Kleenpak sterile disconnector can be rendered sterile by an autoclave cycle of 30 min at 122+/-1 °C.

4.5 Determination of Residual Volume

Introduction

The purpose of this test is to determine if any residual fluid is present on the separated sections following the recommended Kleenpak sterile disconnector disconnection operation as described in Instructions for Use (USD2727).

Summary of the Test Method

Kleenpak sterile disconnector assemblies were submitted to 1 autoclave cycle of 75 min at 130 +/-2 °C or gamma irradiated at 50+/-5 kGy and the residual volume was determined for each unit after the disconnection operation as per the recommended Kleenpak sterile disconnector Instructions for Use.

Table 20

Detail of test units quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number
KPD01HB6 (½ in.)	12	IL1018 IL1027
KPD01HB10 (⅜ in.)	12	T11/00066H T11/00066N T11/00066/L
KPD01HB7 (¼ in.)	12	T11/00067H T11/00067N T11/00067/L

A blot cloth is placed on scale which is set to zero. The assembled Kleenpak sterile disconnector were flushed with water, and then drained. The units were dried externally before being actuated and disconnected.

Any excess of water is wiped and the blot cloth is weighted. The difference is the residual volume of water measured during the disconnection.

Results

Table 21

Kleenpak sterile disconnector residual volume determination results (Autoclaved at 130 +/-2 °C for 1 cycle of 75 min)

Part Number (Connector Size)	Batch Number	Minimum and Maximum Weight Difference (g)	Average Weight Difference (g)* Corresponding Volume (µL)
KPD01HB6 (½ in.)	IL1018	0.009 - 0.020	0.0172 (18)
	IL1027		
KPD01HB10 (⅜ in.)	T11/00067L	0.0162- 0.0289	
	T11/0067N		
	T11/0067H		
KPD01HB7 (¼ in.)	T11/00066L	0.0147- 0.0233	
	T11/00066N		
	T11/00066H		

**the actuation/disconnection system is identical for the 3 variants, the average is then made on all measurements done in the same conditions.*

Table 22

Kleenpak Sterile disconnector residual volume determination results (Gamma irradiation cycle of 50 +/-5 kGy)

Part Number (Connector Size)	Batch Number	Minimum and Maximum Weight Difference (g)	Average Weight Difference (g)* Corresponding Volume (µL)
KPD01HB10 (⅜ in.)	T11/00067L	0.0003- 0.0198	
	T11/0067N		
	T11/0067H		
KPD01HB7 (¼ in.)	T11/00066L	0.0026- 0.0126	0.0082 (9)
	T11/00066N		
	T11/00066H		

**the actuation/disconnection system is identical for the 3 variants, the average is then made on all measurements done in the same conditions.*

Conclusion

The residual volume that has been recorded on Kleenpak sterile disconnector from part number KPD01HB6, KPD01HB7 and KPD01HB10 is 29 µL max. with an average value below 18 µL.

To ensure absence of excessive residual fluid, units should be fully drained prior to the disconnection operation as described in Instructions for Use (USD2727).

5. Extractables

5.1 Introduction

The purpose of this test is to quantify and characterise the extractables from the Kleenpak sterile disconnector. Water and ethanol are chosen as extracting fluids representative of many biopharmaceuticals fluids.

5.2 Summary of the test method

Extractables tests were performed on assembled Kleenpak sterile disconnectors KPD01HB6 subjected to an appropriate pre-treatment.

A total of 12 units from 3 batches were irradiated at 50 +/- 5 kGy; a total of 12 units from 3 batches were submitted to 1 autoclave cycle of 75 min at 130 +/-2 °C.

Table 23

Detail of test units quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number
KPD01HB6 (½ in.)	48 (12 sets of 4 units)	T09/0036H T09/0037N T09/0038L

The extraction was performed on 4 units connected in series. Test fluid was recirculated using a PTFE diaphragm pump and PTFE tubing though the 4 assembled units, so that the extraction was performed on the fluid path only.

The extraction conditions were respectively water at 60-65 °C and ethanol at ambient temperature and extraction time was 24 h.

The following analysis was then performed on the extracts:

	Water	Ethanol	Target
TOC	•		Total organic content
pH	•		Change acidic/alkaline properties of the aqueous solution
Conductivity	•		Presence of ions that could conduct electric current through the fluid, mostly inorganic ions.
Ion Chromatography (IC)	•		Acetate and formate
Non Volatile Residues	•	•	Quantify the total masse of extractables after evaporation of the test fluid.
UV (200 to 400 nm)	•	•	Compounds with chromophores
Direct-injection Gas Chromatography / Mass Spectrometry (GC/MS)	•	•	Semi-volatile molecules such as solvents, lubricants, plasticizers and antioxidants.
Headspace Gas Chromatography / Mass Spectrometry (GC/MS)	•	•	Volatile organic molecules which can come from different sources, such as various productions steps, additives
Derivatization Gas Chromatography / Mass Spectrometry (GC/MS)	•	•	Can allow detecting some organics such as organic acids, as stearic acid, palmitic acid and myristic acid.
High Performance Liquid Chromatography / UV / Mass Spectrometry (HPLC/UV/MS)	•	•	Additives from polymers and their degradation products.
Inductively Coupled Plasma / Mass Spectroscopy (ICP/MS)	•	•	Any metallic extractables

5.3 Results

Kleenpak Sterile Disconnector - Autoclaved

The results of tests performed to quantify and identify the extractables from Kleenpak sterile disconnector having been submitted to 1 x 75 min autoclave cycles at 130 +/-2 °C are given below.

Table 24

Water extraction of autoclaved Kleenpak sterile disconnector (results given for the system of 4 units in series)

Test Method	Detection Limit	Blank Value	Results
NVR (mg)		0.0	< 1.0
TOC (ppm)	–	0.029	< 0.288
pH	–	5.87	5.47
Conductivity (µS/cm)	–	0.583	< 0.976
UV spectroscopy (AU)	0.01	–	No peak detected
IC Acetate (ppm) Formate (ppm)	0.003 0.002	–	< 0.016 < 0.034
FTIR spectroscopy	NA	–	Spectra similar to negative control - minor signal attributed to silicone O-ring (Appendix 1, Figure 3)
Headspace GC/MS (ppm)	0.01	–	No peak detected
Direct injection GC/MS (ppm)	0.1	–	No peak detected
Derivatization GC/MS			
Dibutyl oxalate (ppm)	0.008	–	< 0.025
Butyl benzoate (ppm)	0.003		< 0.021
Butyl myristate (ppm)	0.005		< 0.025
Butyl palmitate (ppm)	0.008		< 0.048
Butyl stearate (ppm)	0.008		< 0.034
LC/UV/MS (ppm) Bisphenol A	0.01 0.025	–	No peak detected Not detected
ICP/MS			
B (ppb)	0.13	-	< 0.42
Na (ppb)	0.03		< 123.61
Mg (ppb)	0.04		< 5.41
Al (ppb)	0.07		< 1.31
K (ppb)	0.02		< 6.04
Ca (ppb)	0.10		< 19.4
Ni (ppb)	0.06		< 0.33
Cu (ppb)	0.05		< 0.15
Zn (ppb)	0.09		< 0.49
Ba (ppb)	0.05		< 1.34
Si (ppb)	1.87		< 108.7

Note: Highest or extreme result is reported in the above table

Table 25

Ethanol extraction of autoclaved Kleenpak sterile disconnector (results given for the system of 4 units in series)

Test Method	Detection Limit	Blank Value	Results
NVR (mg)	-	0.0	21.0
UV spectroscopy (AU)	0.02	-	No peak detected
FTIR spectroscopy	NA	-	Signal attributed to silicone O-ring (Appendix 1, Figure 4)
Headspace GC/MS (ppm)	0.01	-	No peak detected
Direct injection GC/MS (ppm)	0.1	-	No peak detected
Derivatization GC/MS			
Dibutyl oxalate (ppm)	0.008	–	< 0.025
Butyl benzoate (ppm)	0.003		< 0.025
Butyl palmitate (ppm)	0.008		< 0.025
Butyl stearate (ppm)	0.008		< 0.025
LC/UV/MS			
Bisphenol A (ppm)	0.01	–	No peak detected
	0.025	–	Not detected
ICP/MS			
Na (ppb)	1.19	–	< 99.3
Mg (ppb)	0.83		< 10.9
Al (ppb)	0.76		< 14.6
Ca (ppb)	0.50		< 82.3
Fe (ppb)	0.34		< 13.1
Zn (ppb)	0.25		< 10.4
Si (ppb)	20.5		< 3623

Note: Highest or extreme result is reported in the above table

Kleenpak sterile disconnector - Gamma irradiated

The results of tests performed to quantify and identify the extractables from Kleenpak sterile disconnector having been submitted to gamma irradiation at 50 +/- 5 kGy are given below.

Table 26

Water extraction of gamma irradiated Kleenpak sterile disconnector (Results given for the system of 4 units in series)

Test Method	Detection Limit	Blank Value	Results
NVR (mg)	-	0.0	1.0
TOC (ppm)	-	0.039	0.498
pH	-	5.87	5.59
Conductivity (µS/cm)	-	0.577	1.970
UV spectroscopy (AU)	0.01	-	Lower than 0.01 AU
IC			
Acetate (ppm)	0.003	-	< 0.051
Formate (ppm)	0.002		< 0.135
FTIR spectroscopy	NA	-	Spectra similar to negative control - minor signal attributed to silicone O-ring (Appendix 1, Figure 5)
Headspace GC/MS (ppm)	0.01	-	No peak detected
Direct injection GC/MS (ppm)	0.1	-	No peak detected
Derivatization GC/MS		–	
Dibutyl oxalate (ppm)	0.008		< 0.025
Butyl benzoate (ppm)	0.003		< 0.025
Butyl palmitic (ppm)	0.008		< 0.025
Butyl stearate (ppm)	0.008		< 0.025
LC/UV/MS (ppm)	0.01	-	No peak detected
ICP/MS			
B (ppb)	0.13	–	< 1.46
Na(ppb)	0.03		< 8.11
Mg (ppb)	0.04		< 20.11
Al (ppb)	0.07		< 1.88
K (ppb)	0.02		< 2.92
Ca (ppb)	0.10		< 33
Ni (ppb)	0.06		< 0.38
Cu (ppb)	0.05		< 0.15
Zn (ppb)	0.09		< 1.93
Ba (ppb)	0.05		< 3.01
Si (ppb)	1.87		< 194.1

Note: Highest or extreme result is reported in the above table

Table 27

Ethanol extraction of gamma irradiated Kleenpak sterile disconnector (results given for the system of 4 units in series)

Test Method	Detection Limit	Blank Value	Results
NVR (mg)	–	0.0	< 18.6
UV spectroscopy (AU)	0.01	–	< 0.06
FTIR spectroscopy	NA	–	Signal attributed to silicone O-ring (Appendix 1, Figure 6)
Headspace GC/MS (ppm)	0.01	–	No peak detected
Direct injection GC/MS (ppm)	0.1	–	No peak detected
Derivatisation GC/MS			
Dibutyl oxalate (ppm)	0.008	–	< 0.025
Butyl benzoate (ppm)	0.003		< 0.025
Butyl palmitic (ppm)	0.008		< 0.025
Butyl stearate (ppm)	0.008		< 0.025
LC/UV/MS (ppm)	0.01	–	No peak detected
ICP/MS			
B (ppb)	0.39	–	< 1.31
Na(ppb)	1.19		< 8.4
Mg (ppb)	0.76		< 82.6
Al (ppb)	0.83		< 10.5
Ca (ppb)	0.50		< 43.0
Fe (ppb)	0.34		< 8.4
Zn (ppb)	0.25		< 5.7
Si (ppb)	20.5		< 2578

Note: Highest or extreme result is reported in the above table

5.4 Conclusion

Water and ethanol were selected as generic model solvents to evaluate the extractables level that could be generated by Kleenpak sterile disconnector. Water is a polar solvent representative of most aqueous process fluid solutions, while ethanol is a semi-polar solvent that represents a “worst than worst case” for typical bioprocess formulations, including presence of surfactants.

The study was conducted on sets comprising 4 disconnectors part number KPD01HB6 previously gamma-irradiated or autoclaved. Extraction was conducted with water at 60-65 °C and ethanol at 20-25 °C for 24 h.

Extracts were analyzed using analytical techniques aiming to detect various compounds that could be present.

These results apply as well to Kleenpak sterile disconnector variants part number KPD01HB7 and KPD01HB10. Material of constructions and manufacturing processes are identical, therefore the same type of extractable compounds are expected.

The evaluation has been made on the basis of the internal fluid contacting surface and the welding area of each variant. The KPD01HB7 and KPD01HB10 variants have been estimated to present 2.5 scaling up factor (which include a safety margin) compared to the KPD01HB6 variant.

To evaluate the extractables level of KPD01HB7 or KPD01HB10, this scaling up factor should then be taken into account and applied to results presented here in this section.

6. Biological Safety, Particulates and PhysicoChemical Tests

Results detailed below apply to all variants of Kleenpak sterile disconnector, as materials of construction and manufacturing processes are similar.

6.1 Introduction

The purpose of these tests was to evaluate the biological and physicochemical suitability of the materials of construction of the fluid path of the Kleenpak sterile disconnector. The purpose was also to evaluate the suitability of the device for pharmaceutical processes in term of cleanliness.

The materials of construction of the fluid path are as follows:

Connector body	Polysulfone
Plunger	Polysulfone
O-rings	Peroxide cured silicone

6.2 Biological reactivity test, *in vitro* (USP 87)

Introduction

The purpose of this test is to determine the biological reactivity of mammalian cell cultures exposed to materials to which the fluid is exposed when passing through the Kleenpak sterile disconnector.

Summary of the Test Method

Test is performed as per USP <87>, MEM Elution Method Cytotoxity. The samples to be tested were divided in small pieces in order to get the ratio of 4 g per 20 mL of cell culture media (MEM). Samples were prepared and extracted with Serum Supplemented Minimum Essential Medium (MEM). Extractions were performed at 37 °C in a 5 % CO_2 humidified incubator for 24 hours. An aliquot of the extract was pipetted onto a monolayer of L929 cells. These plates were incubated at 37 °C in a 5 % CO_2 humidified incubator for 48 hours.

The extracts are then examined to determine any biological reactivity.

The tests were conducted by Ethox International, Inc., Rush, New York, USA.

Results

Kleenpak sterile disconnector passed the USP tests as described above.

Conclusion

Kleenpak sterile disconnector meets the USP <87> specifications.

6.3 Biological Reactivity Test, *in vivo* (USP 88)

Introduction

The purpose of this test is to determine the biological reactivity following contact with the material to which the fluid is exposed when passing through the Kleenpak sterile disconnector.

Summary of the Test Method

USP Biological Reactivity Tests, *in vivo* for Class VI Plastics (121 °C) as described in the current United States Pharmacopeia Chapter <88> was performed on Kleenpak sterile disconnector devices previously gamma irradiated at 50 +/- 5 KGy.

The tests were conducted by Ethox International, Inc., Rush, New York, USA.

The Biological Reactivity Tests *in vivo* for Class VI-121°C 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 are:

- 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 °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 polysulfone fluid contact material were extracted at 121 °C (250 °F).

Systemic Injection Tests

A Systemic Injection Test was performed to evaluate systemic biological responses of animals to plastics, polymers and biomaterials by a single dose injection. Extracts of the fluid path material of construction (polysulfone) in USP saline (0.9% Sodium Chloride) and 1:20 alcohol: USP saline were prepared and injected intravenously. Extracts of the fluid path material of construction (polysulfone) in Polyethylene glycol and vegetable oil were prepared and injected intraperitoneally.

Intracutaneous Tests

An Intracutaneous Test was 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 were used for these tests.

Implantation Tests

Implantation test was also performed, in order to evaluate animal response in case of direct contact with Kleenpak sterile disconnector construction material.

Results

Kleenpak sterile disconnectors passed the USP <88> tests as described above.

Conclusion

Kleenpak sterile disconnectors meet the USP <88> specifications.

6.4 Particle Release as per USP <788>

Introduction

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

Summary of the Test Method

4 units of 3 batch number were tested individually for particle level by the Light Obscuration Method described in USP <788>. Each unit was rinsed with 30 mL of Water for Injection which is collected in a vial, and the fluid tested for presence of particle equal or higher than 10 µm and 25 µm. Specifications taken into account are the ones given in USP for small containers (<100 mL) which are < 6000 particles per mL > 10 µm and <600 particles per mL >25 µm.

Results

Table 28

Kleenpak sterile disconnector Particle release results

Part Number	Batch Number	Minimum and Maximum Particles Count Per Unit (per 30 mL)	
		> 10 µm	> 25 µm
KPD01HB6	T09/00071	82 - 208	4 - 14
KPD01HB6	T09/00072	110 - 238	12 - 18
KPD01HB6	T09/00073	94 - 318	10 - 22

Conclusion

A 30 mL rinse of Kleenpak sterile disconnectors met the requirements of particulate testing performed as per the specifications of USP <788> (for injectables <100 mL) by the Light Obscuration Particle Count Test Method.

6.5 Endotoxin Test as per USP <85>

Introduction

The purpose of this test was to quantify the bacterial endotoxins level that may be present in Kleenpak sterile disconnector.

Summary of the Test Method

4 units from each of 3 lots were tested individually for endotoxins level using the LAL gel clot method as per USP <85>. Each unit was rinsed with 20 mL of water which is collected in a endotoxin free vial. The undiluted collected fluid is tested for endotoxin.

Results

Table 29

Kleenpak sterile disconnector Endotoxin level

Part Number	Batch Number	Endotoxin Level	
		Per Unit (EU/unit)	Per mL (EU/mL)
KPD01HB6	T09/00071	<0.6	0.03
KPD01HB6	T09/00072	<0.6	0.03
KPD01HB6	T09/00073	<0.6	0.03
KPD01HB6	Positive control (Lysate sensitivity 0.03 EU/mL)	+	NA
KPD01HB6	Negative control	-	NA

Conclusion

Rinse effluents from Kleenpak sterile disconnectors were analyzed for endotoxin content per USP <85> Bacterial Endotoxins Test. The endotoxin level is below the limit of 0.25 /mL (limit for Water For Injection as per current USP).

6.6 Physicochemical Test as per USP <661>

Introduction

The purpose of this test was to evaluate the physicochemical suitability of Kleenpak sterile disconnector for contact with parenterals.

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 the Kleenpak sterile disconnectors were extracted at 70+/-2 °C 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 or acidity of the extract.

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 metal content — detects the presence of metals such as lead, tin, and zinc.

These tests were performed on Kleenpak sterile disconnector giving a surface area sample equivalent to 120 cm^2/20 mL as per USP and previously gamma irradiated at 50 kGy.

Results
Kleenpak sterile disconnectors meet acceptance criteria for all 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.

Conclusion
Kleenpak sterile disconnectors meet the specification of the USP <661> and are therefore appropriate for parenteral use.

7. Shelf Life Studies

7.1 Introduction
Shelf life studies are conducted in order to establish a 3-year shelf life for Kleenpak sterile disconnector after gamma irradiation and a 5-year shelf life for non irradiated products.

Results for a 3 years shelf life (accelerated storage conditions) were available at the time this guide was revised. Results for longer period will be available as developed.

The leak test, burst test, pressure hold test, soiling test and extractables tests (NVR, FTIR for both extraction fluid, and TOC-pH-conductivity for water) tests were performed after the storage in order to demonstrate that performances are maintained.

7.2 Summary of the Test Method
Shelf life studies were performed on Kleenpak sterile disconnector assemblies subjected to gamma irradiation at 50 +/- 5kGy.

After the exposure in accelerated storage conditions, the following tests were performed in order to confirm that the characteristics of the Kleenpak sterile disconnector assemblies are not altered: leak test, burst test, pressure hold test, soiling test and NVR - FTIR tests.

Table 30

Detail of test units quantity and batch number

Part Number (Connector Size)	Quantity	Batch Number	Test
KPD01HB6 (½ in.)	12 12	T10/00030	leak test burst test
	12	T10/00031	pressure hold test soiling test
	12	T10/00032	
	12		NVR -pH - TOC - FTIR

7.3 Results

7.3.1 Leak Test

Kleenpak sterile disconnectors KPD01HB6 were leak tested as per description in section 2.2 in the connected position at the pressure of 3.1 bar (air). All units passed this test.

7.3.2 Burst Test

Kleenpak sterile disconnectors KPD01HB6 were burst tested as per description in section 2.3.

Table 31

Burst pressure of Kleenpak sterile disconnector (gamma irradiated at 50kGy +/-5kGy)

		Burst Pressure	
Part Number	Batch Number	Minimum/Maximum (psig / barg)	Average (psig / barg)
KPD01HB6	T10/00030	193-213 / 13.31 - 14.68	201 / 13.86
KPD01HB6	T10/00031	194 - 207 / 13.38 - 14.27	202 / 13.93
KPD01HB6	T10/00032	194 - 204 / 13.38 - 14.07	198 / 13.66

All units passed this test

7.3.3 Pressure Hold Test

Pressure hold test was performed as per description in section 2.5 on Kleenpak sterile disconnector KPD01HB6. No leak was detected in any samples after 15 min pressure (350 mbar) exposure.

7.3.4 Soiling Test

Soiling test was performed as per description in section 4.3 on Kleenpak sterile disconnector.

Table 32

Soiling test results of connected Kleenpak sterile disconnector (Gamma irradiated at 50 +/- 5 kGy)

		Turbidity in TSB Samples	
Part Number	Sample Number	Male Part Post Disconnection	Female Part Post Disconnection
KPD01HB6	T10/00030	no	no
KPD01HB6	T10/00031	no	no
KPD01HB6	T10/00032	no	no
KPD01HB6	Negative control	no	no
KPD01HB6	Positive control	yes	yes

Concentration of the B. diminuta suspension: 5.4 x 10^7 cfu/mL

7.3.5 Extractables

Table 33

Water extraction of Kleenpak sterile disconnector (Gamma irradiated at 50 +/-5 kGy)
(Results given for the system of 4 units in series)

Test Method	Detection Limit	Blank Value	Results
NVR (mg)	-	0.0	0.9-1.4
TOC (ppm)	-	< 0.02	0.247-0.274
pH	-	5.54	5.78-5.87
Conductivity (µS/cm)	-	1.571	1.803-1.871
FTIR spectroscopy tive	NA	-	Spectra similar to nega-control - minor signal attributed to silicone O-ring

Table 34

Ethanol extraction of Kleenpak Sterile Disconnector (Gamma irradiated at 50 +/-5 kGy)
(results given for the system of 4 units in series)

Test Method	Detection Limit	Blank Value	Results
NVR (mg)	-	0.0	19.5-21.6
FTIR spectroscopy	NA	-	Signal attributed to silicone O-ring

7.4 Conclusion

Samples of Kleenpak sterile disconnector devices KPD01HB6 previously exposed to a storage in accelerated conditions equivalent to 3 year shelf life, were subjected to a leak test, a burst test, a pressure hold test, and a soiling (microbial ingress) test.

All results passed their respective test criteria, indicating that the functionality of the Kleenpak sterile disconnectors units remained intact.

Samples were also submitted to extractables evaluation. NVR results showed a level similar to the results obtained on unstored units. FTIR spectroscopy gave similar spectrum. On the basis of these results, the storage is not expected to have caused any significant modification of the extractables content as identified on unstored Kleenpak sterile disconnectors.

All results are as expected, and apply also to KPD01HB7 and KPD01HB10.

Appendix 1

FTIR spectra

Figure 3

FTIR spectra of water extract Kleenpak sterile disconnector (autoclaved)

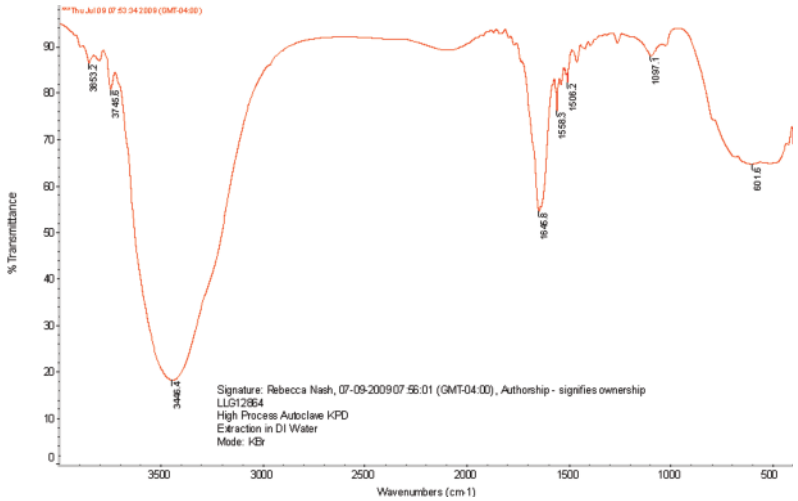


Figure 4

FTIR spectra of ethanol extract Kleenpak sterile disconnector (autoclaved)

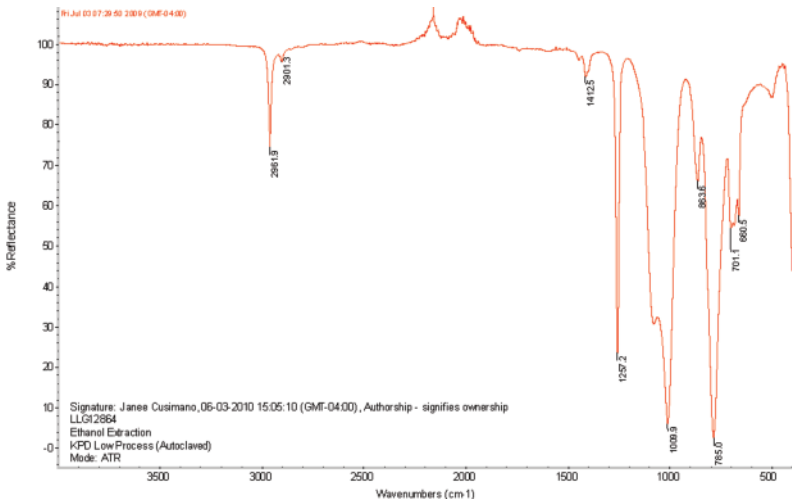


Figure 5

FTIR spectra of water extract Kleenpak sterile disconnector (gamma-irradiated)

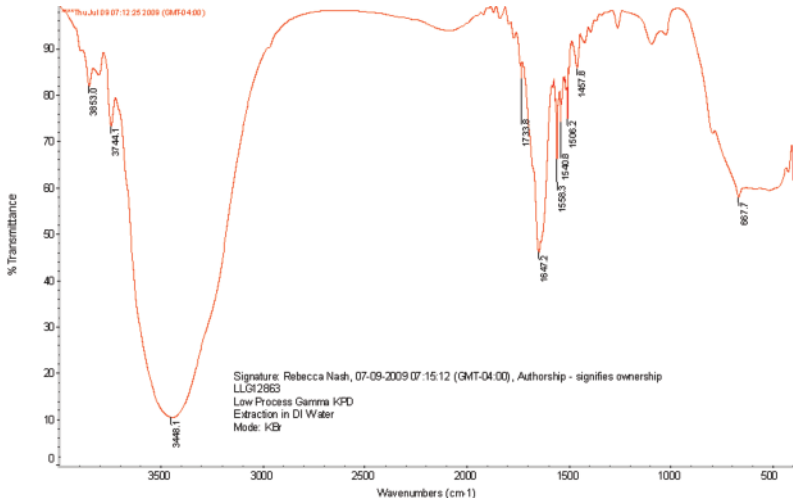
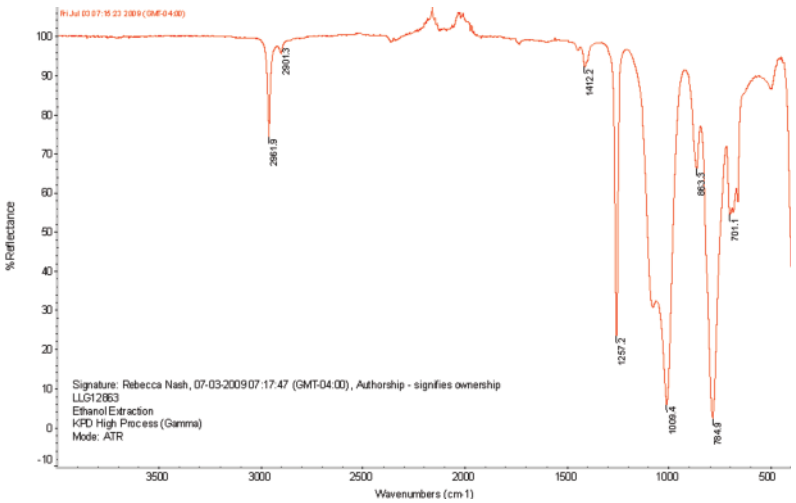


Figure 6

FTIR spectra of ethanol extract Kleenpak sterile disconnector (gamma irradiated)



PALL Life Sciences

Corporate Headquarters
Port Washington, NY, USA
+1 800 717 7255 toll free (USA)
+1 516 484 5400 phone
biopharm@pall.com e-mail

European Headquarters
Fribourg, Switzerland
+41 (0)26 350 53 00 phone
LifeSciences.EU@pall.com e-mail

Asia-Pacific Headquarters
Singapore
+65 6389 6500 phone
sgcustomerservice@pall.com e-mail

Filtration. Separation. Solution.SM

Visit us on the Web at www.pall.com/biopharm
E-mail us at biopharm@pall.com

International Offices
Pall Corporation has offices and plants throughout the world in locations such as: Argentina, Australia, Austria, Belgium, Brazil, Canada, China, France, Germany, India, Indonesia, Ireland, Italy, Japan, Korea, Malaysia, Mexico, the Netherlands, New Zealand, Norway, Poland, Puerto Rico, Russia, Singapore, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, the United Kingdom, the United States, and Venezuela. Distributors in all major industrial areas of the world. To locate the Pall office or distributor nearest you, visit **www.pall.com/contact**.

4/13, PDF, GN13.5631 USTR2734a