PALL Life Sciences

Validation Guide

USTR 2232a

Kleenpak™ Connector For use with 13 mm (½ inch) nominal tubing

*Filtration. Separation. Solution.*SM

CONTENTS

1. Validation Overview

1.1 Introduction

This report contains data applicable to the Pall® **Kleenpak** connector, which can be used for making aseptic connections for 13 mm (½ inch) nominal tubing.

The **Kleenpak** connector is designed to facilitate the sterile connections of tubing and other components in biopharmaceutical applications. The disposable **Kleenpak** connector allows for the dry connection of two separate fluid pathways, while maintaining the sterile integrity of both. The **Kleenpak** connector consists of a male and a female connector, each covered by a vented peel away strip that protects the port and maintains the sterility of the sterile fluid pathway.

The purpose of this report is to document the testing that has been performed to demonstrate the strength and suitability of the **Kleenpak** connector for use in pharmaceutical aseptic connection applications. The testing program includes a demonstration of the ability of the **Kleenpak** connector to provide a sterile fluid pathway even after being soiled with bacterial spores.

Kleenpak connectors are supplied with two different part number sets, reflecting different grades of polycarbonate. Part numbers beginning with ACD (male: ACD02M6; female: ACD02F6) can be used for autoclaving up to 121°C (250°F) and part numbers beginning with KPCHT (male: KPCHT02M6; female: KPCHT02F6) can be used for autoclaving up to 130°C (266°F). All can be subjected to gamma irradiation and the male and female parts can be used interchangeably (the design is identical between ACD and KPCHT series connectors; the only difference is the grade of polycarbonate). The **Kleenpak** connector was first produced in the ACD part number series and the initial validation tests were performed using ACD series samples. Additional tests have been performed on the KPCHT series. All tests are documented in this validation guide.

1.2 Summary of Conclusions

Burst Testing

Twenty-seven ACD series connectors were burst pressure tested after being subjected to gamma irradiation at doses of 50.5 – 54.9 kGy, followed by one 30 minute autoclave cycle at 121°C (250°F). Six KPCHT series connectors were burst pressure tested after being subjected to gamma irradiation at a dose of 25 kGy, followed by two 30 minute autoclave cycles at 135°C (275°F). In addition three ACD series male connectors coupled with KPCHT series female connectors and three ACD series female connectors coupled with KPCHT series male connectors were burst pressure tested after appropriate pretreatment. All **Kleenpak** connectors had burst pressures that were > 19.3 barg (280 psig) giving an acceptable safety factor over the maximum recommended operating pressure of 3 barg (43.5 psig).

Creep Rupture Testing

The **Kleenpak** connector has been designed to be capable of operating at up to 3 barg (43.5 psig) for 168 hours (1 week) in continuous use. The creep rupture data presented using typical **Kleenpak** connectors demonstrates the very large safety margins that have been incorporated into these pressure claims.

Tensile Strength Testing

The **Kleenpak** connector can maintain an integral connection in the event of accidental pulling of the tubing attached to the connector, as demonstrated by tensile strength testing of connected articles after they have been subjected to an appropriate pretreatment (ACD series: gamma irradiation at doses of 50.5 – 54.9 kGy, followed by one 30 minute autoclave cycle at 121°C (250°F); KPCHT series: gamma

irradiation at a dose of 25 kGy, followed by two 30 minute autoclave cycles at 135°C [275°F]). An average of 43 Newtons (9.6 lbf) is required to pull tubing off the hose barb of a connector, while an average force of 245 ± 43 Newtons (55 ± 9 lbf) was required to break the connection. This demonstrates that an integral connection can be maintained in the event of (limited) accidental pulling of the tubing attached to the connector.

Functional Testing (Soiling Test)

The soiling test data presented provide assurance that the **Kleenpak** connector is able to provide a sterile fluid pathway even after being soiled with bacterial spores.

Closure Integrity Testing (Vacuum Leak Test)

The vacuum leak test is based on the guidelines outlined in ASTM D4991-94 (specifying the requirements for packaging process development, validation and control), and constitutes a standard test for demonstrating seal integrity of container closure. The closure integrity test data provided confirms seal integrity of the **Kleenpak** connector.

Water Flow Characteristics

The water flow/pressure drop data presented for connected **Kleenpak** connectors can be used in conjunction with the pressure drop characteristics of other system components (e.g. tubing, filter capsules) to form the basis for sizing the disposable system employing the **Kleenpak** connector.

Extractables Testing

The level of aqueous and ethanol extractables for the ACD and KPCHT series **Kleenpak** connectors are extremely low. The non-volatile residue (NVR) for three connectors tested together was < 0.1 mg after a 24 hour recirculation in water and after a 4 hour recirculation in ethanol for both the ACD and the KPCHT series connectors tested.

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

Shelf Life Studies

Real time shelf life studies are currently in progress and testing supports a shelf life after irradiation of one year. The testing is continuing for establishment of a 24 month shelf life; contact Pall for latest information.

Autoclave Testing

It has been demonstrated that the ACD series **Kleenpak** connector is capable of being autoclaved at a maximum temperature of 121°C (250°F) for 30 minutes and the KPCHT series **Kleenpak** connector is capable of being autoclaved at a maximum temperature of 130°C (266°F) for 30 minutes. Tests with biological indicators demonstrated that the **Kleenpak** connector could be rendered sterile by autoclaving at 121°C (250°F) for 15 minutes.

Biological Reactivity Tests

The fluid path of the ACD and KPCHT series **Kleenpak** connectors were found to meet the requirements of the current United States Pharmacopoeia for Class VI-121°C Plastics.

The fluid path of the ACD and KPCHT series **Kleenpak** connectors were found to meet the requirements of the current United States Pharmacopoeia for Physicochemical tests.

2. Burst Testing

2.1 Introduction

The purpose of these tests was to demonstrate that **Kleenpak** connectors withstand the maximum pressure rating of 3 barg (43.5 psig) with an appropriate safety margin

2.2 Summary of Methods

Burst tests were performed on assembled **Kleenpak** connectors subjected to an appropriate pretreatment. ACD series connectors were subjected to gamma irradiation at doses of 50.5 – 54.9 kGy, followed by one 30 minute autoclave cycle at 121°C (250°F), while KPCHT series connectors were subjected to gamma irradiation at a dose of 25 kGy, followed by two 30 minute autoclave cycles at 135°C (275°F).

Before the burst tests were performed, the male and female parts were connected. The whole assembly was placed in a water bath at 40°C (104°F). The connected **Kleenpak** connectors were filled with water and one end was blanked off. Prior to starting the burst tests, the **Kleenpak** connectors were held within the water for a minimum of 15 minutes to ensure that they had equilibrated with the water bath temperature. The inlet of the water-filled **Kleenpak** connector was then connected to a pressure source and the upstream pressure was gradually increased until failure of the **Kleenpak** connector occurred.

2.3 Results

All **Kleenpak** connector burst tests ended when the connection pulled apart; the connector did not burst.

The results of the burst pressure tests are shown in Tables 2-1 and 2-2. All **Kleenpak** connectors had burst pressures that were > 19.3 barg (280 psig) giving an acceptable safety factor over the maximum recommended operating pressure of 3 barg (43.5 psig).

Table 2-1: Burst Pressures of Connected ACD Series Kleenpak Connectors Pre-treatment: Gamma Irradiation at doses of 50.5 – 54.9 kGy, followed by one 30 minute autoclave cycle at 121°C (250°F)

Pall Kleenpak Connector Sample Number	Burst Pressure	
	Barg	psig
1-1	33.1	480
1-2	38.6	560
1-3	41.4	600
1-4	56.5	820
1-5	44.1	640
1-6	49.6	720
1-7	44.1	640
1-8	42.1	610
1-9	49.6	720

Table 2-1 *(Continued)*

Pall Kleenpak Connector Sample Number	Burst Pressure	
	Barg	psig
1-10	44.1	640
1-11	34.5	500
1-12	37.2	540
1-13	41.4	600
1-14	44.1	640
1-15	41.4	600
2-1	55.2	800
2-2	20.7	300
2-3	42.1	610
2-4	42.1	610
2-5	48.3	700
2-6	49.6	720
2-7	31.7	460
2-8	19.3	280
2-9	42.7	620
2-10	22.1	320
2-11	26.2	380
2-12	21.4	310
Average Burst Pressure	**39.4 barg**	**571.1 psig**

Table 2-2: Burst Pressures of Connected KPCHT Series Kleenpak Connectors Pre-treatment: Gamma irradiation at dose of 25 kGy, followed by two 30 minute autoclave cycles at 135°C (275°F)

Pall Kleenpak Connector Sample Number	Burst Pressure	
	Barg	psig
KPCHT1	31.0	450
KPCHT2	41.1	600
KPCHT3	31.0	450
KPCHT4	39.6	575
KPCHT5	36.2	525
KPCHT6	34.5	500
Average Burst Pressure	**35.6 barg**	**516.7 psig**

Burst pressures of mixed ACD and KPCHT series connected **Kleenpak** connectors were obtained.

ACD series pre-treatment: Gamma irradiation at doses of 50.5 – 54.9 kGy, followed by one 30 minute autoclave cycle at 121°C (250°F)

KPCHT series pre-treatment: Gamma irradiation at dose of 25 kGy, followed by two 30 minute autoclave cycles at 135°C (275°F)

Three ACD series males with three KPCHT series females and three KPCHT series males with three ACD series females were connected and subjected to the burst pressure tests. The burst pressures were greater than or equal to 34.5 bar (500 psi) for the six pairs tested.

2.4 Conclusions

A total of thirty nine **Kleenpak** connectors were burst pressure tested after being subjected to gamma irradiation and autoclave treatment. All **Kleenpak** connectors had burst pressures that were > 19.3 barg (280 psig) giving an acceptable safety factor over the maximum recommended operating pressure of 3 barg (43.5 psig).

3. Creep Rupture Testing

3.1 Introduction

Creep rupture testing was performed in order to demonstrate the strength and stability of the **Kleenpak** connector over extended periods of time whilst under pressure. These tests were performed on the ACD series connectors. Creep rupture testing was not performed on the KPCHT series connectors, since the KPCHT connectors have same design, and the burst pressures of the KPCHT series connectors were similar to the burst pressures of the ACD series connectors.

3.2 Summary of Methods

Prior to the creep rupture testing, the connectors were subjected to gamma irradiation at doses of 50.5 – 54.9 kGy, followed by one 30 minute autoclave cycle at 121°C (250°F).

Before the tests were performed, the male and female parts were connected. For the creep rupture tests the **Kleenpak** connectors were immersed in a water bath held at 40°C (104°F). Prior to starting the tests, the **Kleenpak** connectors were held within the water for a minimum of 15 minutes to ensure that they had equilibrated with the water bath temperature. The parts were connected to a creep rupture rig, designed to maintain set pressures within the inside of the connector until failure or until the test was completed. If the end point was a connector failure, the failure time and mode was noted.

3.3 Results

Kleenpak connectors were tested at 3.4 barg (50 psig) and 6.9 barg (100 psig) and were held at pressure for 1 week (168 hours). Samples at both pressures maintained pressure for one week without leaking or breaking.

3.4 Conclusions

Kleenpak connectors have been designed to be capable of operating at up to 3 barg (43.5 psig) for 168 hours (1 week) in continuous use. The creep-rupture data presented using typical **Kleenpak** connectors demonstrates the very large safety margins that have been incorporated into these pressure claims.

4. Tensile Strength Testing

4.1 Introduction

The purpose of these tests was to demonstrate the ability of the **Kleenpak** connector to maintain an integral connection in the event of (limited) accidental pulling of the tubing attached to the connector.

4.2 Summary of Methods

Tensile strength testing was performed on connected articles after they were subjected to an appropriate pre-treatment. ACD series connectors were subjected to gamma irradiation at doses of 50.5 – 54.9 kGy, followed by one 30 minute autoclave cycle at 121°C (250°F). KPCHT series connectors were subjected to gamma irradiation at a dose of 25 kGy, followed by two 30 minute autoclave cycles at 135°C (275°F).

The tests were performed using a tensile test machine. During the test, tensile force was applied, and the force at which the connector breaks or the connection pulls apart was recorded.

4.3 Results

The tensile test results for five connected ACD series connectors are shown in Table 4-1. Additional test results for a mixture of ACD and KPCHT series connectors are shown in Table 4-2.

Table 4-1: Results of Tensile Strength Testing of ACD Series Kleenpak Connectors

Pall Kleenpak Connector Sample Number	Load at Maximum Tension (Newtons)	Load at Maximum Tension (lbf)
1	271.453	61.025
2	262.196	58.944
3	243.482	54.737
4	302.386	67.979
5	303.075	68.134
Average	**276.386 ± 25.964**	**62.134 ± 5.837**

Table 4-2: Results of Tensile Strength Testing of Mixed ACD and KPCHT Series Kleenpak Connectors

Pall Kleenpak Connector Sample Number	Male Connector	Female Connector	Load at Maximum Tension (Newtons)	Load at Maximum Tension (lbf)
1	ACD	KPCHT	213.626	48.025
2	KPCHT	ACD	184.566	41.492
3	KPCHT	KPCHT	208.542	46.882
4	ACD	ACD	215.089	48.354
Average			**205.456 ± 14.206**	**46.188 ± 3.194**

Additional tests were performed to determine the force required to remove tubing from the hose barb of a **Kleenpak** connector. To perform these tests a special fixture was used to test only the tubing to hose barb on the female side of the connector. The results are reported in Table 4-3.

Table 4-3: Results of Tensile Strength Testing of Tubing on Hose Barb of ACD and KPCHT Series Kleenpak Connectors

Pall Kleenpak Connector Series	Load at Maximum Load (Newtons)	Load at Maximum Load (lbf)	Failure Mechanism
ACD	41.60	9.35	Tubing slipped off hose barb
KPCHT	43.88	9.86	Tubing slipped off hose barb
Average	**42.74**	**9.61**	

4.4 Conclusions

An average of 43 Newtons (9.6 lbf) is required to pull tubing off the hose barb of a connector, while an average force of 245 ± 43 Newtons (55 ± 9 lbf) was required to break the connection. This demonstrates that an integral connection can be maintained in the event of (limited) accidental pulling of the tubing attached to the connector.

5. Functional Testing

5.1 Introduction

The objective of the functional tests (soiling tests) was to provide information on the ability of the **Kleenpak** connector to produce a sterile fluid pathway after being intentionally contaminated with bacterial spores.

5.2 Summary of Methods

A total of 59 **Kleenpak** connectors were used for the tests: 29 ACD series connectors, 20 KPCHT series connectors and 10 ACD and 10 KPCHT series connectors used together.

During the testing of the 29 ACD series connectors, the two components of the connector, the male and female parts, were pre-assembled by STEDIM to 'Flexboy' bags for use in the soiling test. The **Kleenpak** connector/bag assemblies were subjected to gamma irradiation at a dose of 25-35 kGy prior to the test.

During the testing of the KPCHT series, the connectors were attached to glass vessels instead of bags and the assemblies were autoclaved under appropriate conditions. The fluid was transferred by attaching the transfer flask to a low pressure air source, and assembling the test connectors to initiate fluid transfer. ACD series connectors were subjected to one 30 minute autoclave cycle at 121°C (250°F). KPCHT series connectors were subjected to one 30 minute autoclave cycle at 130°C (266°F).

During the functional test, the male and female connectors were soiled with an estimated minimum challenge level of 10^6 CFU of *Geobacillus stearothermophilus* spores per connector. The soiling was accomplished by submerging each male and female connector just up to the flange into a spore suspension of *G. stearothermophilus* suspended in a 4% carboxy methyl celluose (CMC) solution for about 30 seconds. The soiled connectors were then placed upward on glassine paper and allowed to air dry at ambient temperature in a laminar flow hood over night.

To verify the number of spores adhering to the connector, four units were soiled and allowed to dry, per above procedure. The connectors were then suspended in sterile trypticase soy broth (TSB) and sonicated for 15 minutes. After sonication, the TSB suspension was titered for bacterial count. This plate count represents the minimum number of spores that adhered to the connector since it is possible that some of the spores were not removed by the sonication process.

In order to demonstrate that the connector soiling did not impact the ability of the **Kleenpak** connector to provide a sterile pathway, the following procedure was followed for each of the tests.

ACD Series. The supply bag or glass vessel connected to the male connector was first filled with approximately 300 mL of sterile trypticase soy broth (TSB) via septum. The male and female **Kleenpak** connectors were then connected. The clamp on the tubing between the male connector and the supply bag or glass vessel was opened (as appropriate) and the TSB was transferred through the **Kleenpak** connector to the collection bag or glass collection vessel by gravity or low-pressure air source, respectively. After the fluid transfer, the tubing downstream of the **Kleenpak** connector was heat-sealed (if supply bags were used), or aseptically removed and covered with sterile foil (if glassware was used). The collection bag or vessel containing the TSB was incubated for 7 days at 55°C (131°F). Lack of turbidity post the 7 day incubation period indicated absence of any visually detectable microbial growth. To document sterility, the contents of the incubated collection bag or vessel were filtered through a recovery membrane (0.2 micrometer-rated) disc, which was placed on the surface of a trypticase soy agar (TSA) plate and incubated

at 55°C (131°F) for 7 days. Absence of colonies of *G. stearothermophilus* on the recovery membrane (placed on the TSA plate and incubated at 55°C (131°F), 7 days) is evidence of a sterile connection.

KPC Series. The soiling test for KPC connectors was performed only using the glassware set-up, with fluid transfer initiated via low-pressure air source, as described above. Following transfer of the TSB to the glass collection vessel, the contents were immediately passed through a 0.2 µm recovery filter disc, and transferred onto TSA plates. All plates were incubated for 7 days at 55°C (131°F).

Positive and negative controls were performed as part of the soiling tests. A negative control was achieved by eliminating the inoculation of the male and female connectors with the spore suspension. As a positive control, the protective strip was removed and the tip of the plunger on the male connector was inoculated with spore suspension.

Figure 5.1: Male and Female Connector Assemblies Prior to Connection

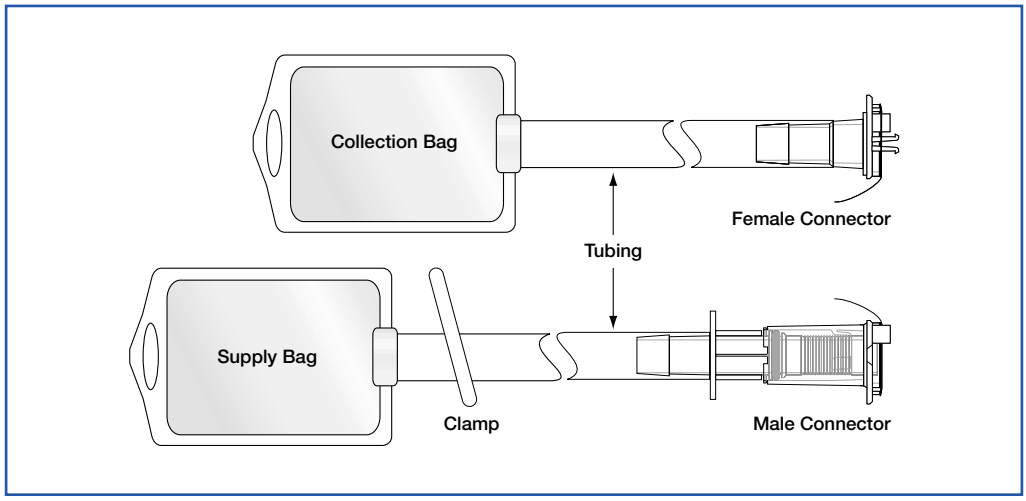


Figure 5.2: Assembly Connected by Kleenpak Connector for Transfer of Trypticase Soy Broth

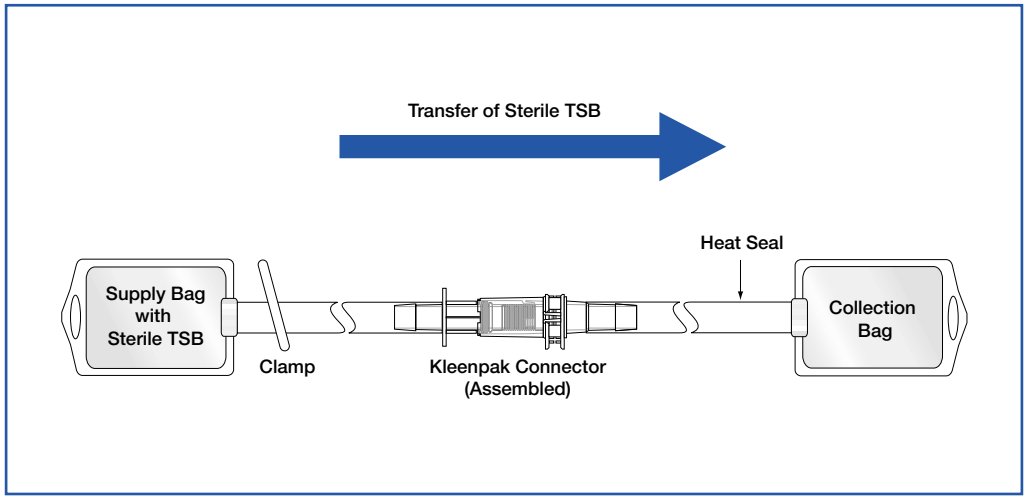
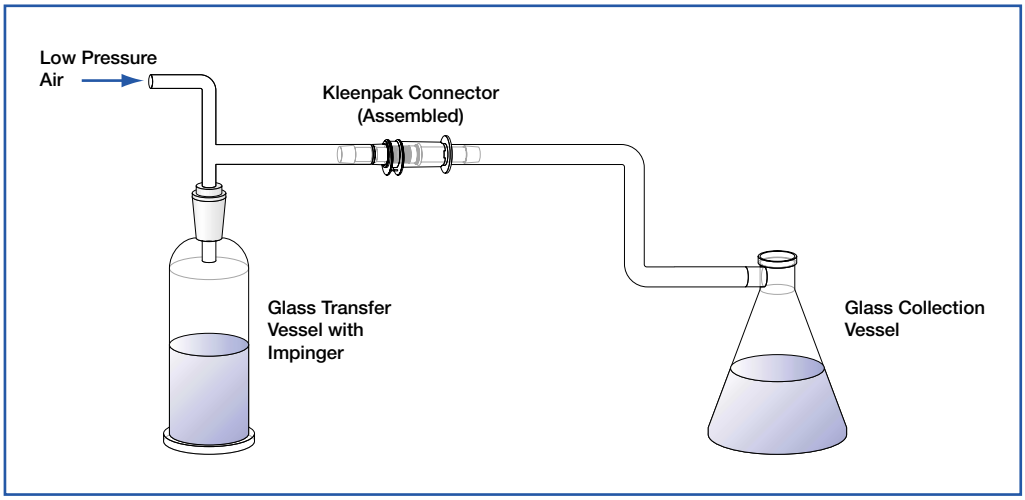


Figure 5.3: Glassware Assembly Connected by Kleenpak Connector for Transfer of Trypticase Soy Broth



5.3 Results

The results of the input verification test are provided in Tables 5-1, 5-7 and 5-9 and the results of the soiling tests are provided in Tables 5-2, 5-3, 5-4, 5-6, 5-8 and 5-10.

Table 5-1: Input Verification Test (IVT) for ACD Series Soiling Tests

Test Number	CFU per Connector*
IVT 1	1.05E+06
IVT 2	9.25E+05
IVT 3	7.20E+05
IVT 4	2.90E+06
Average	**1.40E+06**

* For tests 1-4, CFU is an average of two dilution series

Table 5-2: Soiling Test Results for ACD Series Lot 1

Kleenpak Connector Identification Number	Number of CFU
16	0
19	0
17	0
24	0
28	0
33	0
38	0
41	0
48	0
50	0
Positive Control	
6	Confluent
Negative Control	
7	0

Table 5-3: Soiling Test Results for ACD Series Lot 2

Kleenpak Connector Identification Number	Number of CFU
6	0
15	0
17	0
20	0
25	0
26	0
28	0
37	0
43	0
45	0
Positive Control	
5	Confluent
Negative Control	
19	0

Table 5-4: Soiling Test Results for ACD Series Lot 3

Kleenpak Connector Identification Number	Number of CFU
9	0
15	0
27	0
32	0
33	0
37	0
39	0
40	0
50	0
Positive Control	
8	Confluent
Negative Control	
13	0

Table 5-5: Input Verification Test (IVT) for KPCHT Series Lot 1 Soiling Tests

Test Number	CFU per Connector
IVT 1	3.9E+07
IVT 2	3.6E+07
IVT 3	4.1E+07
Average	**3.9E+07**

Table 5-6: Soiling Test Results for KPCHT Series Lot 1 (Male and Female KPCHT Series Connectors)

Kleenpak Connector Test Number	Number of CFU
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	0
10	0
Positive Control	Confluent
Negative Control	0

Table 5-7: Input Verification Test (IVT) for KPCHT Series Lot 2 Soiling Tests

Test Number	CFU per Connector
IVT 1	2.8E+06
IVT 2	2.5E+06
IVT 3	5.4E+06
Average	**3.6E+06**

Table 5-8: Soiling Test Results for KPCHT Series Lot 2 (Five KPCHT Series Male and Female, three KPCHT Series Male with ACD Series Female, and two ACD Series Male with KPCHT Series Female)

Kleenpak Connector Test Number	Male	Female	Number of CFU
1	KPCHT	KPCHT	0
2	KPCHT	KPCHT	0
3	KPCHT	KPCHT	0
4	KPCHT	KPCHT	0
5	KPCHT	KPCHT	0
6	KPCHT	ACD	0
7	KPCHT	ACD	0
8	KPCHT	ACD	0
9	ACD	KPCHT	0
10	ACD	KPCHT	0
Positive Control	ACD	ACD	Confluent
Negative Control	ACD	ACD	0

Table 5-9: Input Verification Test (IVT) for KPCHT Series Lot 3 Soiling Tests

Test Number	CFU per Connector
IVT 1	2.4E+06
IVT 2	2.9E+06
IVT 3	3.3E+06
Average	**2.9E+06**

Table 5-10: Soiling Test Results for KPCHT Series Lot 3 (Five KPCHT Series Male and Female, two KPCHT Series Male with ACD Series Female, and three ACD Series Male with KPCHT Series Female)

Kleenpak Connector Test Number	Male	Female	Number of CFU
1	KPCHT	KPCHT	0
2	KPCHT	KPCHT	0
3	KPCHT	KPCHT	0
4	KPCHT	KPCHT	0
5	KPCHT	KPCHT	0
6	ACD	KPCHT	0
7	ACD	KPCHT	0
8	ACD	KPCHT	0
9	KPCHT	ACD	0
10	KPCHT	ACD	0
Positive Control	ACD	ACD	Confluent
Negative Control	ACD	ACD	0

5.4 Conclusions

The connectors were soiled with a suspension intended to provide a minimum challenge level of 10^6 CFU of *Geobacillus stearothermophilus* spores per connector, the input verification tests indicated that the challenge was calculated to be $\geq 1.4 \times 10^6$ CFU per connector.

The soiling test data presented provide assurance that the **Kleenpak** connector is able to provide a sterile fluid pathway even after being soiled with bacterial spores.

6. Closure Integrity Testing

6.1 Introduction

The closure integrity test is used to confirm seal integrity of the **Kleenpak** connector.

6.2 Summary of Methods

The vacuum leak test is based on the guidelines outlined in ASTM D4991-94 *Leakage Testing of Empty Rigid Containers by Vacuum Method.* The test method is designed to detect open pathways, channels or voids across the seal area intended as a primary sterile barrier, and to detect pinholes in non-porous materials.

To demonstrate the integrity of container closure, the test was performed with thirty ACD series **Kleenpak** connectors, which were first gamma irradiated at a dose of 50.5 – 54.9 kGy. An additional test was performed with five KPCHT series connectors, which were subjected to gamma irradiation at a dose of 25 kGy, followed by two 30 minute autoclave cycles at 135°C (275°F). The five KPCHT series connectors also had a hole deliberately made in the barrel of the male connector. The hole was made to demonstrate that the o-ring in the male side of the connector is not providing additional protection of the taper seal during the test.

The **Kleenpak** connectors were assembled, sealed at one end and secured to a vacuum pump at the opposite end. The units were then completely immersed at least 2.5 cm (one inch) below the surface of a dye solution. The vacuum was turned on so that the vacuum level increased slowly until a full vacuum was reached (approximately 0.9 bar/ 27 in Hg). The test samples were subjected to this pressure for a 10 minute duration. The test samples were then removed from the dye solution and the outer surface was wiped clean. The samples were examined for any evidence that the dye solution had breached the sterile barrier.

6.3 Results

The results are shown in Tables 6-1 and 6-2.

Table 6-1: Results of Vacuum Leak Test performed with Intact ACD Series Connectors

Pall Kleenpak Connector Lot Number	Number of Connectors Tested	Passed	Failed
1	10	10	0
2	10	10	0
3	10	10	0
Total	**30**	**30**	**0**

Table 6-1: Results of Vacuum Leak Test performed with KPCHT Series Connectors with holes made in the Barrel in the Male side of the Connector

Number of Connectors Tested	Passed	Failed
5	5	0

6.4 Conclusions

The closure integrity test data provided confirms seal integrity of the **Kleenpak** connector.

7. Determination of Water Flow Characteristics

7.1 Introduction

The purpose of these tests was to determine the water flow rates of the **Kleenpak** connector when subjected to different differential pressures.

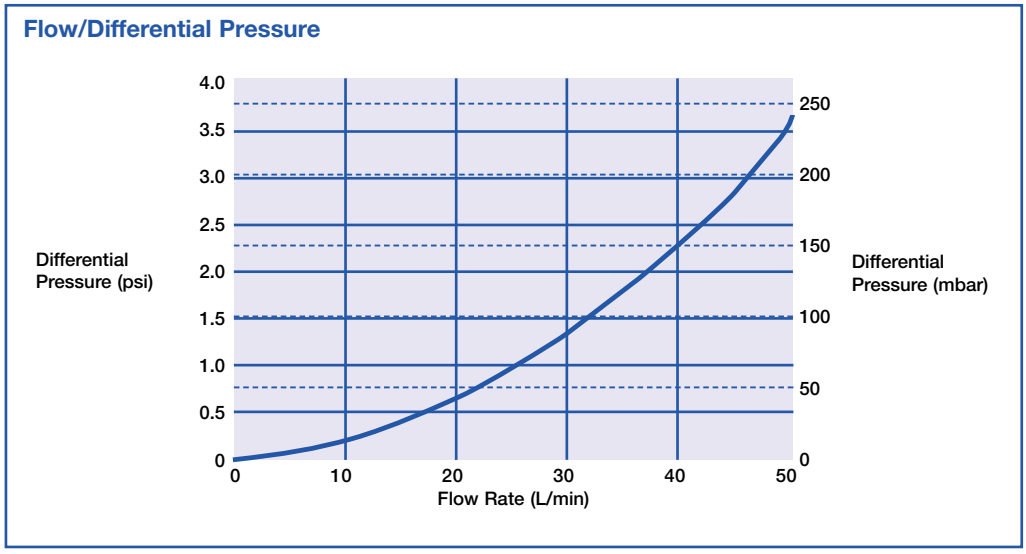
7.2 Summary of Methods

Deionized water was pumped through the assembled ACD series **Kleenpak** connector at various flow rates, and the differential pressure across the **Kleenpak** connector recorded via pressure transducers on the upstream and downstream side of the connection. All of the data were corrected to a standard temperature of 20°C (68°F). The dimensions of the ACD and KPCHT series connectors are the same, therefore this test was performed with the ACD series only and results apply for the KPCHT series.

7.3 Results

The water flow/differential pressure characteristics of the **Kleenpak** connector are shown in Figure 7-1.

Figure 7-1: Water Flow/Differential Pressure Characteristics for Kleenpak Connector



7.4 Conclusions

The water flow/pressure drop data presented for connected **Kleenpak** connectors can be used in conjunction with the pressure drop characteristics of other system components (e.g. tubing, filter capsules and bags) to form the basis for sizing the disposable system employing the **Kleenpak** connector.

8. Extractables Testing

8.1 Introduction

The purpose of these tests was to quantify and characterize the nonvolatile materials that may be extracted from typical **Kleenpak** connectors into biopharmaceutical products by water and ethanol at ambient temperature.

8.2 Summary of Methods

Three typical **Kleenpak** connectors were used for each of the tests. The ACD series connectors were subjected to gamma irradiation at doses of 50.5 – 54.9 kGy, followed by one 30 minute autoclave cycle at 121°C (250°F); the KPCHT series connectors were subjected to gamma irradiation at a dose of 25 kGy, followed by two 30 minute autoclave cycles at 135°C (275°F).

In these tests, the connectors were tested for non-volatile extractables in water. These extractables were analyzed both quantitatively (gravimetric non-volatile residue, NVR) and qualitatively (UV–Vis Spectroscopy). The temperature during the test was 30°C (86°F).

The extraction tests were performed in a flow through arrangement with fluid recirculation so that the extraction was performed on the fluid path only. The flow rate during the tests was 6 liters per minute and the test period was 24 hours. Three connectors were used in series for each recirculation test.

At the end of the 24-hour period, a suitable volume of each extract was evaporated to dryness and the amount of non-volatile residue was determined. The material extracted from the three **Kleenpak** connectors was compared with control samples of water that had been recirculated through the same extraction system but without **Kleenpak** connectors installed.

The Total Organic Carbon (TOC) was determined for the water extract. Additionally, the water extract from the three connectors was subjected to UV absorbance measurements at 240, 280 and 350 nanometers (nm).

Extractables tests were also performed with ethanol (denatured HPLC grade). These tests were performed in a flow through arrangement with fluid recirculation so that the extraction was performed on the fluid path only. The flow rate during the tests was 6 liters per minute and the test period was 4 hours. Three connectors were used in series for each recirculation test.

8.3 Results

The results (Table 8-1) show the typical quantities of non-volatile residue extracted per **Kleenpak** connector using deionized water at ambient temperature. The results reported are typical for the connector. Table 8-2 provides TOC and conductivity measurements and Table 8-3 provides UV absorbance results. Negative and positive controls were performed. The negative control consisted of the test fixture without the **Kleenpak** connector (i.e. tubing and glassware). The positive control was a control to account for evaporation and potassium biphthalate was used.

Table 8-1: Aqueous Extractables for ACD Series Kleenpak Connectors

Connectors were subjected to gamma irradiation at doses of 50.5 – 54.9 kGy, followed by one 30 minute autoclave cycle at 121°C (250°F).

Sample	Male Kleenpak Connector Sample Number	Female Kleenpak Connector Sample Number	Non-volatile Residue (mg) for three Kleenpak Connectors
1	36	90	<0.1 mg
2	98	95	
3	181	109	

Table 8-2: Conductivity and TOC of Aqueous Extractables for ACD Series Kleenpak Connectors

Sample	Conductivity (µS/cm)	TOC (ppb)
Negative Control*	4.15	1672.2 ± 4.3
Positive Control	33.6	25067.8 ± 48.8
Kleenpak Connector Samples 1, 2 and 3	4.11	1096.1 ± 22.6

* Negative controls reflect baseline contaminants associated with test system and consequently are higher than the test samples.

Table 8-3: UV Analysis for ACD Series Connectors: Peak Wavelengths at 240, 280 and 350 nm

Sample	240 nm	280 nm	350 nm
Negative Control*	0.057358	0.045120	0.035767
Positive Control	1.32950	0.28560	<0.001
Kleenpak Connector Samples 1, 2, and 3	<0.001	<0.001	<0.001

* Negative controls reflect baseline contaminants associated with test system and consequently are higher than the test samples.

Table 8-4: Ethanol Extractables for ACD Series Kleenpak Connectors

Sample	Male Kleenpak Connector Sample Number	Female Kleenpak Connector Sample Number	Non-volatile Residue (mg) for three Kleenpak Connectors
1	22	85	<0.1 mg
2	194	200	
3	126	161	

Table 8-5: Aqueous Extractables for KPCHT Series Kleenpak Connectors

Connectors were subjected to gamma irradiation at a dose of 25 kGy, followed by two 30 minute autoclave cycles at 135°C (275°F).

Three Connectors in Series	Non-volatile Residue (mg) for three Kleenpak Connectors
1	<0.1 mg
2	
3	

Table 8-6: TOC of Aqueous Extractables for KPCHT Series Kleenpak Connectors

Sample	TOC (ppb)
Negative Control*	3265.2 ± 90.2
Kleenpak Connector Samples 1, 2 and 3	1603.2 ± 83.6

* Negative controls reflect baseline contaminants associated with test system and consequently are higher than the test samples.

Table 8-7: UV Analysis for KPCHT Series Kleenpak connectors: Peak Wavelengths at 240, 280 and 350 nm

Sample	240 nm	280 nm	350 nm
Negative Control*	0.064346	0.04567	0.030869
Kleenpak Connector Samples 1, 2 and 3	0.050568	0.039337	0.027847

* Negative controls reflect baseline contaminants associated with test system and consequently are higher than the test samples.

Table 8-8: Ethanol Extractables for KPCHT Series Kleenpak Connectors

Connectors were subjected to two 30 minute autoclave cycles at 135°C (275°F).

Three Connectors in Series	Non-volatile Residue (mg) for three Kleenpak Connectors
1	<0.1 mg
2	
3	

8.4 Conclusions

The level of aqueous and ethanol extractables for both ACD and KPCHT series **Kleenpak** connectors are extremely low. The non-volatile residue (NVR) for three connectors tested together was < 0.1 mg after a 24 hour recirculation in water and after a 4 hour recirculation in ethanol for both the ACD and KPCHT series connectors tested.

Conductivity (performed for ACD series connectors only), UV and TOC measurements on the extracted water from the three ACD series connectors and three KPCHT series connectors indicated close agreement to the negative controls for these measurements. All results indicate that extractables are low and at the detection limit of the analysis techniques.

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

9. Shelf Life Studies

9.1 Introduction

Shelf life studies are performed as real time tests. There are four aims for this series of tests:

- **To demonstrate that an adequate safety margin is maintained for the burst pressures of gamma-irradiated Kleenpak connectors following storage at room temperature for up to 24 months**
- **To demonstrate that an adequate safety margin is maintained for the extractables of gamma-irradiated Kleenpak connectors following storage at room temperature for up to 24 months**
- **To demonstrate that Kleenpak connectors provide a sterile fluid pathway following storage for 24 months after exposure to gamma irradiation**
- **To establish a shelf life for the Kleenpak connector after gamma irradiation**

Results of real time shelf life testing for a six month and one year period are available at the time of writing. Interim reports for longer time periods are available upon request. All testing was performed with ACD series connectors.

9.2 Summary of Methods

Samples of typical **Kleenpak** connectors were irradiated at a dose of 50.5 – 54.9 kGy and then stored at ambient temperature for six months. On completion of the storage interval, the connectors were subjected to the following tests.

Burst Pressure Testing

The burst pressures of the **Kleenpak** connectors were determined according to the method described previously in Section 2.

Extractables

Kleenpak connector extractables were determined according to the method described previously in Section 8 for water.

Peel Away Strip Seal

Samples of the **Kleenpak** connector were subjected to a test of the integrity of the seal of the peel away strip to the device. This is a standard manufacturing test that is performed on every lot.

9.3 Results

Six month real time test (all tests performed with ACD series).

Three **Kleenpak** connectors were subjected to a test of the integrity of the seal of the peel away strip to the device and all three passed the test.

The **Kleenpak** connector burst tests ended when the connection pulled apart; the connector did not burst.

The results of the burst pressure tests are shown in Table 9-1. The burst pressures are > 19.3 barg (280 psig), which was the lowest burst pressure for the connectors tested in Section 2 ("zero" shelf life)

Table 9-1: Burst Pressures of Connected Kleenpak Connectors Irradiated at 50.5 – 54.9 kGy (6 month shelf life)

Pall Kleenpak Connector Sample Number	Burst Pressure	
	Barg	Psig
6 months -1	53.8	780
6 months -2	35.8	320

The results of the extractables tests performed on three **Kleenpak** connectors connected in series stored for 6 months following irradiation with doses of 50.5 – 54.9 kGy are shown in Table 9-2. The non volatile residue extracted for water and ethanol were low and comparable to the results for the irradiated samples with zero shelf life, which had extractables < 0.1 mg.

Table 9-2: Extractables for Kleenpak Connectors

Sample	Recirculation Period (Hours)	Non-volatile Residue (mg) for three Kleenpak Connectors
Water	24	0.7 mg
Ethanol	4	<0.1 mg

One year real time test (all tests performed with ACD series connectors).

Three **Kleenpak** connectors were subjected to a test of the integrity of the seal of the peel away strip to the device and all three passed the test.

The **Kleenpak** connector burst tests ended when the connection pulled apart; the connector did not burst.

The results of the burst pressure tests are shown in Table 9-3. The burst pressures are > 19.3 barg (280 psig), which was the lowest burst pressure for the connectors tested in Section 2 ("zero" shelf life).

Table 9-3: Burst Pressures of Connected Kleenpak Connectors Irradiated at 50.5 – 54.9 kGy (1 year shelf life)

Pall Kleenpak Connector Sample Number	Burst Pressure	
	Barg	Psig
1 year -1	59.3	860
1 year -2	56.5	820

The results of the extractables tests performed on three **Kleenpak** connectors connected in series and stored for 1 year following irradiation with doses of 50.5 – 54.9 kGy are shown in Table 9-4. The non-volatile residue extracted for water and ethanol were comparable to the results for the irradiated samples with zero shelf life, which had extractables < 0.1 mg.

Table 9-4: Extractables for Kleenpak Connectors

Sample	Recirculation Period (Hours)	Non-volatile Residue (mg) for three Kleenpak Connectors
Water	24	<0.1 mg
Ethanol	4	<0.1 mg

9.4 Conclusions

Burst pressure tests performed on irradiated connectors that had been stored for six months and 1 year demonstrated that a high safety factor was maintained between the burst strength and the maximum recommended operating pressure.

Extractables tests performed on three irradiated connectors tested in series that had been stored for six months indicated that the non-volatile residue extracted was 0.7 mg when water was used as the extraction fluid and < 0.1 mg for ethanol. The samples stored for 1 year indicated that the non-volatile residue extracted was < 0.1 mg when water was used as the extraction fluid and < 0.1 mg for ethanol.

Samples of the **Kleenpak** connector subjected to a test of the integrity of the seal of the peel away strip to the device indicated that the seal remained intact after six months and after 1 year.

10. Autoclave Testing

10.1 Introduction

The purpose of the autoclave tests was to verify the ability of the **Kleenpak** connector to be rendered sterile by autoclaving and to set maximum conditions for autoclave time and temperature.

10.2 Summary of Methods

To establish autoclave conditions for the ACD series connectors, tests were performed by autoclaving these connectors at a temperature of 121°C (250°F) for a 30 minute cycle. The physical characteristics of the **Kleenpak** connectors were examined after the autoclave tests. In addition, several of the validation tests (Burst pressure, creep rupture, extractables, tensile strength) were performed after the **Kleenpak** connector had been subjected to both gamma irradiation and autoclaving at a temperature of 121°C (250°F) for a 30 minute cycle. This represents a worst case condition, since the **Kleenpak** connector is intended for use with either autoclave or gamma irradiation, but not for both.

To establish autoclave conditions for the KPCHT series connectors, tests were performed by autoclaving these connectors at a temperature of 130°C (266°F) for a 30 minute cycle. The physical characteristics of the **Kleenpak** connectors were examined after the autoclave tests. In addition, several of the validation tests (Burst pressure, extractables, tensile strength) were performed after the **Kleenpak** connector had been subjected to both gamma irradiation and autoclave at a temperature of 135°C (275°F) for a 30 minute cycle. This represents a worst case condition, since the **Kleenpak** connector is intended for use with either autoclave or gamma irradiation, but not for both and the autoclave temperature of 135°C (275°F) is higher than the recommended temperature of 130°C (266°F).

Autoclave tests were also performed with biological indicators *(G. stearothermophilus)*. These tests were performed with the ACD series connectors. For the male connectors, the spore strips were placed in the region between the male spike probe and the body of the male connector and for the female connector, the strips were placed in the fluid path. Spore strips that provided a minimum of 10^6 CFU per connector were used. The connectors were autoclaved at 121°C (250°F) for 15 minutes. The strips were transferred to TSB and allowed to incubate at 55°C (131°F) for seven days. The TSB was then filtered through a 0.2 micrometer membrane disc and plated onto trypticase soy agar (TSA) and incubated for 7 days at 55°C (131°F). Absence of microbial growth was indicative of the ability of the **Kleenpak** connector to be rendered sterile by autoclaving.

10.3 Results

Physical examination, as well as additional tests post autoclaving at 121°C (250°F) for a 30 minute cycle indicates that the ACD series **Kleenpak** connector can be autoclaved at these conditions. The same tests applied to the KPCHT series connector indicate that this connector series can be autoclaved at 130°C (266°F) for 30 minutes.

The results (Table 10-1) show the results of the autoclave spore strip tests.

Table 10-1: Autoclave Spore Strip Tests

Pall Male Kleenpak (ACD Series) Connector Sample Number	Number of CFU	Pall Female Kleenpak (ACD Series) Connector Sample Number	Number of CFU
115	0	9	0
121	0	66	0
128	0	127	0
166	0	174	0

10.4 Conclusions

The ACD series **Kleenpak** connector has been demonstrated to be capable of being autoclaved at a maximum temperature of 121°C (250°F) for 30 minutes, while the KPCHT series connector has been demonstrated to be capable of being autoclaved at a maximum temperature of 130°C (166°F) for 30 minutes. Tests with biological indicators demonstrated that the **Kleenpak** connector could be rendered sterile by autoclaving at 121°C (250°F) for 15 minutes.

11. Biological Safety Tests

11.1 Introduction

The purpose of these tests was to evaluate the biological suitability of the materials of construction of the fluid path of the **Kleenpak** connector. The materials of construction are as follows:

Connector Body*:	Polycarbonate
Plunger:	Polycarbonate
Rubber Grommets:	Thermoplastic elastomer
O-Ring:	Internally lubricated ethylene propylene
Protective Cap:	Polypropylene
Peel-away Strip:	Hydrophobic Polyethersulfone

* Fluid path

11.2 Summary of Methods

Tests include USP Biological Reactivity Tests, *in vivo* for Class VI Plastics (121°C) as described in the current *United States Pharmacopeia* Chapter <88> and USP Physicochemical Tests for Plastics, as described in Chapter <661> of the *United States Pharmacopoeia.* These tests will be conducted only on the fluid contact material of construction (polycarbonate).

The tests were conducted by STS duoTEK , Inc., Rush, New York, USA.

Biological Reactivity Tests

The Biological Reactivity Tests *in vivo* for Class VI-121°C Plastics as described in the *United States Pharmacopoeia* include:

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

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

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

The USP states that extracts may be prepared at one of three standard conditions: 50°C (122°F) for 72 hours, 70°C (158°F) for 24 hours, or 121°C (250°F) for 1 hour. The most stringent condition not resulting in physical changes in the plastic is recommended, therefore the polycarbonate fluid contact material were extracted at 121°C (250°F).

Acute Systemic Injection Tests

An Acute Systemic Injection Test will be performed to evaluate the potential of a single injection of an extract to produce systemic toxicity. Extracts of the fluid path material of construction (polycarbonate) in Sodium Chloride Injection and 1-in-20 Solution of Ethanol in Sodium Chloride Injection will be injected intravenously. Cottonseed oil extract and Polyethylene Glycol 400 extracts will be injected intraperitoneally.

Intracutaneous Tests

An Intracutaneous Test will be performed to evaluate the potential of 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 tests will also performed, in order to subject the fluid path material of construction to the most stringent conditions included in the USP.

Physicochemical Test

Containers composed of plastics that are intended for packaging products for parenteral use must meet the requirements of Physicochemical Testing - Plastics found in the current USP24. These tests are designed to measure the properties of impurities extracted from plastics when leached with extraction medium over a specified period and temperature. The value of these tests becomes important to insure the efficacy of product within the container.

The tests under USP <661> are:

- **Non Volatile Residue (NVR) - measures organic/inorganic residues soluble in extraction media**
- **Residue On Ignition - this test is performed when the NVR is greater than 15 milligrams**
- **Buffering Capacity - measures the alkalinity or acidity of the extract**
- **Heavy Metals - detects the presence of metals such as lead, tin, zinc, etc.**

11.3 Results

The components of the ACD series and KPCHT series **Kleenpak** connector passed all of the tests specified.

11.4 Conclusions

The fluid path components of the **Kleenpak** connector meet the requirements of the USP for Class VI -121°C Plastics and the Physicochemical tests.

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