PALL Life Sciences

Validation Guide

USTR 2568

Kleenpak™ Sterile Connectors

Shelf-life Studies
An Addendum to Pall Publication USTR 2232: Validation Guide for Kleenpak™ Sterile Connectors for use with 13 mm (½-inch) nominal tubing

*Filtration. Separation. Solution.*SM

Contents

1. Introduction

This shelf life study has been completed on the Kleenpak™ sterile connector[1] as a continuation of the shelf life studies detailed in USTR 2322. The aims of this series of tests are:

- To demonstrate that an adequate safety margin is maintained for burst pressure and extractables, and that a sterile fluid path can be maintained following storage for up to 3 years (using accelerated aging method) or up to 2 years (real time) after exposure to gamma irradiation;
- To establish a shelf life for the Kleenpak sterile connector after gamma irradiation.

On completion of each storage interval, the Kleenpak sterile connector was subjected to the following tests:

- Burst Testing
- Extractables Test
- Peel Strip Seal Test
- Functional (Soiling) Test

The data collected support a three year shelf life claim for the Kleenpak sterile connector.

[1] This product is not sold sterile. For use in making sterile connections, each connector must be fitted in a closed single-use assembly that has been subjected to a validated sterilizing process.

2. Summary of Methods

2.1 Kleenpak Sterile Connector Sample Information

Kleenpak sterile connectors (Part Numbers KPCHT02M6, KPCHT02F6, ACD02M6, and ACD02F6) were tested. Prior to storage, samples were subjected to gamma irradiation doses of 48.5 – 57.0 kiloGray (kGy). Certificates of gamma irradiation treatment are on file.

2.2 Storage

For real-time aging, following irradiation, the Kleenpak sterile connectors were stored at ambient temperature for up to 2 years. Additional Kleenpak sterile connectors were stored using accelerated aging method as established in ASTM F 1980-02; storage area temperature and humidity were monitored and maintained to 55 °C ± 2 °C, and less than 20 percent, respectively, and Kleenpak sterile connectors were stored for 38, 76, or 114 days to simulate storage at ambient conditions for 1, 2, and 3 years, respectively. Kleenpak sterile connectors were evaluated as described below.

2.3 Burst Pressure Test

The burst pressures of the assembled Kleenpak sterile connectors were determined according to the method described previously in Section 2 of USTR 2232.

2.4 Extractables Test

The extractables test was performed using methods consistent with that in Section 8 of USTR 2232. In summary, a male and female Kleenpak sterile connector were assembled together. Three assembled Kleenpak sterile connectors were then connected in series, and following a 24-hour recirculation with deionized water or 4-hour recirculation with 95% ethanol at a flow rate of 6 L/min, extractables levels were analyzed quantitatively (gravimetric non-volatile residue, NVR).

2.5 Peel Strip Seal Test

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

2.6 Functional (Soiling) Test

The soiling test was performed using methods consistent with that in Section 5 of USTR 2232. In summary, for test samples and input verification control samples, male and female Kleenpak sterile connectors were soiled by exposing Kleenpak sterile connectors up to the flange in a spore

solution of *Geobacillus stearothermophilus.* The positive assay control Kleenpak sterile connector set were exposed to the spore solution after first removing the protective peel strip. The negative assay control was not exposed to the spore solution. A volume (300 mL) of Trypticase◆ Soy Broth (TSB) was then transferred through all assembled Kleenpak sterile connectors, aseptically collected, and then passed through a sterile recovery filter (0.2 µm rated). This filter was then plated onto Trypticase Soy Agar (TSA), and incubated at 55 °C for 7 days. After incubation, each plate was inspected for growth of *Geobacillus stearothermophilus.*

3. Summary of Results

3.1 Burst Testing

When irradiated Kleenpak sterile connectors were subjected to the burst test, the data demonstrated that the minimum burst pressure measured for any of the test samples was 28 barg (391 psig). This is more than 10 times greater than the maximum recommended operating pressure. This indicates that storage period of up to 3 years does not adversely affect the burst pressure of the device.

3.2 Extractables Test

When irradiated Kleenpak sterile connectors (three in series) were subjected to the extractables tests, the data demonstrated that the non-volatile residue was < 1 mg when either water or ethanol was used as the extraction fluid, which is consistent with the claims for the device. This indicates that a storage period of up to 3 years does not adversely affect the extractables level of the device.

3.3 Peel Strip Seal Test

When irradiated Kleenpak sterile connectors were subjected to the peel strip seal test, each of the connected Kleenpak sterile connectors passed the test, regardless of storage period applied to the devices. This indicates that a storage period of up to 3 years does not adversely affect the seal of the strip to the device.

3.4 Functional (Soiling) Test

When irradiated Kleenpak sterile connectors were subjected to functional (soiling) tests, the data demonstrated that pre-sterilized fluid passed through the assembled pre-sterilized Kleenpak sterile connectors yielded zero colonies, which is consistent with the claims for the device. This indicates that a storage period of up to 3 years does not adversely affect the ability of the Kleenpak sterile connector to maintain a sterile fluid path.

4. Evaluation of Kleenpak Sterile Connectors (Post-Gamma Irradiation) after 1 Year of Storage

4.1 Burst Test

4.1.1 Test Summary

The Kleenpak sterile connector burst tests ended when the connection separated. The minimum burst pressure obtained in these tests was 29.7 barg (431 psig). The burst pressure data are summarized in Table 1.

4.1.2 Test Results

Table 1

Burst Pressures of Assembled Kleenpak Sterile Connectors following 1 Year of Storage (Simulated or Real Time)

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Quantity of Kleenpak Sterile Connectors Tested	Minimum Burst Pressure[1] barg	psig
1 year (Simulated)	KPCHT02M6	615802	9	38.1	55
	KPCHT02F6	613602, 603402, 604502	9	38.1	552
1 year (Simulated)	ACD02M6	608002	9	29.7	431
	ACD02F6	608202	9	29.7	431
1 year (Real Time)	KPCHT02M6	532802	5	33.9	492
	KPCHT02F6	532602	5	33.9	492
1 year (Real Time)	ACD02M6	609302, 611002, 612402	12	35.0	514
	ACD02F6	608202, 609502	12	35.0	514

[1] *Pressures recorded indicate the pressure required to separate the connection.*

4.2 Extractables Test

4.2.1 Test Summary

Extractables testing performed on the three gamma irradiated Kleenpak sterile connectors tested in series indicated that the non-volatile residue was less than 1 mg when either water or ethanol was used as the extraction fluid. The results of the extractables tests are summarized in Table 2. Values presented have been adjusted for background. The limit of detection of the testing is 0.1 mg.

4.2.2 Test Results

Table 2

Aqueous and Ethanol Extractables Levels for Assembled Kleenpak Sterile Connectors following 1 Year of Storage (Simulated or Real Time)

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Replicate Set of 3 Kleenpak Sterile Connectors	Aqueous Extractables (Non-volatile Residue [mg per 3 Kleenpak Sterile Connectors])	Ethanol Extractables (Non-volatile Residue [mg per 3 Kleenpak Sterile Connectors])
1 year (Simulated)	KPCHT02M6	615802	1	ND[1]	< 1.0
	KPCHT02F6	603402, 604502	2	ND[1]	< 1.0
		613602	3	ND[1]	< 1.0

Table 2 *Continued*

Aqueous and Ethanol Extractables Levels for Assembled Kleenpak Sterile Connectors following 1 Year of Storage (Simulated or Real Time)

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Replicate Set of 3 Kleenpak Sterile Connectors	Aqueous Extractables (Non-volatile Residue [mg per 3 Kleenpak Sterile Connectors])	Ethanol Extractables (Non-volatile Residue [mg per 3 Kleenpak Sterile Connectors])
1 year (Simulated)	ACD02M6	608002	1	ND[1]	< 1.0
	ACD02F6	608202	2	ND[1]	< 1.0
			3	ND[1]	< 1.0
1 year (Real Time)	KPCHT02M6	532802	1	< 1.0	< 1.0
	KPCHT02F6	532602	2	< 1.0	< 1.0
			3	< 1.0	< 1.0
1 year (Real Time)	ACD02M6	609302, 611002,	1	< 1.0	< 1.0
		612402	2	< 1.0	< 1.0
	ACD02F6	609502, 608202	3	< 1.0	< 1.0

[1] *ND: Not detectable*

4.3 Peel Strip Seal Test

4.3.1 Test Summary

All Kleenpak sterile connectors passed the test. The results of the Peel Strip Seal Test are presented in Table 3: Results of Peel Strip Seal Testing on Kleenpak Sterile Connectors following 1 Year of Storage (Simulated or Real Time).

4.3.2 Test Results

Table 3

Results of Peel Strip Seal Testing on Kleenpak Sterile Connectors following 1 Year of Storage (Simulated or Real Time)

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Quantity of Kleenpak Sterile Connectors Tested	Test Results	
				Pass	Fail
1 Year (Simulated)	KPCHT02M6	615802	9	9	0
	KPCHT02F6	603402	9	9	0
	ACD02M6	608002	9	9	0
	ACD02F6	608202	9	9	0
1 Year (Real Time)	KPCHT02M6	532802	5	5	0
	KPCHT02F6	532602	5	5	0
	ACD02F6	608202	6	6	0
		608002	6	6	0
	ACD02M6	609302	4	4	0
		611002	5	5	0
		612402	5	5	0

4.4 Functional (Soiling) Test

4.4.1 Test Summary

For the Kleenpak sterile connectors tested, no colonies were detected in fluid transferred through any of the sets, apart from the positive control. The results of the functional (soiling) tests performed on Kleenpak sterile connectors are presented in Table 4: Results of Functional (Soiling) Tests on Assembled Kleenpak Sterile Connectors following 1 Year of Storage (Simulated or Real Time).

4.4.2 Test Results

The input verification control samples confirmed that a minimum of 1.0×10^6 CFU of the test organism adhered to the Kleenpak sterile connectors. The negative control yielded 0 CFU of test organism, and the positive control yielded recovery of the test organism.

Table 4

Results of Functional (Soiling) Tests on Assembled Kleenpak Sterile Connectors following 1 Year of Storage (Simulated or Real Time)

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Quantity of Kleenpak Sterile Connectors Tested	CFU Detected per Kleenpak Sterile Connector Set
1 year (Simulated)	KPCHT02M6	615802	6	0
	KPCHT02F6	603402, 613602, 604502	6	0
1 year (Simulated)	ACD02M6	608002	4	0
	ACD02F6	608202	4	0
1 year (Real Time)	KPCHT02M6	532802	5	0
	KPCHT02F6	532602	5	0
1 year (Real Time)	ACD02M6	609302, 611002, 612402	3	0
	ACD02F6	609502, 608202	3	0

5. Evaluation of Kleenpak Sterile Connectors (Post-Gamma Irradiation) after 2 Years of Storage

5.1 Burst Test

5.1.1 Test Summary

The Kleenpak sterile connectors burst tests ended when the connection separated. The minimum burst pressure obtained in these tests was 33.2 barg (481 psig). The burst pressure data are summarized in Table 5.

5.1.2 Test Results

Table 5

Burst Pressures of Assembled Kleenpak Sterile Connectors following 2 Years of Storage (Simulated or Real Time)

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Quantity of Kleenpak Sterile Connectors Tested	Minimum Burst Pressure[1] barg	psig
2 years (Simulated)	KPCHT02M6	615802	9	44.9	651
	KPCHT02F6	613602, 603402, 604502	9	44.9	651
2 years (Simulated)	ACD02M6	608002	9	33.2	481
	ACD02F6	608202	9	33.2	481
2 years (Real Time)	KPCHT02M6	532802	8	34	499
	KPCHT02F6	532602	8	34	499

[1] *Pressures recorded indicate the pressure required to separate the connection.*

5.2 Extractables Test

5.2.1 Test Summary

Extractables testing performed on the three gamma irradiated Kleenpak sterile connectors tested in series indicated that the non-volatile residue was less than 1 mg when either water or ethanol was used as the extraction fluid. The results of the extractables tests are summarized in Table 6: Aqueous and Ethanol Extractables Levels for Assembled Kleenpak Sterile Connectors following 2 Years of Storage (Simulated or Real Time). Values presented have been adjusted for background. The limit of detection of the testing is 0.1 mg.

5.2.2 Test Results

Table 6

Aqueous and Ethanol Extractables Levels for Assembled Kleenpak Sterile Connectors following 2 Years of Storage (Simulated or Real Time)

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Replicate Set of 3 Kleenpak Sterile Connectors	Aqueous Extractables (Non-volatile Residue [mg per 3 Kleenpak Sterile Connectors])	Ethanol Extractables (Non-volatile Residue [mg per 3 Kleenpak Sterile Connectors])
2 years (Simulated)	KPCHT02M6	615802	1	< 1.0	< 1.0
	KPCHT02F6	603402, 604502,	2	< 1.0	< 1.0
		613602	3	< 1.0	< 1.0
2 years (Simulated)	ACD02M6	608002	1	< 1.0	< 1.0
	ACD02F6	608202	2	< 1.0	< 1.0
			3	< 1.0	< 1.0

Table 6 *Continued*

Aqueous and Ethanol Extractables Levels for Assembled Kleenpak Sterile Connectors following 2 Years of Storage (Simulated or Real Time)

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Replicate Set of 3 Kleenpak Sterile Connectors	Aqueous Extractables (Non-volatile Residue [mg per 3 Kleenpak Sterile Connectors])	Ethanol Extractables (Non-volatile Residue [mg per 3 Kleenpak Sterile Connectors])
2 years (Real Time)	KPCHT02M6	532802	1	< 1.0	< 1.0
	KPCHT02F6	532602	2	< 1.0	< 1.0
			3	< 1.0	< 1.0
2 years (Real Time)	ACD02M6	608002	1	< 1.0	< 1.0
	ACD02F6	608202	2	< 1.0	< 1.0
			3	< 1.0	< 1.0

5.3 Peel Strip Seal Test

5.3.1 Test Summary

All of the Kleenpak sterile connectors passed the test. The results of the Peel Strip Seal Test are presented in Table 7: Results of Peel Strip Seal Test on Kleenpak Sterile Connectors following 2 Years of Storage (Simulated or Real Time).

5.3.2 Test Results

Table 7

Results of Peel Strip Seal Test on Kleenpak Sterile Connectors following 2 Years of Storage (Simulated or Real Time)

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Quantity of Kleenpak Sterile Connectors Tested	Test Results	
				Pass	Fail
2 Years (Simulated)	KPCHT02M6	615802	9	9	0
	KPCHT02F6	603402, 613602, 604502	9	9	0
2 Years (Simulated)	ACD02M6	608002	9	9	0
	ACD02F6	608202	9	9	0
2 Years (Real Time)	KPCHT02M6	532802	8	8	0
	KPCHT02F6	532602	8	8	0

5.4 Functional (Soiling) Test

5.4.1 Test Summary

For Kleenpak sterile connectors tested, no colonies were detected in fluid transferred through any of the sets, apart from the positive control. The results of the functional (soiling) tests performed on Kleenpak sterile connectors are presented in Table 8: Results of Soiling Tests on Assembled Kleenpak Sterile Connectors following 2 Years of Storage (Simulated or Real Time).

5.4.2 Test Results

The input verification control samples indicated that a minimum of 4.0 x 10^6 CFU of the test organism adhered to the Kleenpak sterile connectors. The negative control yielded 0 CFU of test organism, and the positive control yielded recovery of the test organism.

Table 8

Results of Soiling Tests on Assembled Kleenpak Sterile Connectors following 2 Years of Storage (Simulated or Real Time)

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Quantity of Kleenpak Sterile Connectors Tested	CFU Detected per Kleenpak Sterile Connectors Set
2 years (Simulated)	KPCHT02M6	615802	6	0
	KPCHT02F6	603402, 613602, 604502	6	0
2 years (Simulated)	ACD02M6	608002	5	0
	ACD02F6	608202	5	0
2 years (Real Time)	KPCHT02M6	532802	5	0
	KPCHT02F6	532602	5	0
2 years (Real Time)	ACD02M6	608002	3	0
	ACD02F6	608202	3	0

6. Evaluation of Kleenpak Sterile Connectors (Post-Gamma Irradiation) after 3 Years of Storage

6.1 Burst Test

6.1.1 Test Summary

The Kleenpak sterile connector burst tests ended when the connection separated. The minimum burst pressure obtained in these tests was 28 barg (391 psig). The burst pressure data are summarized in Table 9.

6.1.2 Test Results

Table 9

Burst Pressures of Assembled Kleenpak Sterile Connectors following Accelerated Aging to Simulate 3 Years of Real Time Storage

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Quantity of Kleenpak Sterile Connectors Tested	Minimum Burst Pressure[1]	
				barg	psig
3 years (Simulated)	KPCHT02M6	615802	9	33.0	478
	KPCHT02F6	613602, 603402, 604502	9	33.0	478
3 years (Simulated)	ACD02M6	608002	9	28.0	391
	ACD02F6	608202	9	28.0	391

[1] *Pressures recorded indicate the pressure required to separate the connection.*

6.2 Extractables Test

6.2.1 Test Summary

Extractables testing performed on the three gamma irradiated Kleenpak sterile connectors tested in series indicated that the non-volatile residue was less than 1 mg when either water or ethanol was used as the extraction fluid. The results of the extractables tests are summarized in Table 10: Aqueous and Ethanol Extractables for Assembled Kleenpak Sterile Connectors following Accelerated Aging to Simulate 3 Years of Storage. Values presented have been adjusted for background. The limit of detection of the testing is 0.1 mg.

6.2.2 Test Results

Table 10

Aqueous and Ethanol Extractables for Assembled Kleenpak Sterile Connectors following Accelerated Aging to Simulate 3 Years of Storage

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Replicate Set of 3 Kleenpak Sterile Connectors	Aqueous Extractables (Non- volatile Residue [mg per 3 Kleenpak Sterile Connectors)	Ethanol Extractables (Non-volatile Residue [mg per 3 Kleenpak Sterile Connectors)
3 years (Simulated)	KPCHT02M6	615802	1	< 1.0	< 1.0
	KPCHT02F6	603402, 604502,	2	< 1.0	< 1.0
		613602	3	< 1.0	< 1.0
3 years (Simulated)	ACD02M6	608002	1	< 1.0	< 1.0
	ACD02F6	608202	2	< 1.0	< 1.0
			3	< 1.0	< 1.0

6.3 Peel Strip Seal Test

6.3.1 Test Summary

All Kleenpak sterile connectors passed the test. The results of the Peel Strip Seal Test are presented in Table 11.

6.3.2 Test Results

Table 11

Results of Peel Strip Seal Test on Kleenpak Sterile Connectors following Accelerated Aging to Simulate 3 Years of Real Time Storage

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Quantity of Kleenpak Sterile Connectors Tested	Test Results	
				Pass	Fail
3 Years (Simulated)	KPCHT02M6	615802	9	9	0
	KPCHT02F6	603402	9	9	0
3 Years (Simulated)	ACD02M6	608002	9	9	0
	ACD02F6	608202	9	9	0

6.4 Functional (Soiling) Test

6.4.1 Test Summary

For Kleenpak sterile connectors tested, no colonies were detected in fluid transferred through any of the sets, apart from the positive control. The results of the functional (soiling) tests performed on Kleenpak sterile connectors are presented in Table 12.

6.4.2 Test Results

The input verification control samples indicated that a minimum of 4.9 x 10^6 CFU of the test organism adhered to the Kleenpak sterile connectors. The negative control yielded 0 CFU of test organism, and the positive control yielded recovery of the test organism.

Table 12

Results of Soiling Tests on Assembled Kleenpak Sterile Connectors following Accelerated Aging to Simulate 3 Years of Real Time Storage

Storage Time	Kleenpak Sterile Connector Part Number	Kleenpak Sterile Connector Lot Number	Quantity of Kleenpak Sterile Connector Tested	Detected per Kleenpak Sterile Connector Set
3 years (Simulated)	KPCHT02M6	615802	6	0
	KPCHT02F6	603402, 613602, 604502	6	0
3 years (Simulated)	ACD02M6	608002	5	0
	ACD02F6	608202	5	0

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