PALL Life Sciences

Validation Guide

USTR 2532

Kleenpak™ HT Sterile Connectors – Extension of the Autoclave Cycle Sterilization Time

An Addendum to Pall publication USTR-2232:
'Validation Guide for Kleenpak™ Sterile Connector for use with 13 mm (½ in.) nominal tubing'

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1. Objective

This study provides data applicable to Pall High-Temperature Kleenpak™ sterile connectors (Kleenpak HT sterile connector)[1] with ½ in. hose barbs or fitted with ¼, ⅜ or ⅝ in. hose barb adapters. This data was collected to demonstrate that the Kleenpak HT sterile connector maintains all functionality after sterilization by autoclaving at 1 x 75-minute cycles at 130 °C with a suitable safety margin.

Table 1 Part Numbers of Kleenpak HT Sterile Connectors with Hose Barb Adapters

Description	Part Number
Male, Kleenpak HT sterile connectors with ½ in. hose barb	KPCHT02M6
Female, Kleenpak HT sterile connectors with ½ in. hose barb	KPCHT02F6
Male, Kleenpak HT sterile connectors with ¼ in. hose barb adapter	KPCHT02M7
Female, Kleenpak HT sterile connectors with ¼ in. hose barb adapter	KPCHT02F7
Male, Kleenpak HT sterile connectors with ⅜ in. hose barb adapter	KPCHT02M10
Female, Kleenpak HT sterile connectors with ⅜ in. hose barb adapter	KPCHT02F10
Male, Kleenpak HT sterile connectors with ⅝ in. hose barb adapter	KPCHT02M11
Female, Kleenpak HT sterile connectors with ⅝ in. hose barb adapter	KPCHT02F11

To qualify the new Kleenpak HT sterile connector configurations, the following tests were performed:

- Burst testing
- Tensile strength
- Functional (soiling) testing
- Seal integrity

Extractables, tensile strength of a completed connection, water flow, creep-rupture, autoclave testing, shelf life studies, and biological safety testing were not repeated because the new autoclave conditions will not affect the results presented in the *Validation Guide for the Kleenpak Sterile Connector for use with tubing of ½ in. nominal tubing* (USTR 2232) and in the *Validation Guide for Kleenpak Sterile Connectors* with *Hose Barb Adapters* (USTR 2451). All testing has been performed on the Kleenpak HT sterile connectors with ⅜ in. adapters. Testing on Kleenpak HT sterile connectors with ¼ in. and ⅝ in. hose barb adapters was not conducted because materials of construction and assembly methods are the same as those for the ⅜ in. hose barb adapters.

Additionally, testing was not performed on the Kleenpak HT sterile connector for ½ in. tubing (P/N KPCHT02M6, KPCHT02F6) because the ⅜ in. Kleenpak HT sterile connector body is the same material of construction.

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

2. Materials and Methods

All testing on the Kleenpak HT sterile connector with hose barb adapters utilize the same methods as used for the Kleenpak HT sterile connector outlined in Validation Guide USTR 2232. These high-temperature Kleenpak sterile connectors with hose barb adapters were autoclaved for 2 x 75-minute cycles to 130 °C prior to testing in order to incorporate an adequate safety margin for claiming 1 x 75-minutes cycles at 130 °C.

3. Results

3.1 Burst Testing

3.1.1 Objective

The purpose of these tests was to demonstrate that the Kleenpak HT sterile connector subjected to autoclave sterilization can withstand the maximum pressure rating of 3 barg (43.5 psig) with an appropriate safety margin.

Burst tests were performed on individual and assembled male and female Kleenpak HT sterile connectors with hose barb adapters subjected to autoclave sterilization by 2 x 75-minute autoclave cycles at 130 °C.

3.1.2 Results

The results of the burst tests are shown in Tables 2 and 3.

Table 2 Burst Pressures of Individual Kleenpak HT Sterile Connectors with ⅜ in. Hose Barb Adapters

Sample No.	Part No.	Pre-Treatment	Burst Pressure (barg)	Burst Pressure (psig)
1	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	81.5	1182
2	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	68.5	993
3	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	75.7	1098
4	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	76.3	1106
5	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	68.3	991
		Average	**74**	**1074**
1	KPCHT02M10	Autoclave at 130 °C for 2 x 75-minute cycles	89.6	1299
2	KPCHT02M10	Autoclave at 130 °C for 2 x 75-minute cycles	90.3	1310
3	KPCHT02M10	Autoclave at 130 °C for 2 x 75-minute cycles	100.2	1454
4	KPCHT02M10	Autoclave at 130 °C for 2 x 75-minute cycles	81.4	1181
5	KPCHT02M10	Autoclave at 130 °C for 2 x 75-minute cycles	87.5	1263
		Average	**89.8**	**1303**

Table 3 Burst Pressures of Connected Kleenpak HT Sterile Connectors with ⅜ in. Hose Barb Adapters

Sample No.	Male Part No.	Female Part No.	PreTreatment	Burst Pressure (barg)	Burst Pressure (psig)
1	KPCHT02M10	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	43.5	631
2	KPCHT02M10	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	53.8	780
3	KPCHT02M10	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	38.8	563
4	KPCHT02M10	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	36.7	532
5	KPCHT02M10	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	44.1	639
			Average	**43.4**	**629**

3.1.3 Conclusions

All sterilized Kleenpak HT sterile connectors with hose barb adapters had burst pressures that were ≥ 36.7 barg (532 psig) giving an acceptable safety factor over the maximum recommended operating pressure of 3 barg (43.5 psig), following 1 x 75-minute autoclave cycle at 130 °C with safety margin.

3.2 Tensile Strength

3.2.1 Objective

The purpose of these tests was to demonstrate the strength of the adhesive joint between the hose barb adapter and the relevant male or female Kleenpak HT sterile connector. All test samples were autoclaved to 130 °C for a 2 x 75-minute autoclave cycles prior to test to provide an adequate safety margin.

3.2.2 Results

The results are shown in Table 4.

Table 4 Results of Tensile Strength Testing of Kleenpak HT Sterile Connectors with ⅜ in. Hose Barb Adapters

Sample No.	Part No.	Pre-Treatment	Tensile Result (N)	Tensile Result (lbf)
1	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	1488	334.5
2	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	1334	300.0
3	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	1568	352.5
4	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	1515	340.6
5	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	1861	418.3
		Average	**1553**	**349.2**
1	KPCHT02M10	Autoclave at 130 °C for 2 x 75-minute cycles	1879	422.4
2	KPCHT02M10	Autoclave at 130 °C for 2 x 75-minute cycles	1540	346.3
3	KPCHT02M10	Autoclave at 130 °C for 2 x 75-minute cycles	1366	307.2
4	KPCHT02M10	Autoclave at 130 °C for 2 x 75-minute cycles	1247	280.4
5	KPCHT02M10	Autoclave at 130 °C for 2 x 75-minute cycles	1357	305.1
		Average	**1478**	**332.3**

3.2.3 Conclusions

A minimum force of 1247 N (280.4 lbf) was required to break the bond between hose barb adapter and Kleenpak HT sterile connector, which demonstrates the strength of Kleenpak sterile HT connectors built with adhesively bonded adapters following a 1 x 75-minute autoclave cycle at 130 °C with safety margin.

3.3 Functional (Soiling) Testing

3.3.1 Objective

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

3.3.2 Results

The results of the input verification test are provided in Table 5 and the results of the soiling tests are provided in Table 6.

Table 5 Input Verification Test (IVT) for Soiling Tests for Kleenpak HT Sterile Connectors with ⅜ in. Hose Barb Adapters

Test Number	CFU per Connector*
IVT 1	4.5E + 06

* CFU is an average of two (2) dilution series

Table 6 Soiling Test Results for Kleenpak HT Sterile Connectors with ⅜ in. Hose Barb Adapters

KPC Identification	CFU per Connector
Test sample 1	0
Test sample 2	0
Test sample 3	0
Positive Control	TNTC*
Negative Control	0

* TNTC = too numerous to count

3.3.3 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 ≥ 4.5 x 10^6 CFU per connector.

The soiling test data presented provide assurance that the sterilized Kleenpak HT sterile connector is able to provide a sterile fluid pathway even after being soiled with bacterial spores, following a 1 x 75-minute autoclave cycle at 130 °C with safety margin.

3.4 Seal integrity

3.4.1 Objective

The seal integrity test is used to confirm seal integrity of the adhesive joint between the ⅜ in. hose barb adapter and the relevant male or female Kleenpak HT sterile connector. The method is outlined in ASTM D4991-94 Leakage Testing of Empty Rigid Containers by Vacuum Method.

Tests were performed on assembled male and female Kleenpak HT sterile connector with hose barb adapters subjected to autoclave sterilization by 2 x 75-minute autoclave cycles at 130 °C to provide an adequate safety margin.

3.4.2 Results

The results are shown in Table 7.

Table 7 Results of the Seal Integrity Test

Sample No.	Part No.	Pre-Treatment	Number Tested*	Number Passed**
1	KPCHT02M10	Autoclave at 130 °C for 2 x 75-minute cycles	5 connections	5 connections
1	KPCHT02F10	Autoclave at 130 °C for 2 x 75-minute cycles	5 connections	5 connections

* Connectors were tested in assembled condition; i.e., male and female joined together and male plunger actuated.
** No penetration of the dye containing solution was observed during testing

3.4.3 Conclusions

The seal integrity test data confirms seal integrity and demonstrates the ability of the Kleenpak HT sterile connector with hose barb adapters to prevent liquid product leakage during operation (e.g. product transfer), following a 1 x 75-minute autoclave cycle at 130 °C with safety margin.

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