PALL Life Sciences

Validation Guide

USTR 2451a

Kleenpak™ Sterile Connectors with Hose Barb Adapters

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

Filtration. Separation. Solution.SM

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1 Objective

This study provides additional experimental data applicable to Pall High-Temperature Kleenpak™ Sterile connectors (Kleenpak Sterile connector)[1] fitted with hose barb adapters.

Table 1 Kleenpak (KPCHT) Sterile Connector with Hose Barb Adapters

Description	KPCHT with ⅜ in. Hose Barb Part Number	KPCHT with ¼ in. Hose Barb Part Number
Male	KPCHT02M10	KPCHT02M7
Female	KPCHT02F10	KPCHT02F7

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

- Burst Testing
- Creep-Rupture
- Tensile Strength
- Seal Integrity
- Water Flow
- Extractables
- Biological Safety

Additional autoclave and functional (soiling) testing was not repeated, as the new configurations will not affect the results presented in the *Kleenpak Sterile Connector Validation Guide* (USTR 2232).

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 sterile connector with hose barb adapters employed the same methods as used for the Kleenpak sterile connector outlined in Validation Guide USTR 2232. All testing has been performed on the Kleenpak sterile connectors with ⅜ in. adapters. Some tests were performed on ¼ in. hose barb adapters where applicable. Where applicable, the Kleenpak sterile connectors were gamma-irradiated (48 – 55 kGy) and/or autoclaved to 135 °C for 2 x 30 minutes prior to test.

3 Test Results

3.1 Burst Testing

3.1.1 Objective

The purpose of these tests was to demonstrate that the adhesive joint between the hose barb adapter and the relevant male or female Kleenpak sterile connector withstands the maximum pressure rating of 3 barg (43.5 psig) with an appropriate safety margin.

Burst tests were performed on male and female Kleenpak sterile connector with hose barb adapters subjected to gamma irradiation at doses of 48 – 55 kGy or autoclaved to 135 °C for 2 x 30 minutes.

3.1.2 Results

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

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

Sample No.	Part No.	Pre-Treatment	Burst Pressure (barg)	Burst Pressure (psig)
1	KPCHT02F10	Gamma 51.7 kGy	79.6	1155
2	KPCHT02F10	Gamma 51.7 kGy	64.3	932
3	KPCHT02F10	Gamma 51.7 kGy	76.0	1105
4	KPCHT02F10	Gamma 51.7 kGy	75.2	1091
5	KPCHT02F10	Gamma 51.7 kGy	74.4	1079
		Average	**74**	**1072**
1	KPCHT02M10	Gamma 51.7 kGy	81.1	1176
2	KPCHT02M10	Gamma 51.7 kGy	83.0	1204
3	KPCHT02M10	Gamma 51.7 kGy	62.8	911
4	KPCHT02M10	Gamma 51.7 kGy	71.8	1042
5	KPCHT02M10	Gamma 51.7 kGy	73.7	1069
		Average	**74**	**1080**
1	KPCHT02F10	Autoclave 135 °C (2 x 30 minutes)	72.3	1049
2	KPCHT02F10	Autoclave 135 °C (2 x 30 minutes)	83.8	1215
3	KPCHT02F10	Autoclave 135 °C (2 x 30 minutes)	88.0	1276
4	KPCHT02F10	Autoclave 135 °C (2 x 30 minutes)	72.3	1049
5	KPCHT02F10	Autoclave 135 °C (2 x 30 minutes)	75.8	1099
		Average	**78**	**1138**
1	KPCHT02M10	Autoclave 135 °C (2 x 30 minutes)	85.2	1236
2	KPCHT02M10	Autoclave 135 °C (2 x 30 minutes)	75.4	1093
3	KPCHT02M10	Autoclave 135 °C (2 x 30 minutes)	106.0	1537
4	KPCHT02M10	Autoclave 135 °C (2 x 30 minutes)	78.2	1134
5	KPCHT02M10	Autoclave 135 °C (2 x 30 minutes)	93.9	1362
		Average	**88**	**1272**

Table 3 Burst Pressures of Individual Kleenpak Sterile Connectors with ¼ in. Hose Barb Adapters

Sample No.	Part No.	Pre-Treatment	Burst Pressure (barg)	Burst Pressure (psig)
1	KPCHT02F7	Gamma 51.1 kGy	90.5	1312
2	KPCHT02F7	Gamma 51.1 kGy	101.5	1472
3	KPCHT02F7	Gamma 51.1 kGy	92.7	1344
4	KPCHT02F7	Gamma 51.1 kGy	101.4	1471
5	KPCHT02F7	Gamma 51.1 kGy	94.6	1372
		Average	**96.1**	**1394**
1	KPCHT02M7	Gamma 51.1 kGy	80.6	1169
2	KPCHT02M7	Gamma 51.1 kGy	87.7	1272
3	KPCHT02M7	Gamma 51.1 kGy	70.8	1027
4	KPCHT02M7	Gamma 51.1 kGy	84.6	1227
5	KPCHT02M7	Gamma 51.1 kGy	87.1	1264
		Average	**82.2**	**1192**
1	KPCHT02F7	Autoclave 135 °C (2 x 30 minutes)	81.2	1178
2	KPCHT02F7	Autoclave 135 °C (2 x 30 minutes)	78.4	1137
3	KPCHT02F7	Autoclave 135 °C (2 x 30 minutes)	81.1	1176
4	KPCHT02F7	Autoclave 135 °C (2 x 30 minutes)	76.4	1108
5	KPCHT02F7	Autoclave 135 °C (2 x 30 minutes)	78.0	1131
		Average	**79.0**	**1146**
1	KPCHT02M7	Autoclave 135 °C (2 x 30 minutes)	77.4	1122
2	KPCHT02M7	Autoclave 135 °C (2 x 30 minutes)	78.0	1132
3	KPCHT02M7	Autoclave 135 °C (2 x 30 minutes)	90.4	1311
4	KPCHT02M7	Autoclave 135 °C (2 x 30 minutes)	94.9	1376
5	KPCHT02M7	Autoclave 135 °C (2 x 30 minutes)	93.5	1356
		Average	**86.8**	**1259**

3.1.3 Conclusions

All sterilized Kleenpak sterile connectors with hose barb adapters had burst pressures that were ≥ 62.8 barg (911 psig) giving an acceptable safety factor over the maximum recommended operating pressure of 3 barg (43.5 psig).

3.2 Creep-Rupture

3.2.1 Objective

The purpose of these tests was to demonstrate that the adhesive joint between the ⅜ in. hose barb adapter and the relevant male or female Kleenpak sterile connector remains stable over extended periods of time while under pressure.

Creep rupture tests were performed on male and female Kleenpak sterile connectors with hose barb adapters subjected to gamma irradiation at doses of 48 – 55 kGy or autoclaved to 135 °C for 2 x 30 minutes.

3.2.2 Results

The sterilized Kleenpak sterile connectors with hose barb adapters were tested at 3.5 bar (50.8 psi) at a temperature of 40 °C and were held at pressure for 1 week (168 hours). Samples were maintained at pressure for 1 week without leaking or breaking.

3.2.3 Conclusions

Kleenpak sterile 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 for typical Kleenpak sterile connectors demonstrates the very large safety margins that have been incorporated into these pressure claims.

Testing with ¼ in. hose barb adapters was not carried out as materials of construction and assembly methods are the same as for the ⅜ in. hose barb adapters, and burst pressures were considered to be similar.

3.3 Tensile Strength

3.3.1 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 sterile connector.

All test samples were gamma irradiated with doses of 48 – 55 kGy or autoclaved to 135 °C for 2 x 30 minutes prior to test.

3.3.2 Results

The tensile strength test results are shown in Table 4: Results of Tensile Strength Testing of Kleenpak Sterile Connectors with ⅜ in. Hose Barb Adapters and Section 5: Results of Tensile Strength Testing of Kleenpak Sterile Connectors with ¼ in. Hose Barb Adapters.

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

Test	Part No.	Pre-Treatment	Tensile Result (N)*	Tensile Result (lbf)**
1	KPCHT02F10	Gamma 51.7 kGy	1337	300.5
2	KPCHT02F10	Gamma 51.7 kGy	1897	426.6
3	KPCHT02F10	Gamma 51.7 kGy	1829	411.1
4	KPCHT02F10	Gamma 51.7 kGy	1907	428.6
5	KPCHT02F10	Gamma 51.7 kGy	1664	374.1
		Average	**1727**	**388**
1	KPCHT02M10	Gamma 51.7 kGy	1753	394.1
2	KPCHT02M10	Gamma 51.7 kGy	2028	455.9
3	KPCHT02M10	Gamma 51.7 kGy	1952	438.9
4	KPCHT02M10	Gamma 51.7 kGy	1774	398.8
5	KPCHT02M10	Gamma 51.7 kGy	1870	420.4
		Average	**1875**	**422**
1	KPCHT02F10	Autoclave 135 °C (2 x 30 minutes)	1350	303.6
2	KPCHT02F10	Autoclave 135 °C (2 x 30 minutes)	1444	324.7
3	KPCHT02F10	Autoclave 135 °C (2 x 30 minutes)	1538	345.8
4	KPCHT02F10	Autoclave 135 °C (2 x 30 minutes)	1405	315.9
5	KPCHT02F10	Autoclave 135 °C (2 x 30 minutes)	1772	398.3
		Average	**1502**	**338**
1	KPCHT02M10	Autoclave 135 °C (2 x 30 minutes)	1453	326.7
2	KPCHT02M10	Autoclave 135 °C (2 x 30 minutes)	1758	395.2
3	KPCHT02M10	Autoclave 135 °C (2 x 30 minutes)	1637	367.9
4	KPCHT02M10	Autoclave 135 °C (2 x 30 minutes)	1529	343.7
5	KPCHT02M10	Autoclave 135 °C (2 x 30 minutes)	1451	326.2
		Average	**1566**	**352**

* N = Newtons
** lbf = pounds-force

Table 5 Results of Tensile Strength Testing of Kleenpak Sterile Connectors with ¼ in. Hose Barb Adapters

Test	Part No.	Pre-Treatment	Tensile Result (N)*	Tensile Result (lbf)**
1	KPCHT02F7	Gamma 51.1 kGy	1847	415.2
2	KPCHT02F7	Gamma 51.1 kGy	1941	436.3
3	KPCHT02F7	Gamma 51.1 kGy	2003	450.2
4	KPCHT02F7	Gamma 51.1 kGy	1907	428.6
5	KPCHT02F7	Gamma 51.1 kGy	1824	410.1
		Average	**1904**	**428.1**
1	KPCHT02M7	Gamma 51.1 kGy	1829	411.1
2	KPCHT02M7	Gamma 51.1 kGy	1527	343.2
3	KPCHT02M7	Gamma 51.1 kGy	1868	419.9
4	KPCHT02M7	Gamma 51.1 kGy	2110	474.4
5	KPCHT02M7	Gamma 51.1 kGy	1463	328.8
		Average	**1759**	**395.5**
1	KPCHT02F7	Autoclave 135 °C (2 x 30 minutes)	1630	366.4
2	KPCHT02F7	Autoclave 135 °C (2 x 30 minutes)	1694	380.8
3	KPCHT02F7	Autoclave 135 °C (2 x 30 minutes)	1586	356.6
4	KPCHT02F7	Autoclave 135 °C (2 x 30 minutes)	1625	365.3
5	KPCHT02F7	Autoclave 135 °C (2 x 30 minutes)	1410	317.0
		Average	**1589**	**357.2**
1	KPCHT02M7	Autoclave 135 °C (2 x 30 minutes)	1066	239.7
2	KPCHT02M7	Autoclave 135 °C (2 x 30 minutes)	1508	339.1
3	KPCHT02M7	Autoclave 135 °C (2 x 30 minutes)	1916	430.7
4	KPCHT02M7	Autoclave 135 °C (2 x 30 minutes)	1170	262.9
5	KPCHT02M7	Autoclave 135 °C (2 x 30 minutes)	1637	367.9
		Average	**1459**	**328.1**

* N = Newtons
** lbf = pounds-force

3.3.3 Conclusions

A minimum force of 1066 N (240 lbf) was required to break the bond between hose barb adapter and Kleenpak sterile connector, which demonstrates the strength of Kleenpak sterile connectors built with adhesively bonded adapters.

3.4 Seal Integrity

3.4.1 Objective

The seal integrity test was used to confirm the seal integrity of the adhesive joint between the ⅜ in. hose barb adapter and the relevant male or female Kleenpak 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 sterile connectors subjected to gamma irradiation at doses of 48 – 55 kGy or autoclaved to 135 °C for 2 x 30 minutes.

3.4.2 Results

The results are shown in Table 6:

Table 6 Results of the Seal Integrity Test

Test No.	Part No.	Pre-Treatment	Number of Connections Tested*	Number of Connections Passed**
1	KPCHT02M10	Gamma 51.7 kGy	5	5
	KPCHT02F10	Gamma 51.7 kGy	5	5
2	KPCHT02M10	Autoclave 135 °C (2 x 30 minutes)	5	5
	KPCHT02F10	Autoclave 135 °C (2 x 30 minutes)	5	5

* Note: Sterile 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 closure integrity test data provided confirms seal integrity and demonstrates the ability of the Kleenpak sterile connector to prevent liquid product leakage during operation (e.g., product transfer).

Testing of Kleenpak sterile connectors with ¼ in. hose barb adapters was not carried out as materials of construction and assembly methods are the same as for the Kleenpak sterile connectors with ⅜ in. hose barb adapters.

3.5 Water Flow

3.5.1 Objective

The purpose of these tests was to determine the water flow rates of fully assembled male and female Kleenpak sterile connectors with hose barb adapters when subjected to different differential pressures.

3.5.2 Results

The water flow/differential pressure characteristics of the assembled Kleenpak sterile connector with hose barb adapters are shown in Figures 1 and 2.

Figure 1 Water Flow/Differential Pressure Characteristics for the Kleenpak Sterile Connector with ⅜ in. Hose Barb Adapters

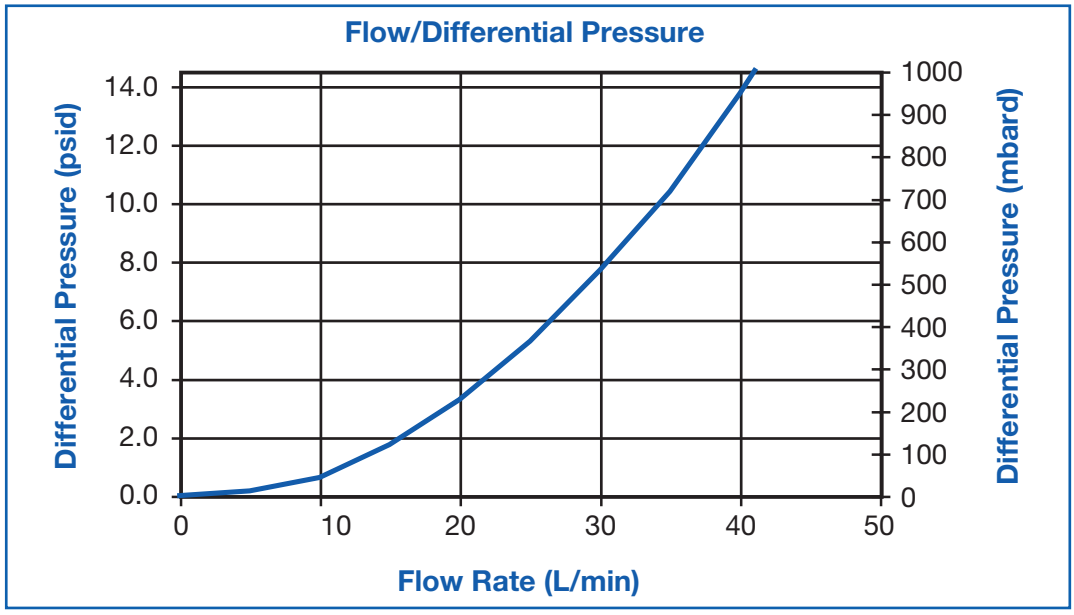
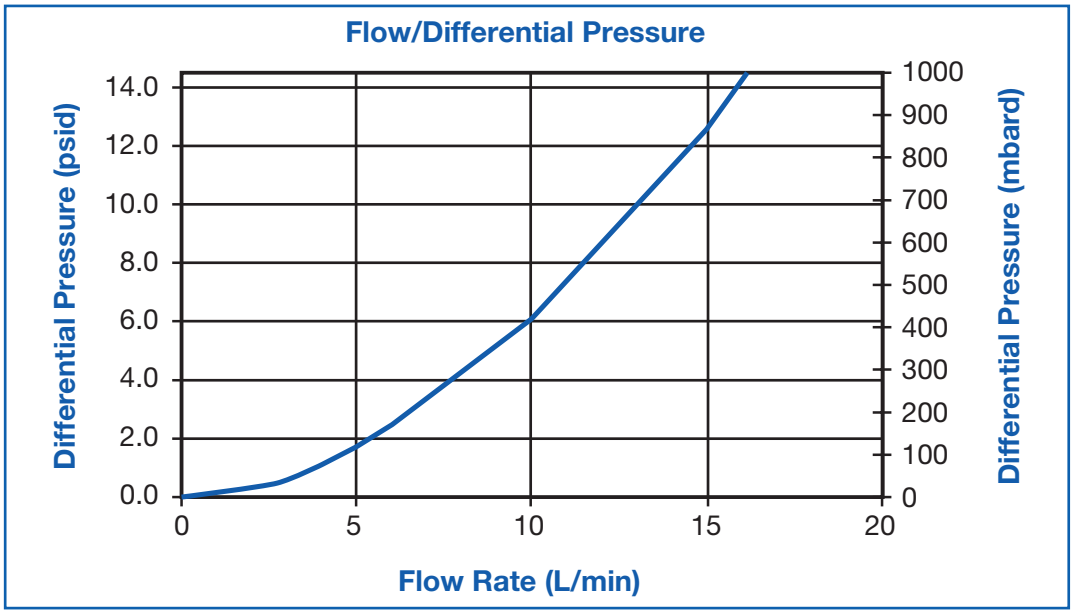


Figure 2 Water Flow/Differential Pressure Characteristics for the Kleenpak Sterile Connector with ¼ in. Hose Barb Adapters



3.5.3 Conclusions

The water flow/pressure drop data presented for assembled Kleenpak sterile connectors with hose barb adapters 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 these configurations of Kleenpak sterile connector.

3.6 Extractables

3.6.1 Objective

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

3.6.2 Results

The results from the aqueous extractables test performed on the Kleenpak sterile connector with ⅜ in. hose barb adapters are shown in Table 7: Aqueous Extractables for Kleenpak Sterile Connectors with ⅜ in. Hose Barb Adapters. The results from the ethanol extractables test performed on the Kleenpak sterile connector with ⅜ in. hose barb adapters are shown in Table 8: Ethanol Extractables for Kleenpak Sterile Connectors with ⅜ in. Hose Barb Adapters.

Table 7 Aqueous Extractables for Kleenpak Sterile Connectors with ⅜ in. Hose Barb Adapters

Test No.	Male Part No.	Female Part No.	Pre-Treatment	Non-volatile Residue (mg) for Three Sterile Connectors
1	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
2	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
3	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg

Table 8 Ethanol Extractables for Kleenpak Sterile Connectors with ⅜ in. Hose Barb Adapters

Test No.	Male Part No.	Female Part No.	Pre-Treatment	Non-volatile Residue (mg) for Three Sterile Connectors
1	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
2	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
3	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg
	KPCHT02M10	KPCHT02F10	Gamma 51.7 kGy and Autoclave 135 °C (2 x 30 minutes)	< 1 mg

Control Samples	Non-volatile Residue (mg)
Negative (per 1000 mL)	0.9
Positive	99.5

3.6.3 Conclusions

The level of aqueous and ethanol extractables for a Kleenpak sterile connector with hose barb adapters, irradiated with doses of 48 – 55 kGy followed by autoclaving to 135 °C for 2 x 30 minutes, are extremely low. The non-volatile residue extracted was not quantifiable when water and ethanol were used as the extraction fluid.

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

Testing of Kleenpak HT connectors with ¼ in. hose barb adapters was not carried out because materials of construction and assembly methods are the same as those for the Kleenpak HT sterile connectors with ⅜ in. hose barb adapters.

4 Biosafety Test Results

4.1 Biological Reactivity Tests

4.1.1 Objective

The purpose of these tests was to evaluate the Biological suitability of the materials of construction of the fluid part of the Kleenpak sterile connector.

The Biological Reactivity Tests in vivo for Class VI-121 °C plastics are described in the United States Pharmacopoeia (USP <88>), and 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)

4.1.2 Results

The components of the Kleenpak sterile connector with hose barb adapters passed all of the tests specified. Contact Pall for more information.

4.1.3 Conclusions

The fluid path components of the Kleenpak sterile connector with ⅜ in. hose barb adapters meet the requirements of the USP <88> Biological Reactivity, in vivo for Class VI – 121 °C Plastics. Testing of Kleenpak sterile connectors with ¼ in. hose barb adapters was not carried out as materials of construction and assembly methods are the same as for Kleenpak sterile connectors with ⅜ in. hose barb adapters.

4.2 Physicochemical Tests

4.2.1 Objective

Containers, composed of plastics, that are intended for use in packaging parenteral products, and which are in direct contact with that product, must meet the Physicochemical Testing — Plastics requirements set forth in USP <24>. This test is designed to evaluate the presence of material that might be leached or extracted from the plastic container into the parenteral by the use of specific solvents at defined temperatures.

Although the Kleenpak sterile connector is not a container, nor can it be used as a container for parenterals, it can be utilized as a part of a container system and for that reason Pall has conducted this test to provide data on the Kleenpak sterile connector in these applications where a parenteral could inadvertently be left in direct contact with the Kleenpak sterile connector for an extended period.

The tests under USP <661> are:

- Non-Volatile Residue (NVR) — measures organic/inorganic residues soluble in extraction media
- Residue on ignition — 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, and zinc

4.2.2 Results

The components of the Kleenpak sterile connector passed all of the tests specified.

4.2.3 Conclusions

The fluid path components of the Kleenpak sterile connector meet the requirements of the physicochemical tests detailed in USP <24>.

Testing with ¼ in. hose barb adapters was not carried out as materials of construction and assembly methods are the same as for the ⅜ in. hose barb adapters.

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