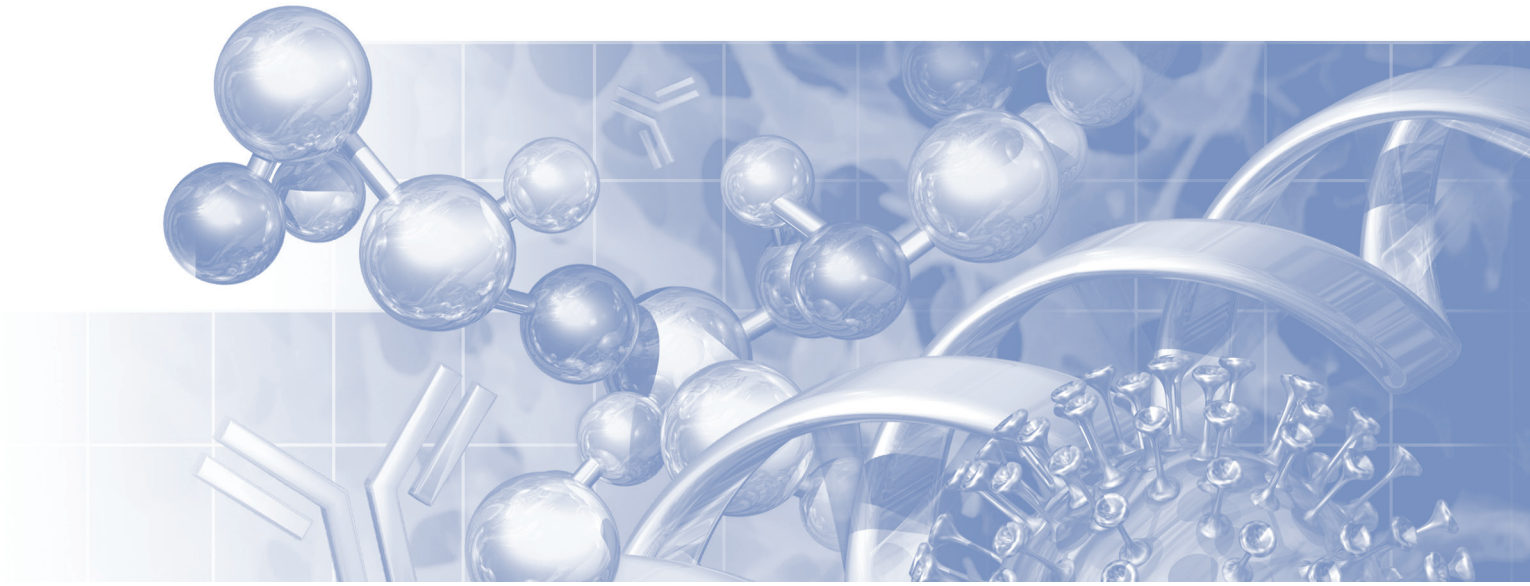
PALL Biotech

White Paper

USD3164b

Genderless Sterile Connection Technology - A Quality By Design (QbD) Approach For Greater Sterility Assurance From Manufacturing To Use



*Filtration. Separation. Solution.*SM

1. Introduction

One of the major trends in pharmaceutical manufacturing over the last decade has been the replacement of traditional, reusable cleanable components by single-use disposable alternatives. This transition has been possible due to the development of a wider range of single-use products for both upstream and downstream processing as well as for formulation and filling.

The increasing scope and availability of single-use technology has also presented the opportunity to design and assemble multi-component manifolds and systems that can offer benefits such as elimination of in-process cleaning and steam sterilization, faster processing times, more flexible manufacturing and lower risk of operator error. Most importantly, single-use pre-sterilized systems can also offer potentially a higher level of sterility assurance and a lower risk of product contamination by eliminating some of the operational procedures of traditional processing. The importance of this benefit was highlighted by the FDA's product recalls in 2015 where 78% of recalls were attributed to lack of sterility assurance or to contamination of the drug product, with the primary reason being failure to follow written procedures.

A key requirement for multi-component, single-use sterile systems is the need to make safe and secure connections between the various manifolds and attachments using a sterile connector – and without the need for aseptic handling under laminar airflow in classified environments. In this publication, we describe the approach to the design, development and validation of a new sterile connector, the Pall Kleenpak® Presto Sterile Connector, with a unique design and manufacturing procedure that ensures the robustness and full traceability of every connector. The contribution of this feature to the integrity assurance of single-use systems is discussed as well as other properties of the connector that facilitate the management of sterile connections.

2. User Requirement Specifications (URS) for Single-Use Sterile Connectors

Before embarking on the design of a new sterile connector:

- We investigated the properties of the connectors already available for single-use systems
- We obtained feedback from end-users on their experiences with current products and their 'wish lists' for features in an enhanced connector
- We had also accumulated experience and feedback from the industry on our Kleenpak sterile connector range.

Our findings revealed not only some features of existing products that were important to retain, as well as others that were seen as problematic but most importantly, some requirements that were novel and challenging from a product development perspective.

From the data, we were able to prepare a generic User Requirement Specification that contained five critical quality attributes:

- High sterility assurance
- Traceability and security of supply
- Error-free intuitive operation
- Suitability for a wide range of applications
- Biological safety and regulatory compliance

Our design and development plan to satisfy these user requirements focused on two key elements:

- Enhancement of the features that had been shown to be effective in existing single-use sterile connectors
- Development of unique features and manufacturing methods to provide additional benefits to the end-user.

2.1 User Requirement - High Sterility Assurance

Assurance of integrity and sterility during installation and actuation of the sterile connector was the most critical requirement in the development plan. To achieve this target, we were able to build on the manufacturing and application experience gained over a 15-year period from the Pall Kleenpak range of sterile connectors. The key feature of this established design is the incorporation of a sterile peel strip barrier on the faces of the two component parts and the simultaneous withdrawal of these peel strips during actuation. Assurance of integrity and sterility was achieved by validation of the manufacturing process and final product, and subsequent production quality controls to ensure consistent quality and performance. Further details on the validation of this sterile connector can be found in the validation guide.

Figure 1

Kleenpak sterile connector

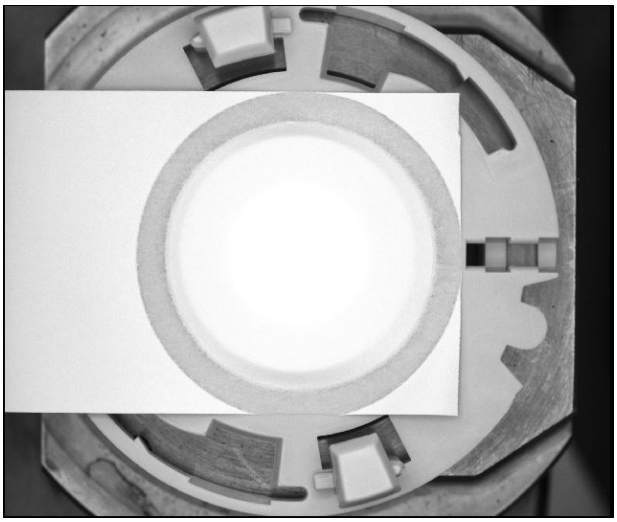
Sample testing of this style of connector from routine production lots for integrity and sealing of the peel strip was able to confirm the suitability of the design for achievement of high sterility assurance and has been supported by successful performance in a range of applications.

However, for a new design of sterile connector, the possibility of performing during manufacturing some form of automated testing on every connector to check both the integrity of weld of the weld and of the peel strip, was set as a clear target for the project. This approach is consistent with the aims of the BioProcessing Systems Alliance (BPSA) who have developed a guide on the Design, Control, and Monitoring of Single-use Systems for Integrity Assurance, with special emphasis on the role of the manufacturer and supplier to provide assurances on the integrity of single-use components and assemblies.

Figure 2

Kleenpak Presto sterile connector

Figure 3
Camera image of peel strip and connector



2.1.1 Development of Image Analysis System

For testing the critical peel strip interface of the new connector, shown in Figure 2, an image analysis system was developed and evaluated. The 'Vision System' involved the use of high resolution cameras, specialized tools with robotic, automated operation and purpose-designed software and databases for image analysis and identification of any defects. The Vision System was developed and programmed to identify the following critical features:

- Absence of peel strip defects
- Weld integrity
- Seal integrity
- Alignment of components

2.1.2 Absence of Peel Strip Defects

The image analysis system was designed to produce a high resolution camera image of the peel strip and body of every connector, as shown in Figure 3.

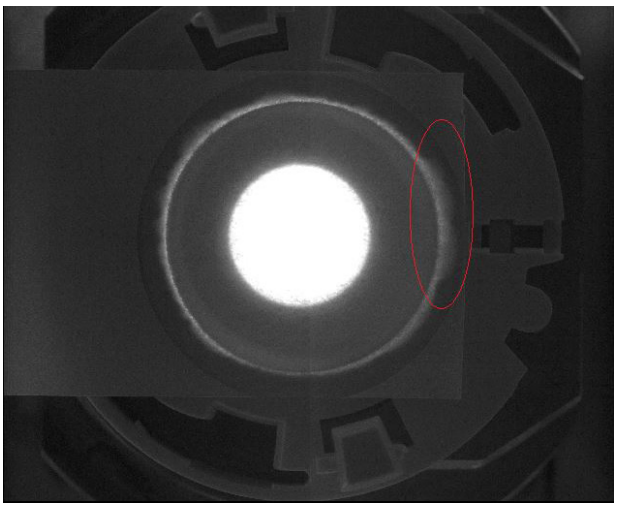
The objective was to develop the capability to detect and highlight automatically any defined defects, as programmed in the software. The defect would be tagged by crosshairs and other symbols on the recorded image, and the connector automatically discarded from the production line during routine manufacture.

The Vision System was programmed to identify three types of defect.

Hole ≥ 30 µm
Results from qualification studies showed that the image analysis system could achieve a detection sensitivity for a hole in the peel strip equal to or greater than 30 µm. The image analysis system operates by first cross checking the back light to ensure its activity and to prevent false positives from occurring. The system then checks for brighter than average area within the halo or along the halo edge (Figure 4). If detected the device fails and is rejected. The significance of the size of the hole on the risk to microbial ingress and sterility assurance is discussed in a later section.

Figure 4

Image of backlit peel strip. In this example the backlit bright areas (circled) highlight a weld failure and a central hole in the peel strip.



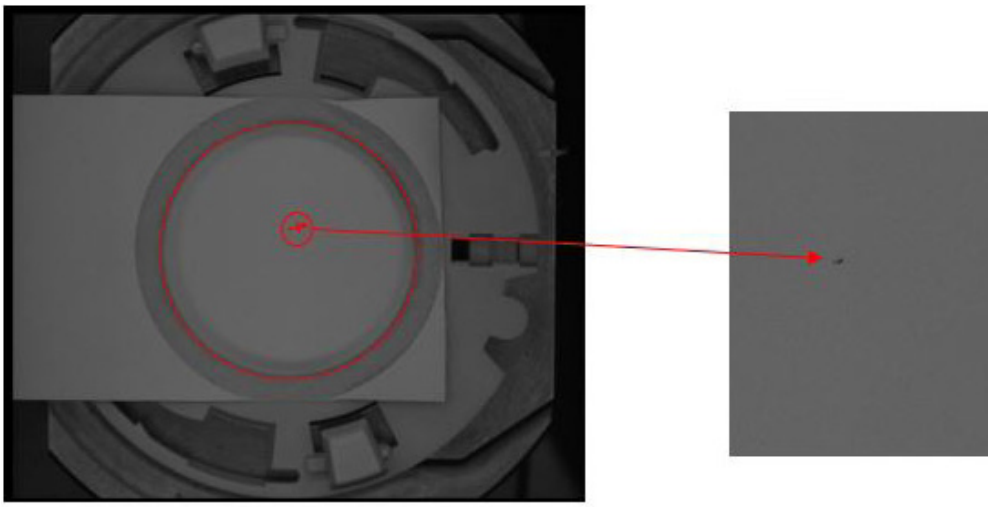
Fibers or particulates on peel strip

The detection system was also shown to identify fibers or particulates on the peel strip inside of the welded area, which is used to indicate the cleanliness of rest of membrane.

The typical images obtained for these types of defects are presented in Figure 5.

Figure 5

Camera images of peel strip defects



2.1.3 Weld Performance

This procedure was designed to identify either a gross gap in the peel strip weld to the body, or a peeling of the peel strip from the weld.

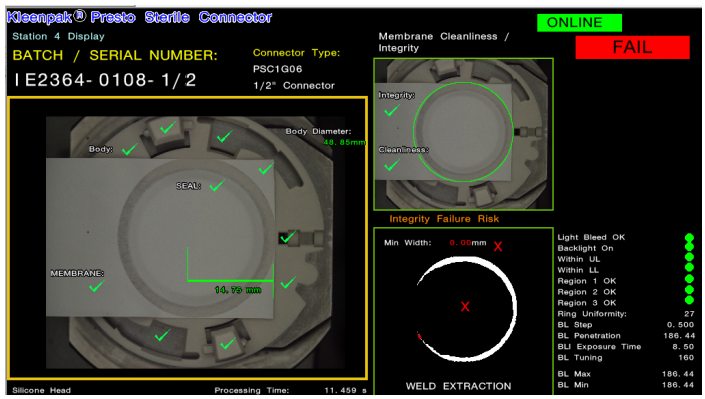
For example, in Figure 6, the absence of gaps in the white extracted weld on the black background confirmed the completeness of the weld. The pass result is shown by the ‘WELD COMPLETE’ confirmatory message being displayed. If the weld is damaged black areas show on the white welded zone and the on-screen image displays a ‘FAIL’ and failure mode notification message (Figure 7).

Figure 6

On-screen image and ‘PASS’ notification highlighting completeness of the weld
Note: Serial number is simulated and does not represent an actual device serial number.

Figure 7

On-screen image and ‘FAIL’ notification highlighting weld failure mode
Note: Serial number is simulated and does not represent an actual device serial number.



2.1.4 Seal Integrity

The objective of this imaging procedure was to identify the presence of a seal between the peel strip and the body. In Figure 8, this has been confirmed by the white seal ring.

The image was compared to a reference image and if the two images matched, a tick is to displayed to designate a pass (Figure 8). If a mismatch was detected then a cross is displayed to designate a fail.

Figure 8

On-screen image of intact seal

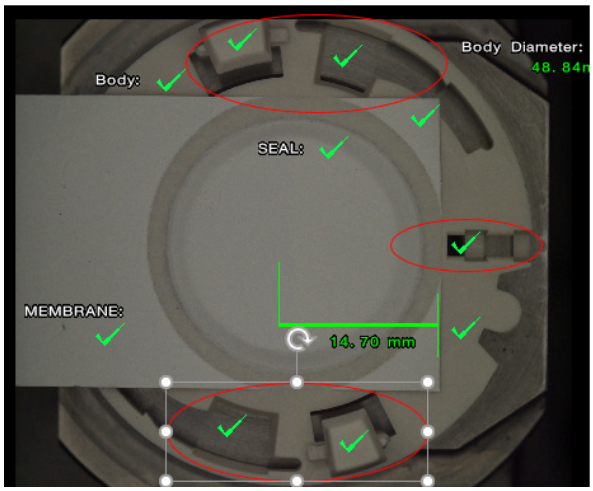
2.1.5 Alignment of Components

Tools and software were also designed into the Vision system for other dimensional measurements that could provide quality controls for consistency of manufacture and product release. These measurements included:

- Peel strip alignment
  Edge detection tools for peel strip alignment were designed which could measure the peel strip position, and check that peel strip was not covering any of the critical areas highlighted in Figure 9. The tools were also designed to detect the absence of the peel strip.

Figure 9

Peel strip alignment detection



- Critical feature positioning
  A tool was developed to determine the positioning of the connector critical features - membrane, lugs and tab slot. Comparison of these measurements would then confirm correct weld and body orientation and alignment.

After performance qualification, the imaging and measurement system was incorporated into an automated assembly line for large scale production of the sterile connectors with 100% inspection for weld integrity and peel strip defects. Control of the manufacturing process was achieved using bespoke software which could also store securely the images and measurements from the Vision system to meet other user requirements such as traceability and regulatory compliance, as described in the following sections.

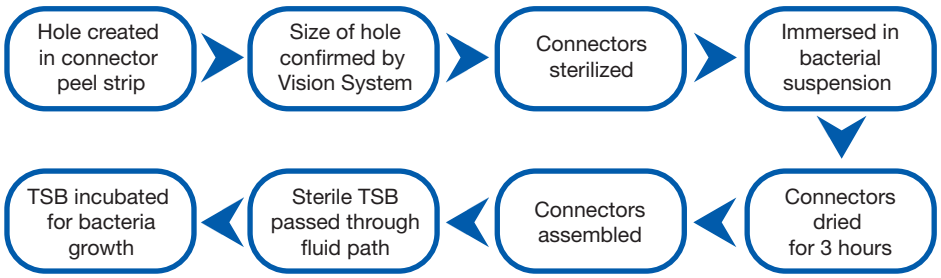
2.1.6 Bacterial Challenge Tests

Having established an imaging system that could detect defects of 30 µm size and greater, it was essential to determine whether there was a critical defect size above which microbial ingress may occur. In this way, a level of sterility assurance could be determined for connectors released by manufacturing and meeting the specified integrity standards, as determined by 100% testing of the connectors by the Vision system.

The test sequence for the immersion bacterial challenge tests, which are also described as soiling tests, is shown in Figure 10.

Figure 10

Test sequence for bacterial challenge test on connectors



The test conditions are presented in Table 1.

Table 1

Test conditions for bacterial challenge tests on sterile connectors

Feature	Properties
Hole diameters in peel strip	30 µm, 60 µm, 120 µm
Challenge organism	*Brevundimonas diminuta*
Challenge media	4% CMC
Bacterial concentration	>1 x 10^6 cfu/mL
Growth media	Tryptone Soya Broth (TSB)
Volume of media	25 mL

Table 2

A summary of the results

Hole Size	Vision System Detectable	Number of Devices / Assemblies Tested	Results from Bacterial Challenge Test	Comments
30 microns	Yes	30 devices – 15 assemblies	100% Pass	
60 microns	Yes	30 devices – 15 assemblies	100% Pass	
120 microns	Yes	30 devices – 15 assemblies	100% Pass	
200 microns	Yes	30 devices – 15 assemblies	2 pairs of failing devices detected	ID was that of the test organism – i.e. *B. Diminuta*

Results from these studies were as follows:

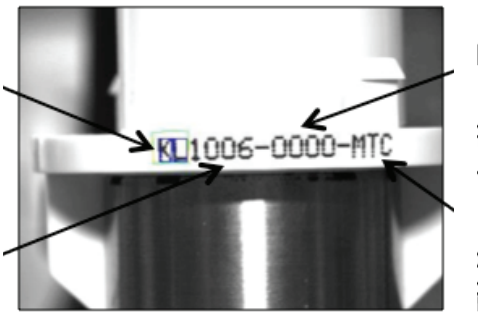
- Negative controls using connectors with intact peel strips showed no growth in the incubated TSB broth
- Positive controls using connectors without peel strips showed bacterial growth as expected, confirming suitability of the incubation conditions
- Connectors with hole sizes of up to 120 µm did not show any microbial ingress, even for the diminutive rod-shaped organism *Brevundimonas diminuta* used in this study, with dimensions of approximately 0.3 µm x 0.8 µm
- The conditions in this study could be described as 'worst case' compared to those conditions which the connector would be exposed to in typical applications and therefore indicate a high safety margin against bacterial ingress in normal use
- This safety margin, together with the fact that the Vision System will reject connectors with defect sizes of 30 µm and greater, demonstrate a very high level of sterility assurance for the connector.

2.2 User Requirement Specification – Traceability and Security of Supply

Having established the vision system to provide assurance of sterility and integrity for the new connector, it was necessary to develop technology and procedures for printing essential information onto each connector to satisfy the user requirement for product traceability. Most single-use components provide some form of traceability, typically restricted to part numbers and lot numbers with sample tests on each lot for integrity and other properties.

Figure 11
Printed part number

Figure 12
Printed information on connector body



Our development brief was to provide enhanced traceability. In addition to the pre-printed part number, shown in Figure 11, the following details were printed on the body of the connector during manufacture, as shown in Figure 12:

- Manufacturing site code
- Batch/lot number
- Unique serial number
- Assembly variant code

An Optical Character Recognition (OCR) system was incorporated into the manufacturing assembly machine which sends details of printed information to the Vision system. This information is loaded into the OCR registers in two parts:

1. The batch number

2. The serial number and assembly variant

Characters are then extracted from the collected image and compared to the:

- Vision system's character library
- Registered characters.

Serial numbers that have been successfully recognized are highlighted with a green box whereas illegible or incorrect serial numbers will show a red box.

By interfacing the OCR system with the images and measurements from 100% inspection of the connectors, the manufacturing quality control can be traced and audited for every device to provide the highest possible level of traceability and security of supply.

Figure 13
Assembled connector

2.3 User Requirement Specification - Error-free, Intuitive Operation

A simple and rapid operating procedure for a single-use connector was considered by users to be an important user requirement when replacing aseptic connection methods using more complex and time-consuming procedures. Such methods, for example, may require classified environmental conditions, glove boxes, specialized equipment such as tube welders or highly skilled operators.

To achieve an error-free intuitive operation, a peel strip-based sterile barrier, as used successfully in the Kleenpak sterile connector, was retained. This type of design allows sterile connections to be made in unclassified environments, thus eliminating some of the restrictions for operators typically associated with cleanroom operations.

The incorporation of tamper-resistant caps gave additional security between point of manufacturing and point of use.

For the new connector, the two connecting faces were designed to be genderless rather than male/female faces. This universal feature could simplify the installation options and standard operation procedures for the connectors, especially in systems using a large number of connectors. The design also provided:

- A simple connection procedure with only three steps
- A short connection time.

The requirement for an error-free operation was also assisted by geometric features which ensure that the two parts can only be joined together in the correct way.

We also specified that the force required during actuation to remove the peel strip and to engage and lock the two parts together was not excessive and did not require operators with high manual strength. In addition, the locking mechanism was designed to ensure that, after connection, the assembly could not be separated accidentally or intentionally by the operator. A clear visual indication of a successful connection removes any doubt that the step has been completed by the operator.

2.4 User Requirement Specification - Wide Range of Applications

The user requirement for suitability in a wide range of applications in upstream, downstream and final formulation/filling presented some significant challenges regarding the chemical, physical and mechanical properties of the device. A summary of the main features of the validated product are shown in Table 3.

Table 3

Summary of features and properties of Kleenpak Presto sterile connectors

Feature	Properties
Materials of construction – fluid contact	Polyethersulfone (PES)[1]
Pressure rating	3 bar g for 90 days 4 bar g for 2 days
Operating temperature range	2 °C to 60 °C
Storage temperature	Down to -80 °C
Autoclaving	130 °C for 75 minutes
Gamma irradiation	Up to 50 kGy
Shelf life	Currently 6 months – ongoing tests Target - Irradiated (sterile): 3 years - Non-irradiated: 5 years
Operating pH range	pH 2 to pH 12
Connection sizes	¼ in., ⅜ in., ½ in., and ⅝ in. hose barb ½ in. mini-sanitary sterile connector

1. Free of bisphenol-A (BPA)

2.4.1 Chemical and pH Compatibility

Polyethersulfone (PES) was chosen as the material of construction as it offers a very wide chemical and pH compatibility, as well as suitability for gamma irradiation up to 50 kGy and steam sterilization up to 130 °C.

The wide chemical and pH compatibility of the connector was confirmed by our validation studies with the liquids shown in Table 4.

Table 4

Chemical compatibility studies on sterile connectors

Liquid	Concentration	Temperature	Time
Sodium hydroxide	2 M (pH 12)	25 °C	21 days
Hydrochloric acid	pH 2	25 °C	21 days
Dimethylacetamide (DMA)	20%	40 °C	24 hours
Dimethylsulfoxide (DMSO)	25%	25 °C	24 hours
Polyethylene glycol (PEG)	30%	25 °C	24 hours
Tween 80	1%	25 °C	24 hours
Water for injection (WFI)		40 °C	24 hours
Ethanol	100%	40 °C	24 hours

The results show compatibility to extremes of pH as well as to surfactants and solvents. Compatibility with DMA and DMSO makes the connector particularly suitable for use in processing antibody drug conjugates and for stem cell cryopreservation applications.

2.4.2 Operating Pressure and Temperature Range

The construction and welding methods were developed to enable the connector to be used in high pressure systems. The pressure rating of 3 barg for 90 days was established by validation studies including:

- Air leak test for integrity of the connected device at 3 barg, including samples subjected to -80 °C before connection
- Burst pressure tests in which all connected samples withstood pressures of up to 16.7 barg without bursting

These pressure tests also established that the connector could operate at up to 4 barg for shorter periods, including pulsating pressure systems such as found in tangential flow filtration (TFF) applications.

The operating temperature range was established from validation studies with the connected devices held at 2 °C and 60 °C at pressures up to 4 barg, as shown in Table 5.

Table 5

Temperature studies on sterile connectors

Temperature	Hold Pressure	Time	Sterilization Method
2 °C	4 barg	2 days	Gamma irradiation
2 °C	3 barg	90 days	Autoclave, Gamma irradiation & both
60 °C	4 barg	2 days	Gamma irradiation
60 °C	3 barg	90 days	Autoclave, Gamma irradiation & both

The studies confirmed that the specified temperature range was applicable, regardless of the method of sterilization and the exposure time to the test conditions.

Suitability for applications involving sub-zero temperatures, such as cryopreservation, was confirmed by freezing studies. Devices were held at -80 °C for 7 days, thawed, connected and subjected to leak, burst and creep testing. All tests passed the acceptance criteria.

2.4.3 Choice of Connection Options

In order to make the connector suitable for a wide range of tubing and system sizes, variants with hose barb connections from ¼ inch to 5/8 inch diameters were developed and manufactured, as well as a ½ inch mini-sanitary sterile connection.

A Tyvek◆ bellows accessory also enables use of the ½ inch hosebarb option for probe insertion into biocontainers, mixers and bioreactors.

Figure 14

Connector with Tyvek bellows for probe

Figure 15

Connector installed for probe

Figure 16

Color-coded caps for connection

As all variants are genderless and possess the same universal peel strip face, it is possible to join different hose barb connection diameters on the same connector, which can be especially convenient when stepping up or down on tubing diameters.

Selection and installation of the correct connector diameter is facilitated by the color-coded end caps for different diameters, as shown in Figure 16. This feature also provides a simple, clear confirmation to the end-user of the connection diameters installed on pre-sterilized systems from the supplier. The universal peel strip face and interchangeable connection diameters can also assist in reducing the quantity of part numbers for both the supplier and the end-user.

2.4 User Requirement Specification – Biological Safety and Regulatory Compliance

Biological safety is often raised by end-users due to concerns over the predominant use of plastic components in single-use systems and the potential influence of extractables and leachable materials on the pharmaceutical drug product. Regulatory compliance is also seen as a critical requirement when new technology or processes are being introduced and submitted for approval by regulatory authorities.

These considerations were taken into account during design and development of the connector and in the preparation of the validation program.

The qualification tests for biological safety of the connectors are listed in Table 1 in Section 3.1.4.

The data show compliance with the various USP tests for endotoxins, particulates, plastic packaging cytotoxicity and biological reactivity.

In this context, biological safety was also assessed for the following materials:

- The polyethersulfone polymer used as the material of construction is free of Bisphenol-A (BPA) which can be present in plastic resins. Its potential toxicity has been of concern, especially for foetuses and young children.
- All materials are free of animal-derived components (ADC) in view of the potential risk of TSE and BSE prion contamination

The URS for biological safety and regulatory compliance is also covered by the Certificate of Conformance, released for each lot number, which certifies the following:

- 100% of devices have been tested for defects in peel strip and integrity of the welds
- All materials are free of animal derived components (ADC) which may be considered to be specified TSE or BSE risk materials
- Polyethersulfone is certified free of Bisphenol-A (BPA)
- Fluid path materials listed in Title 21 US Code of Federal Regulations
- The components meet the requirements under USP <661> Containers. Physiochemical tests - Plastics
- Material of construction compliant to USP <87> Cytotoxicity and USP <88> Biological reactivity
- Release tests in conformance to USP <788> (Particulates) and USP <85> (Endotoxins)

3. Discussion and Conclusions

This project has established that a successful development of a single-use component such as a sterile connector must be based on a sound understanding of the user requirements for the device. In order to meet the user requirement specifications, special attention was given to the primary function of the device - the absolute requirement to maintain a sterile fluid path for sterile connections, and ideally in the widest possible range of applications and conditions.

At the same time, there was an opportunity to consider other features that might enhance the performance or suitability of the device. Some were very simple features such as color coding of variants or other properties that could simplify operation of the connector such as a minimizing the number of steps to three in the connection sequence or incorporation of error proofing and fail safe features into the device.

However, we were also able to consider an opportunity to introduce new technology into the manufacturing process that addressed a fundamental issue – assurance of integrity. The Vision system made it possible to test every connector automatically for weld integrity and peel strip defects and to incorporate this facility with an Optical Character Recognition system to provide a unique feature for sterile connectors of full traceability for every device.

With the additional qualification studies on bacterial challenge, it has been possible to 'raise the bar' with regard to levels of integrity assurance and sterility assurance for sterile connections – and without imposing additional constraints on the end-user. This project has also reinforced the critical role that the supplier or manufacture of single-use devices must play in achieving these high assurance levels for the end-user and in assisting in the safe and effective introduction of single-use systems into pharmaceutical manufacturing.

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