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Technical Brief

Steam-In-Place Feasibility Study for Lynx® CDR 1.5-inch Female Sterile-to-Sterile Connectors

Summary

This technical brief describes the mechanical strength of Lynx® CDR connectors during extreme steaming conditions as well as their ability to be steam sterilized. The mechanical strength of the connectors was evaluated at temperatures higher than conventional steaming temperatures. Steam sterilization was evaluated at nominal steam in place conditions.

Introduction

Many biopharmaceutical manufacturers have adopted single-use technologies for media, buffer, and intermediate storage. However, due to batch volumes and/or existing capabilities, they have retained the ability to prepare media and buffers in stainless steel tanks. Our family of Lynx® connectors, including Lynx® S2S, Lynx® ST, and Lynx® CDR connectors, are designed for various applications common in biomanufacturing.

The Lynx® ST connector can be used for sterile liquid transfer from stainless steel systems to single-use systems. This is achieved through the pre-sterilization of the single-use system, either by gamma radiation or by autoclave, which is connected to the Lynx® ST connector. The Lynx® ST connector is then connected via a TC flange to a stainless steel system where it is steam sterilized. Unlike the Lynx® ST connector, which is typically connected once and then disconnected from a stainless system, the Lynx® CDR connector enables connection, disconnection and reconnection to the system up to six times while preserving the sterility of the flow path. It can be connected/disconnected when dry and also if the lines are pressurized and filled with liquid. Because of this flexibility, it is an ideal connector to use in hybrid applications where both stainless steel and single-use systems and assemblies are used.

Objective

The objective of this study was to assess the steam-in-place (SIP) sterilization feasibility for Lynx® CDR 1.5-inch TC female connectors to support hybrid applications. The mechanical strength of the connectors during steaming at elevated temperatures and pressures was evaluated. The connectors' ability to be sterilized, defined as steam reaching all inside surfaces of a connector and ability to reach and maintain sterilization temperatures in hard to reach locations, was also assessed.

Methods

Pre- and Post-SIP Testing

PRE-SIP INTEGRITY TESTING

Lynx® CDR connectors were integrity tested prior to each SIP cycle using a Sentinel Blackbelt™ leak detection instrument from Cincinnati Test Systems. The testing was based on measuring pressure decay at 60 psig as a function of time and used our standard Lynx® CDR connector integrity test methods.

POST SIP-INTEGRITY TESTING

Post-SIP cycle integrity testing was performed after each SIP cycle; as follows:

- Female component of a Lynx® CDR connector was connected to a male part of a Lynx® CDR connector under pressure of 10 psig
- Flow rate of reverse osmosis (RO) water was set to reach a pressure of 60 psig and water was flowed through the connector for 2 minutes

- Female and male components of a connector were disconnected under a pressure of 10 psig. The faceplate of the female component of the connector was opened to expose the interior of the valve and the area was inspected for the presence of liquid.

Steam-In-Place Cycle

Gamma and autoclave presterilized Lynx® CDR 1.5-inch TC female connectors (CDRF1TN05) were evaluated to determine if they could withstand conditions during a SIP cycle. The connectors were attached to a stainless steel manifold in the closed position and evaluated for:

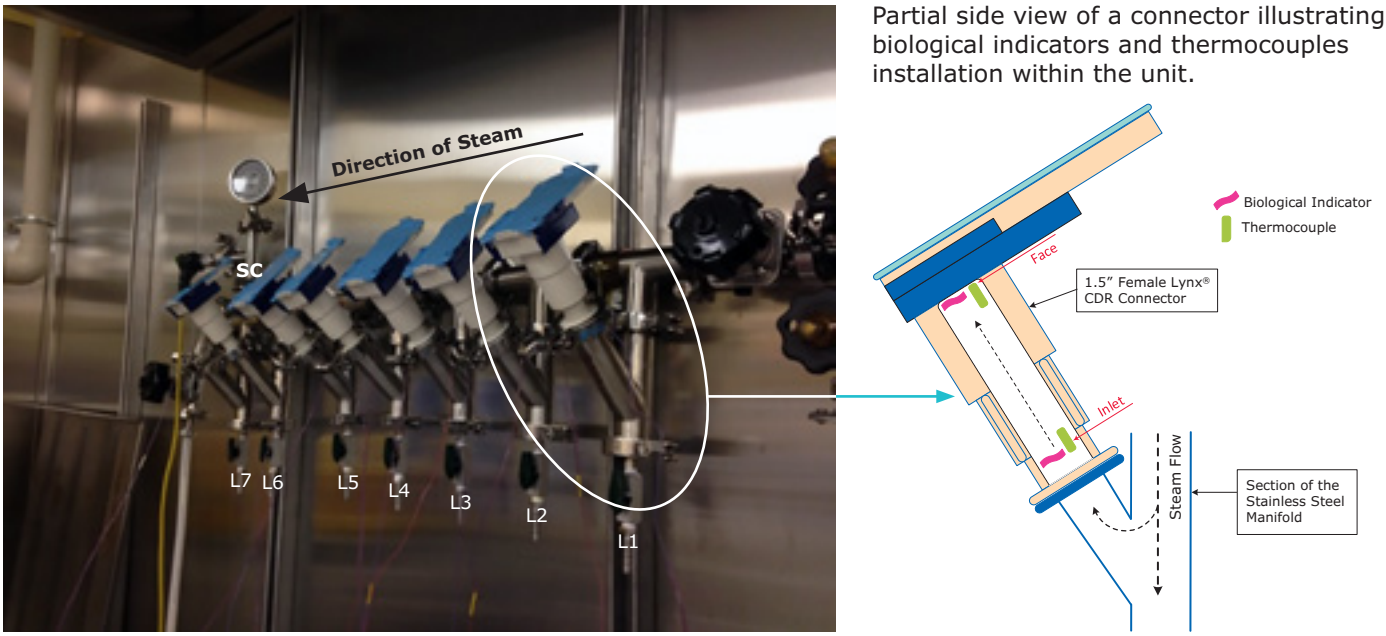
1. Mechanical strength at target temperature of: 140 °C for 60 minutes (~ 37 psig)
2. Sterility at target temperature minimum of 121 °C for 30 minutes
3. Ability to be sterilized in horizontal and vertical orientations to simulate connections to a large stainless steel vessel at target temperature minimum of 121 °C for 30 minutes.

Biological indicators (BIs) and thermocouples were used to evaluate the success of the steaming cycle; BIs were installed inside each connector to demonstrate sterilization and thermocouples were used to monitor and record the temperature during steaming cycles.

One pair of biological indicators and thermocouples were installed in each connector prior to steaming: one at the far (face) closed end of the connector, and one at the connector's inlet opening as illustrated in Figure 1.

At the end of each SIP cycle, the steam source was turned off. When the pressure in the system reached approximately 10 psig, sterile air was introduced into the manifold to ensure 10 psig positive pressure and sterility throughout the cool-down step. When the system reached approximately 40 °C, the connectors were disconnected from the stainless steel manifold one at a time. The biological indicators were retrieved from each connector and placed into recovery media to incubate at 60 °C for a minimum of 48 hours and 7 days. During this time, the samples were observed for growth. In addition, the following controls were included in every SIP cycle, incubated at 60 °C and monitored for growth:

- System control: Biological indicator was located at the far end of the manifold near where the steam exits the system
- Positive control: Biological indicator never exposed to steam
- Recovery media control (not exposed to steam): to prove that media was not contaminated



Partial side view of a connector illustrating biological indicators and thermocouples installation within the unit.

Figure 1. System Setup: Stainless Steel Manifold; Installation of Biological Indicators and Thermocouples within a Connector

L1-L6: Location ID used for connectors during steaming

L7: No connector installed at this location; L7 was capped and the valve closed during steaming

SC: System control located at the end of the stainless steel manifold near the steam exit from the system

Results

1. Mechanical Strength Evaluation of Lynx® CDR Connectors at Target Temperature 140 °C, 60 minutes

Gamma Irradiated Connectors

Six gamma irradiated connectors containing biological indicators and thermocouples were steamed-in-place (SIP) at a temperature between 140-144 °C for 60 minutes. Biological indicators retrieved from each connector and the system after the SIP cycle were incubated at 60 °C for seven days and did not show any growth, which demonstrated sterility of each connector. Integrity testing before and after SIP confirmed integrity of all connectors and demonstrated the mechanical strength of Lynx® CDR connectors.

Autoclaved Connectors

Six autoclaved Lynx® CDR connectors containing biological indicators and thermocouples were steamed-in-place at a temperature between 140-144 °C for 60 minutes. Enveloped biological indicators retrieved from each connector and the system after the SIP cycle were incubated at 60 °C for seven days and did not show any growth, which demonstrated sterility of each connector. Integrity testing before and after SIP confirmed integrity of all connectors and demonstrated the mechanical strength of Lynx® CDR connectors.

2. Lynx® CDR Connectors Sterility Evaluation, Target Temperature Minimum of 121 °C, 30 minutes

Although the elevated temperature and extended SIP cycle time are the worst-case scenario for evaluating mechanical strength of a connector, these conditions are not the worst-case scenario for demonstrating the ability to achieve sterility. Therefore, to demonstrate the sterility under worst-case conditions, the target temperature was decreased to a minimum of 121 °C and steam exposure time was decreased to 30 minutes.

Three autoclave and three gamma pre-treated connectors were included in the steaming cycle. Actual temperature during the cycle was between 120–131 °C. Biological indicators were recovered from each connector and incubated at 60 °C for up to seven days and did not indicate any growth, demonstrating the complete sterilization of all connectors.

3. Simulating Lynx® CDR Connectors Attached to a Large Stainless Steel Vessel , Target Temperature Minimum of 121 °C, 30 minutes

An additional SIP cycle was performed to simulate the connectors attached to a large stainless steel vessel. In all previous SIP cycles, the connectors were attached to the SIP system at a 45° angle (see Figure 1) which is an optimal angle for efficient condensate removal. Large stainless steel vessels such as buffer tanks or bioreactors might only have an option to install the connectors horizontally or vertically. To evaluate if sterility can still be achieved, the connectors were connected to a 20 L stainless steel pressure vessel, one in a horizontal and one in a vertical orientation and were steamed at temperatures of 121 - 127 °C for 30 minutes. Biological indicators were retrieved after the cool-down step, placed into recovery media, incubated at 60 °C, and observed for growth up to seven days. No growth was observed, demonstrating that sterility was achieved even though one of the connectors (horizontal orientation) was installed at a suboptimal drainage angle.

Conclusion

The Lynx® CDR connectors are ideally suited for use in single-use, stainless steel, and hybrid systems, providing manufacturers with a secure and robust connection and disconnection during sterile liquid transfer.

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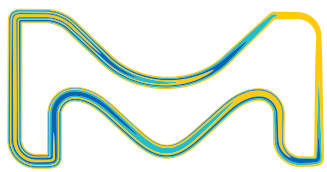

Lit. No. MK_TB3819EN Ver. 1.0
2019-21023
01/2020