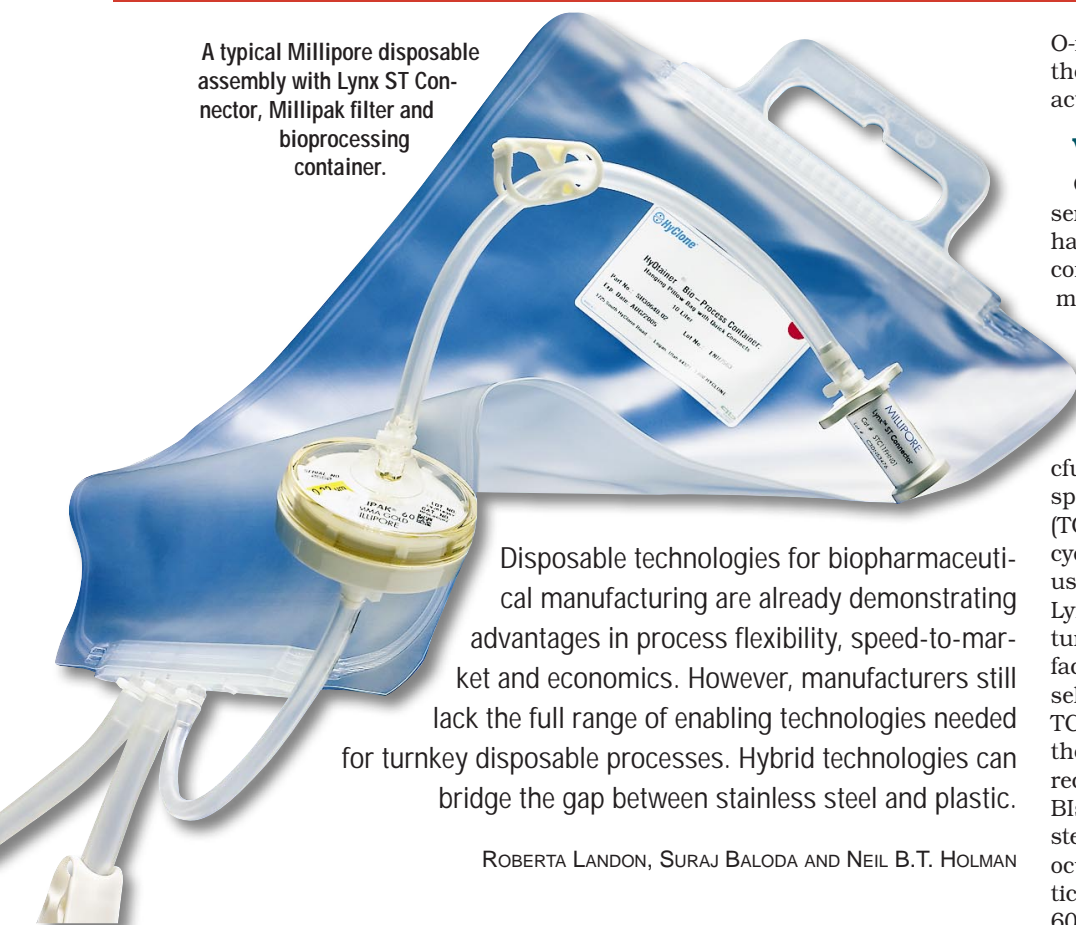


# Bridging the gap

A case study in the validation of hybrid connectors

A typical Millipore disposable assembly with Lynx ST Connector, Millipak filter and bioprocessing container.



Disposable technologies for biopharmaceutical manufacturing are already demonstrating advantages in process flexibility, speed-to-market and economics. However, manufacturers still lack the full range of enabling technologies needed for turnkey disposable processes. Hybrid technologies can bridge the gap between stainless steel and plastic.

ROBERTA LANDON, SURAJ BALODA AND NEIL B.T. HOLMAN

Over the next few years, until a full range of scalable solutions is available for disposable manufacturing processes, hybrid technologies will be needed to bridge the gap between traditional stainless steel and disposable technologies. This article covers a hybrid connector technology, the Millipore Lynx ST connector, and its validation.

The Lynx ST is a single-use, "steam to" connector that incorporates a valve and provides a sterile connection between stainless steel and disposable systems. One side of the connector has a Tri-Clamp fitting that connects to stainless steel piping or a tank. On the other side is a hose barb fitting that connects to a disposable assembly. A typical pre-sterilized disposable assembly comprises of equipment such as filters, bioprocessing containers and tubing, as required by the ap-

plication. The Lynx ST connector is already attached to the assembly. The operator connects the Tri-Clamp to the stainless steel piping or tank, steam-sterilizes the exposed Tri-Clamp face and allows it to cool.

## Simple twist-and-pull action

Fluid flow can then be started by opening the connector, using a simple twist-and-pull action. To stop the flow, the operator twists and pushes the connector back to the closed position. The connector can be actuated many times in a single-use application.

Ultem, a polyetherimide resin, is the main material of construction for the Lynx ST connector. It provides the strength required to resist gamma irradiation and steam sterilization at temperatures up to 135 °C.

Other materials of construction include three silicone O-rings which maintain a sterile fluid path in the closed position. The O-ring at the Tri-Clamp face moves into the connector body when the flow path is opened. The patent-pending double

O-ring seal on the hose barb side protects the fluid path from contamination during actuation.

## Validating the Tri-Clamp face

Once the pre-sterilized disposable assembly, including the Lynx ST connector, has been removed from its packaging and connected to the stainless steel equipment, steaming of the Lynx ST Tri-Clamp face is required before fluid flow begins. This steaming step is a point of validation to ensure that it is carried out correctly.

Biological indicators (BIs,  $1.2 \times 10^6$  cfu of *Geobacillus stearothermophilus* spores) and calibrated thermocouples (TCs) were used to validate the steaming cycle. Two BIs and calibrated TCs were used at the Tri-Clamp face of each of five Lynx ST units for testing at two temperatures: 121 °C and 135 °C. The Tri-Clamp face of the connector was exposed to the selected temperature for 30 minutes. The TCs were continuously monitored during the steaming cycles and the data was recorded in real time. After steaming, the BIs were aseptically removed from the steaming face of each of the test units, inoculated individually in 10 ml sterile Trypticase Soy Broth (TSB) and incubated at  $60 \pm 2$  °C for seven days.

None of the TSB test tubes with the BIs either from the 121 °C or 135 °C steaming cycles showed any growth. On the other hand, as expected, the positive control (TSB with unexposed spore strip) showed growth under similar incubation conditions. Thus, the results demonstrated that an absolute kill of  $\geq 10^6$  spores of *G. stearothermophilus* was achieved after 30 minutes at 121 °C and 135 °C, indicating that standard steaming practice ensures sterility of the Tri-Clamp face of the Lynx ST connector. Furthermore, the TC data also showed that the face of the test units reached and maintained the required steaming temperatures throughout the study.

## Validating actuation integrity

During the process of opening and closing (actuating) the valve of the Lynx ST connector, the integrity of the fluid path is protected by the double O-ring seal. To ensure that fluid path sterility is maintained, the cam slot of the Lynx ST connector was challenged with  $\geq 10^6$  cfu of *Brevundimonas diminuta* cells grown in saline lactose broth (SLB).

The authors are all with Millipore Corporation, Bedford, MA/USA. Roberta Landon is Worldwide Product Manager for Disposable Technology. Dr Suraj Baloda is Group Manager for Process Microbiology & Sterile Applications. Neil B.T. Holman is Group Product Manager for Disposable Technologies.

The cells were grown in SLB to create worst-case challenge conditions. 100 µl of the SLB culture were inoculated in the cam slot by keeping the connector in a vertical position with the steaming face pointing downwards. After waiting for 2–3 minutes, the units were actuated multiple times. This was followed by flushing 500 ml of sterile TSB through the fluid path and collecting the liquid in sterile flasks. The test samples, as well as positive and negative control flasks, were incubated at 30 °C and observed for growth due to the downstream passage of organisms contaminating the fluid path. The results showed no growth in the test flasks after seven days of incubation. This proves that there was no downstream passage of the organisms in the Lynx ST connector.

### Validating the Tri-Clamp O-ring

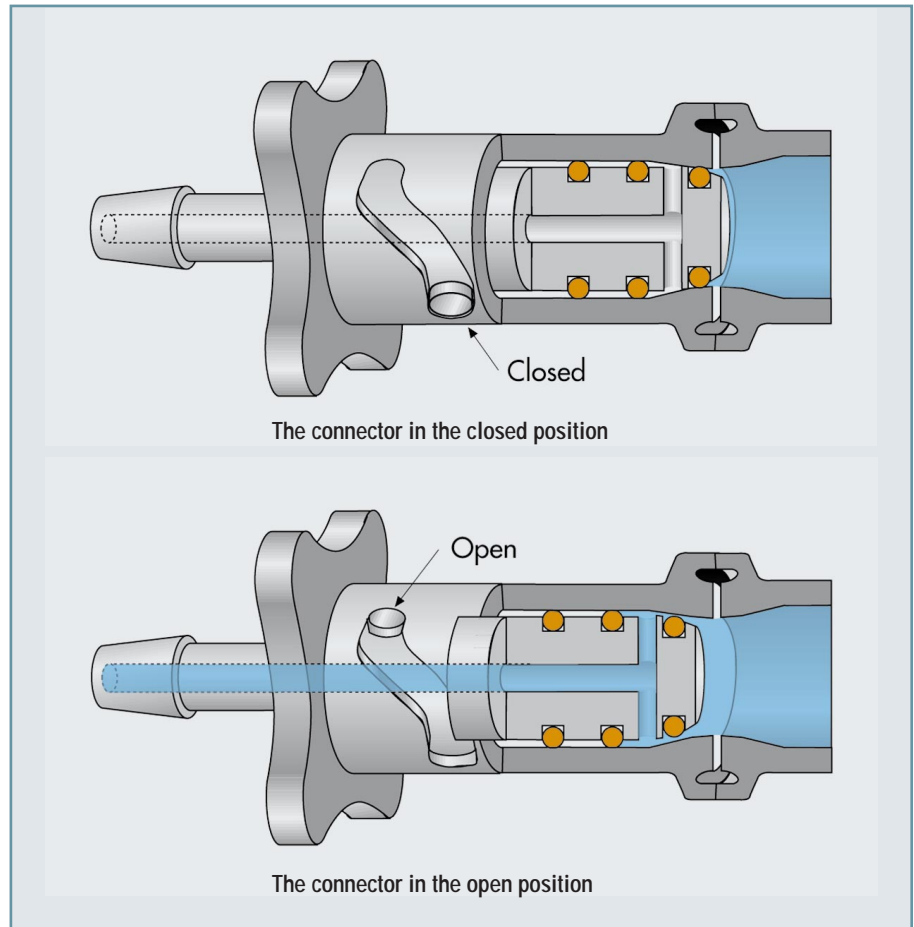
The Tri-Clamp face of the Lynx ST connector was challenged microbiologically by simulating high-pressure situations such as pressure hold steps prior to steam-in-place (SIP). A liquid suspension (100 µl) of the challenge organism ( $\geq 10^6$  cfu of *B. diminuta* or *G. stearothermophilus*) was dried on the face of the Lynx ST connector and a pressure of 30 psi (3 cycles of 15 minutes each) was applied to the face seal under aseptic conditions. At the end of the last cycle, without actuating (opening) the connector, the hose barb end of the fluid path was flushed three times with around 1 ml of sterile TSB each time and the flush media inoculated in sterile TSB tubes. Depending on the type of BI used, the tubes were incubated for seven days at 30 °C or 60 °C together with their respective positive and negative controls. This challenge to the Tri-Clamp face showed no microbial growth in the TSB tubes, proving that there was no passage of BIs across the face seal into the fluid path when 30 psi pressure is applied to the Tri-Clamp face.

### Validating irradiation

Since the Lynx ST connector would be part of an assembly with other components, the entire assembly would need to be validated for sterility according to AAMI/ISO guidelines. To prove that the Lynx ST connector is capable of sterilization by gamma irradiation, testing was conducted with a challenge organism.

For further information:

Roberta Landon  
 Millipore Corporation  
 290 Concord Road  
 Billerica, MA 01821  
 USA  
 Tel.: +1-978-715-4321  
 Website: [www.millipore.com](http://www.millipore.com)  
 e-mail: [roberta\\_landon@millipore.com](mailto:roberta_landon@millipore.com)



Pictures: Millipore

This was indicated by achieving a total kill of  $\geq 10^6$  inoculum of *Bacillus pumilus* (ATCC 27142) spores in the fluid path of the connector. The innermost region of the connector was shown to be transparent to 25 kGy of gamma irradiation.

These studies show that Lynx ST hybrid technology can provide the critical link required between stainless steel and disposable equipment until fully-disposable solutions are available. Millipore embraces this technology and provides the industry

with pre-sterilized disposable assemblies that have Lynx ST connectors. Through this unique ability to offer turnkey solutions, Millipore is helping customers simplify processes and shift some of the burden of assembly production, including sterility validation, to the supplier. As the industry continues to evolve towards complete disposable manufacturing processes, the Lynx ST connector, with its robust and validated design, is ideal to bridge the gap between stainless steel and disposables. ■

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