Millipore®
Filtration, Separation
& Preparation

Application Note

Use of the Lynx® CDR Connector in a non-classified “gray space” environment

Introduction

Today, sterile connectors are used in a variety of different spaces ranging from highly classified, controlled environments, such as ISO® 5 (Class 100) clean rooms, to non-classified, uncontrolled “gray space” environments. Current disposable connector technology, which allows users to only make a single sterile-to-sterile connection per device, requires complex assemblies in situations where multiple connections are required. Additionally, only dry connections can be made.

The Lynx® CDR Connector offers users the ability to connect, disconnect, and reconnect, while maintaining a sterile flow path, for up to six connect/disconnect cycles. This first-of-its-kind, multi-use connector eliminates the need for tube-welding and complicated manifold systems, resulting in increased process simplicity, speed, and flexibility. Additionally, connections and/or disconnections can be made wet and with the flow path under pressure, adding to the flexibility of the approach.

In this study, the Lynx® CDR Connector was utilized during two typical bioprocessing steps (media preparation and bioreactor fill) where multiple connections/disconnections are required. The results demonstrate the Lynx® CDR Connector’s ability to maintain a sterile flow path during multiple connect/disconnect/reconnect cycles in a non-classified “gray space” laboratory.

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Process Overview

The experiment was performed in an unregulated gray space environment, carried out in three phases over a 16-day period (two days for media preparation, filtration, and storage, followed by a 14-day cell culture simulation). The experiment was designed such that each Lynx® CDR Connector was actuated multiple times, and the connectors on the media storage bags were utilized in multiple process steps, as outlined in Table 1. Female connectors were used on both the mixer and bioreactor in order to enable connection of the male connectors on the storage bags to both systems.

1. Media Preparation

Media was prepared in a Mobius® mixer open to the environment.

2. Sterile Filtration & Storage

The media was passed through a sterilizing-grade filter, and Lynx® CDR Connectors were used to fill two separate media storage bags (one stored at ambient temperature, the other at 2-8°C).

3. Bioreactor Fill

Lynx® CDR Connectors were used to make a total of six media additions to a bioreactor incubated at 37°C, alternating between the media stored at ambient temperature and in the cold. Media additions made from storage bag #2 were performed immediately after it was removed from the cold environment in order to demonstrate that Lynx® CDR Connections can be made between two connector halves of differing temperature. Between additions, the Lynx® CDR Connectors were left connected on the benchtop after ambient media additions, and were disconnected after cold media additions, in order to demonstrate that the connector can maintain a sterile flow path in both states.

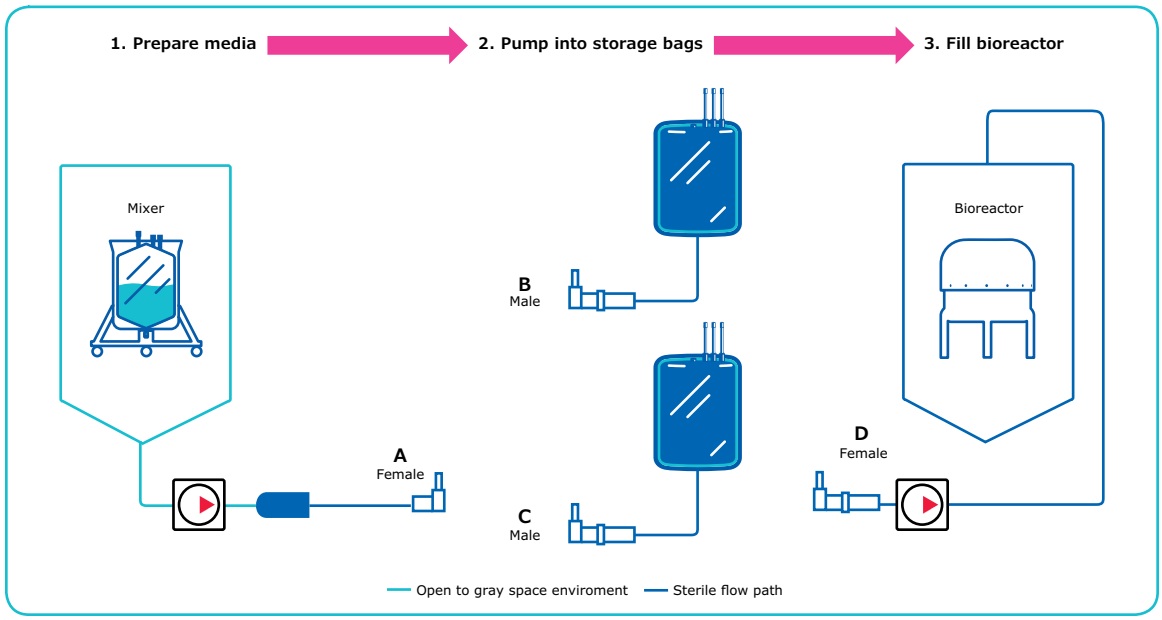


Figure 1.

Experimental process flow diagram for media prep and bioreactor fill. Lynx® CDR Connectors (A, B, C and D) were used to make several connections/disconnections while maintaining a sterile flow path.

Utilization of the Lynx® CDR connectors in the media prep and bioreactor fill steps

Table 1.

Lynx® CDR Connector (Refer to Figure 1)	# of actuations during media prep	# of actuations during bioreactor fill	Total # of actuations
A – Mixer (female)	2	0	2
B – Bag #1 (male)	1	3	4
C – Bag #2 (male)	1	3	4
D – Bioreactor (female)	0	6	6

Media Preparation & Filtration into Storage Bags

50 L of OptiCHO™ AGT™ powdered media was prepared in a Mobius® Mix 10-50 system that was open to the environment. Once the media were fully dissolved, 50 mL samples were taken to serve as controls for growth.

A pre-sterilized, single-use assembly containing an Opticap® XL600 with Millipore Express® SHC 0.5/0.2 µm membrane and a female Lynx® CDR Connector was attached to the drain line of the Mobius® Mix Bag. A sterile-to-sterile connection was made between the female Lynx® CDR Connector on the mixer bag and the male Lynx® CDR Connector on the first storage bag. 25 L of media was pumped into the storage bag, using a peristaltic pump. The Lynx® CDR Connection to the first storage bag was disconnected, and this process was repeated to fill the second storage bag with media.

After 48 hours at room temperature, the positive and negative control samples and storage bags containing sterile-filtered media were visually inspected for contamination; the results are shown in Table 2. Storage bag #1 was then set on the benchtop next to the bioreactor (ambient storage), and storage bag #2 was stored at 2–8°C (cold storage).

Figure 2.

Mixer drain line connected to the sterile filter assembly and fed through a peristaltic pump, ready to make a sterile-to-sterile connection with the Lynx® CDR Connector to an empty storage bag.

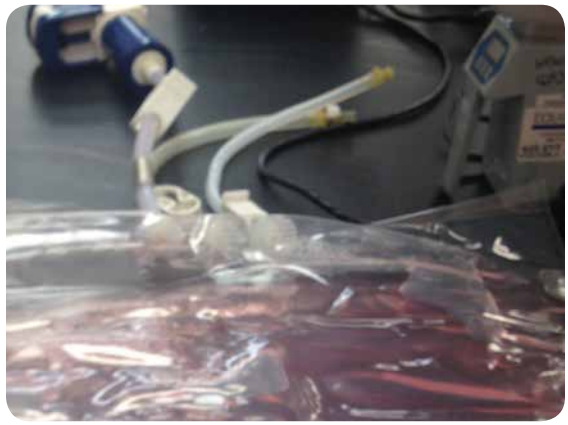


Figure 3.

Pumping 25 L of media from the Mobius® Mix 10-50 system through the sterilizing filter, the sterile Lynx® CDR Connection, and into the sterile storage bag.

Figure 4.

Storage bags filled with sterile media and stored at ambient temperature for 48 hours prior to the cell culture process simulation.

Growth results for control samples and storage bags

Table 2.

Item	Growth/No Growth
Negative Control	No Growth
Positive Control	Growth
Storage Bag #1 (stored at ambient temperature)	No Growth
Storage Bag #2 (stored at 2-8°C)	No Growth

Figure 5.

The Mobius® 50 L Bioreactor with Flexware® assembly installed, ready to connect to the storage bag via Lynx® CDR Connection.

Figure 6.

Pumping media into the bioreactor from storage bag #1.

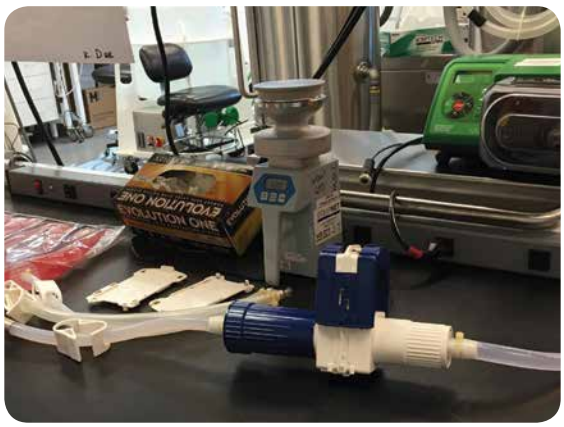
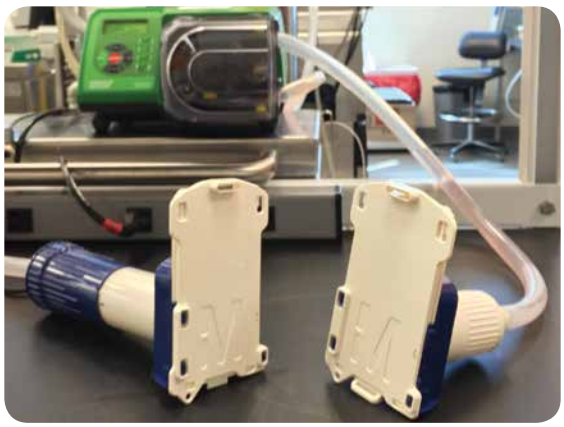


Figure 7.

The Lynx® CDR Connectors remained unconnected on the bench after the final media addition.



Bioreactor Fill

A Mobius® 50 L Bioreactor with Flexware® assembly was modified to include a filling line with a female Lynx® CDR Connector to enable multiple additions of media via connections/disconnections with the media storage bags. For the first media addition, the male Lynx® CDR Connector on storage bag #1 was connected to the female Lynx® CDR Connector on the bioreactor, and 10 L of media was pumped into the bioreactor, using a peristaltic pump. The bioreactor impeller and temperature control were then initiated at 50 RPM and 37°C, respectively. The bioreactor and media storage bag remained connected via the Lynx® CDR Connector for 48 hours prior to the next planned media addition.

After 48 hours of simulated cell culture, the second media-containing bag was removed from cold storage. The first storage bag was disconnected. Storage bag #2 was immediately connected to the bioreactor, demonstrating that a Lynx® CDR Connection can be made between a cold and a warm connector. Note that the same connector on the bioreactor was used to make this new connection. Ten liters of cold media was then pumped into the bioreactor. The Lynx® CDR Connection to storage bag #2 was then disconnected, and the storage bag was returned for storage at 2-8°C. The female Lynx® CDR Connector on the bioreactor remained disconnected and resting on the benchtop for two days prior to the next planned media addition.

Alternating additions of media from storage bag #1 (incubated under ambient conditions) and storage bag #2 (incubated in the cold) were repeated every 2-3 days until the media bags were empty, for a total of six additions (three from each storage bag). The state of the Lynx® CDR Connectors between additions alternated between remaining connected on the benchtop and being immediately disconnected in order to demonstrate that a sterile fluid path can be maintained under both conditions. The bioreactor was visually inspected for contamination before each addition was made (Table 3). After the last media addition, the Lynx® CDR Connector on the bioreactor was disconnected and left resting on the benchtop; the experiment ended on simulated cell culture process day 14, after which 5 L of media was removed from the bioreactor and subjected to sterility testing to confirm that no ingress of microorganisms occurred during the experiment.

Figure 8.

Mobius® Bioreactor on day 14 of the cell culture process simulation. The media is clear, indicating no contamination.

Bioreactor addition results

Table 3.

Lynx® CDR Actuation #						
Process day	# of incubation days in bioreactor	Storage bag #1 (RT)	Storage bag #2 (4°C)	Bioreactor	Addition volume (L)	Growth/ No Growth
2	0	2	-	1	10	No Growth
5	3	-	2	2	10	No Growth
7	5	3	-	3	10	No Growth
9	7	-	3	4	10	No Growth
12	10	4	-	5	5	No Growth
14	12	-	4	6	5	No Growth
16	14	-	-	-	-	No Growth

Media Sterility Testing

The 5 L of media removed from the bioreactor was vacuum-filtered through five Microfil® V 0.45 µm filtration devices (one liter of media was filtered through each device). After vacuum-filtration, the membrane filters were removed from the filtration units and placed individually on five Tryptic Soy Agar plates. The plates were placed in an incubator set at 30 ± 2°C for seven days. After seven days, all five plates were observed and assessed as negative for growth.

Conclusions

- The Lynx® CDR Connector maintained a sterile flow path in a “gray space” lab for the duration of a 16-day simulated cell culture run.
- During this experiment, all four Lynx® CDR Connectors were used to make multiple connections/disconnections.
- Two Lynx® CDR Connectors were used in multiple process steps.
- Successful connection was made with Lynx® CDR Connector halves at different temperatures.
- The growth media remained sterile after processing with the Lynx® CDR Connector.

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