Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Technical Brief

Choosing the right sterile connector based on design and sterility test results

Background

The biotech industry has witnessed a rapid adoption of single-use technology in bioprocess manufacturing. Concurrently, there is a strong push to reduce operating costs by moving manufacturing from traditional stainless steel equipment in a clean room to single-use disposable solutions in an unclassified environment. As contamination risk increases exponentially in an unclassified area, it becomes imperative to ensure a validated sterile flow path for single-use solutions. Pre-sterilized single-use connectors perform the critical function of connecting two pre-sterilized single-use components in a non-sterile environment, while maintaining fluid path sterility.

As a result of the increasing use of single-use connectors, many new types of connectors have entered the marketplace. At the same time, their use has expanded beyond the typical upstream buffer and media applications, into high value-added downstream applications like ultrafiltration/dialfiltration and final filtration and filling. This makes it extremely important that the choice of a single-use connector should take into consideration the following factors:

Robustness of the connector design

By design, a sterile connector should protect the fluid path by never exposing it to the open environment. The Lynx® S2S connector employs a solid plug, not a membrane or peel strip, which creates a gap for ingress of contamination during the connection process. As a result, the Lynx® S2S flowpath remains completely closed, minimizing the risk of bacterial intrusion.

Sterility claims made by the vendor

When making sure that a single-use connector maintains a sterile fluid path, it is important to verify that the methods used to support that claims are valid, reproducible and provide meaningful results. It is equally critical to ascertain that the selected connector will secure the fluid path at a sterility assurance level (SAL) of 10^{-6} under the most rigorous conditions.

Lynx® S2S Connectors

The Lynx® S2S connector is a single-use, single actuation device. It comprises a male and female coupling set (Figure 1) designed to maintain sterility during the connection of two independent sterile fluid paths. Both units of the connector use a hose barb fitting to connect desired bags, containers or assemblies to the fluid path. Each fluid path is then sterilized by either gamma irradiation or by autoclave, in preparation for a sterile transfer of media, buffer or even final drug product.

The connector's process contact materials are constructed of high temperature gamma stable polysulfone with an over-molded silicone gasket, allowing for autoclaving up to 130°C for 30 minutes and/or gamma irradiation up to 45 kGy.

The male and female coupling sets of the Lynx® S2S connector use solid plugs with silicone o-ring seals and gaskets for containment. This design provides high-pressure sealing, and a tortuous path that assures zero passage of bacteria. After the couplings have been autoclaved and/or gamma irradiated, two sterilized assemblies can be connected by a series of simple steps in any environment.

During the connection process, the plugs that will become contaminated in a non-classifed area are isolated by moving them up and away from the fluid path within the female coupling body. This process assures a sterile fluid path (SAL of 10^{-6}) for the liquid to flow through the connection.

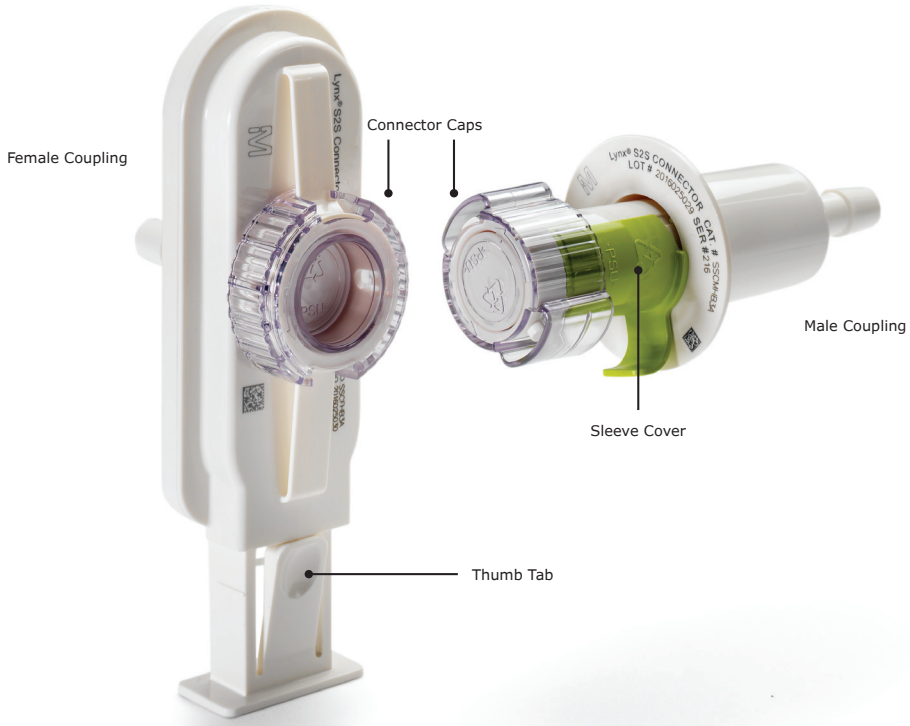

Figure 1.

Aerosol Challenge Studies

An aerosolized bacterial challenge test is used to simulate worst-case environmental contamination. This test challenges the Lynx® S2S connectors with greater than one million bacteria (in colony forming units) per device, thus providing the most stringent testing in the industry. In this test, 80 connector sets, 10 each of Lynx® S2S connector sets and 10 each of seven competitors, were challenged with an aerosolized suspension of *Brevundimonas diminuta* at equal to or greater than 10^6 colony forming units per connector set. They were connected and actuated in this aerosolized environment. Sterile media was transferred through the connectors into sterile receiving vessels, then assayed to determine the presence or absence of growth. Device controls were employed to assure proper execution and function of the test. The negative device control consisted of a connector coupling set that was connected and actuated within the isolator chamber without being exposed to the challenge organism; the positive device control consisted of a connector coupling set with its barrier film, membrane or plugs removed prior to being exposed to the challenge organism, and then connected and actuated. The collection vessels, negative and positive controls were incubated and scored for presence or absence of growth over a seven-day period. Samples exhibiting growth were confirmed to be the test microorganism *Brevundimonas diminuta*.

Results

All 10 Lynx Connectors, 5 each from Test Run #1 and Test Run #2, did not exhibit growth, and therefore passed the test. Competitors devices, Test Run #3 through Test Run #14, showed growth in 50% to 100% of the devices tested. Negative and positive control results were as expected. Negative controls, which were not exposed to the bacterial challenge, had no growth. Positive controls, where the barrier plug was removed from both the male and the female half of the connector, had growth of the challenge organism. See Table 1 below for the results.

Table 1: Aerosol Challenge Test Results

Test Run #1:

Lynx® S2S Connector Test Set #1	Results
Negative Control	No Growth
1	No Growth
2	No Growth
3	No Growth
4	No Growth
5	No Growth
Positive Control	**Growth**

Test Run #2:

Lynx® S2S Connector Test Set #2	Results
Negative Control	No Growth
6	No Growth
7	No Growth
8	No Growth
9	No Growth
10	No Growth
Positive Control	**Growth**

Test Run #3:

Competitor A Test Set #1	Results
Negative Control	No Growth
1	No Growth
2	No Growth
3	**Growth**
4	**Growth**
5	**Growth**
Positive Control	**Growth**

Test Run #4:

Competitor A Test Set #2	Results
Negative Control	No Growth
6	**Growth**
7	**Growth**
8	**Growth**
9	**Growth**
10	**Growth**
Positive Control	**Growth**

Test Run #5:

Competitor B Test Set #1	Results
Negative Control	No Growth
1	**Growth**
2	**Growth**
3	**Growth**
4	No Growth
5	**Growth**
Positive Control	**Growth**

Test Run #6:

Competitor B Test Set #2	Results
Negative Control	No Growth
6	**Growth**
7	**Growth**
8	**Growth**
9	**Growth**
10	No Growth
Positive Control	**Growth**

Test Run #7:

Competitor C Test Set #1	Results
Negative Control	No Growth
1	**Growth**
2	**Growth**
3	**Growth**
4	**Growth**
5	**Growth**
Positive Control	**Growth**

Test Run #8:

Competitor C Test Set #2	Results
Negative Control	No Growth
6	**Growth**
7	**Growth**
8*	No Effluent Collected
9	**Growth**
10	**Growth**
Positive Control	**Growth**

*Peel strips broke during testing. Was unable to flow liquid through device.

Test Run #9:

Competitor D Test Set #1	Results
Negative Control	No Growth
1	**Growth**
2	**Growth**
3	**Growth**
4	**Growth**
5	**Growth**
Positive Control	**Growth**

Test Run #10:

Competitor D Test Set #2	Results
Negative Control	No Growth
6	**Growth**
7	**Growth**
8	**Growth**
9	**Growth**
10	**Growth**
Positive Control	**Growth**

Table 1: Aerosol Challenge Test Results (continued)

Test Run #11:

Competitor E Test Set #1	Results
Negative Control	No Growth
1	**Growth**
2	**Growth**
3	**Growth**
4	**Growth**
5*	**Invalid Test**
Positive Control	**Growth**

*Peel strip not bonded to the connector therefore flow path was open prior to test

Test Run #12:

Competitor E Test Set #2	Results
Negative Control	No Growth
6*	**Invalid Test**
7	**Growth**
8*	**Invalid Test**
9*	**Invalid Test**
10	**Growth**
Positive Control	**Growth**

*Peel strip not bonded to the connector therefore flow path was open prior to test

Test Run #13:

Competitor F Test Set #1	Results
Negative Control	No Growth
1	**Growth**
2	**Growth**
3	**Growth**
4	**Growth**
5	**Growth**
Positive Control	**Growth**

Test Run #14:

Competitor F Test Set #2	Results
Negative Control	No Growth
6	**Growth**
7	**Growth**
8	No Growth
9	No Growth
10	No Growth
Positive Control	**Growth**

Test Run #15:

Competitor G Test Set #1	Results
Negative Control	No Growth
1	No Growth
2	No Growth
3	**Growth**
4	**Growth**
5	No Growth
Positive Control	**Growth**

Test Run #16:

Competitor G Test Set #2	Results
Negative Control	No Growth
6	No Growth
7	**Growth**
8	**Growth**
9	**Growth**
10	No Growth
Positive Control	**Growth**

Conclusion

The design of the Lynx® S2S connector has demonstrated a quality and robustness that will provide significant benefits to the industry by facilitating sterile transfer of fluids with the highest degree of security and safety. One of Mobius® flexible bioprocessing solutions, the Lynx® S2S connector delivers a safer and more robust connection for all unit operations within the biopharmaceutical manufacturing process.

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