Quick Disconnect Aseptic Study

Bacterial / Sporal Ingress – Aerosol and Submersion

Background

Quick disconnect connectors that can interrupt flow and provide a low leak / leak free seal have been available for some time. As designs have matured and a better understanding of customer needs has materialized, specialized configurations have been produced to meet the needs of medical, pharmaceutical, automotive, food and beverage, and general-purpose applications to name a few.

With respect to medical and pharmaceutical applications, the incorporation of quick disconnect fittings has become more prevalent with the adoption of single use systems in lieu of the traditional sterilizable, reusable systems. Autoclavable stainless steel and glass have been replaced with sterilizable plastics and rubbers. The new single use systems are typically preassembled in a cleanroom environment and packaged in materials which will provide a sustained sterile barrier. Sterilization of these systems is typically accomplished with radiation (gamma or E-beam) and ethylene oxide gas (EtO) conditioning.

After these systems are sterilized and put into service, fluid fills the system through filter ports designed to minimize bacterial/sporal transfer to the sterile inner volume of the system. These systems will then undergo a series of fluid transfers which are defined by the system use protocol and used in cleanroom environments, under sterile hoods, or in simple grey space. There are an infinite number of variables that need to be considered for the component manufacturer to provide sterile barrier verification and validation. One approach would be to state that the sterile boundary validation is the responsibility of the end user. Another more ***conservative approach*** is to utilize a model that provides an elevated challenge such that once validated, no real-world scenario will be worst in application.

Additionally, systems traditionally use quick connection fittings that permit system assembly and use. The fittings allow for multiple systems, each individually sterilized, to be connected together prior to system use, ease of assembly during production of the system, and/or the ability to provide a disconnection for collection container storage and manipulation once the system is no longer needed and the fluid needs to be stored. The later becomes the next goal as the ***disconnection of the fitting*** while maintaining the sterile boundary at the connector is desired. SeriesLock® was secondarily tested to prove this function with simulated system use parameters and a disconnection at the end of use. To further challenge the disconnection claim, the test system was reconnected and disconnected two more times.

The *Conservative Approach* to Validation

The conservative approach is what challenged SeriesLock® with PSN Labs, Erie PA, executing all test protocols. To prove a sterile boundary is maintained during simulated, realistic use, the system consisted of:

	In Normal Practice	Conservative Approach
Component Sterilization	Clean-room, pre-assembled kits terminally sterilized	Individually sterilized components
System Assembly	Cleanroom assembled and packaged prior to sterilization	System assembled in grey space with pre-sterile components
Bacterial / Sporal Assault During Use	Typical bacterial loading due to room bioburden and operator exposure (i.e. sneezing, coughing, touching) .	Assembled circuit submerged in a heavy bacterial load slurry or Aerosol attacked with multiple direct shots per extended period of time.

Once again, the goal is to be able to state that the challenge environment was so severe that no real-world application will be worse and as such, the quick disconnect fitting is not a source of system contamination.

Test Definitions and Setup

SeriesLock® connectors were challenge tested with the objective of assessing the connector's integrity against bacterial and spore ingress contamination using exaggerated conditions. The following three scenarios were utilized:

1. Bacterial Aerosol
2. Bacterial Submersion
3. Sporal Suspension Submersion with fluid transfer between two containers

Each testing scenario utilized gamma sterilized SeriesLock® connectors. All other test system components were steam sterilized at 134°C for 30-45 minutes. Additionally, the TSB (Tryptic Sterile Broth) solutions were autoclaved at 121°C for 15-20 minutes before being subjected to testing. Two challenge solutions were utilized for the three ingress test methods detailed below:

1. Aerosol and submersion aerosol challenge tests were composed of 750mL of nutrient broth and 3 vials of *Staphylococcus aureus* (ATCC 12600 – Lot number 70025858).
2. The submersion challenge test with fluid transfer between to containers test was composed of a spore suspension containing *Geobacillius stearothermophilus* (ATCC 7953) in 0.1% Carboxy-Methyl Cellulose.

Bacterial Aerosol:

Bacterial aerosol testing was completed to simulate an aerosolized bacterial transfer to the SeriesLock® connector's surfaces. This test utilized a peristaltic pump to transfer TSB from an upstream reservoir thru the SeriesLock connector and into a collection reservoir. Both upstream and downstream sterile connection containers were used to collect upstream and downstream fluid samples for further analysis.

Prior to initiating TSB flow thru the SeriesLock® connector, the connected system with connector in place was subjected to a directed aerosolized spray sequence every five (5) minutes for one (1) hour. Each spray sequence utilized five (5) individual sprays meant to simulate five (5) human sneezes.

After applying the aerosol solution for the hour, the peristaltic pump transferred the TSB from the upstream reservoir, thru the SeriesLock® Connector, and into the downstream reservoir. As the TSB was pumped, both upstream and downstream collection containers collected fluid samples for further analysis.

Samples for analysis were plated at 1:10, 1:100, and 1:1000 concentrations on 3M petrifilm plates and incubated for twenty-four (24) hours at 35°C. **No growth** was found on either upstream or downstream plates indicating the SeriesLock® connector did not allow bacterial ingress and that the system remained sterile throughout its use.

Bacterial Submersion:

Bacterial submersion testing is considered the worst-case testing scenario to provide confidence that the connection fidelity is adequate to block bacterial intrusion during all normal use conditions. This test utilized a peristaltic pump to transfer TSB from an upstream reservoir thru the SeriesLock® connector and into a collection reservoir. Both upstream and downstream sterile connection containers were used to collect upstream and downstream fluid samples for further analysis.

Prior to initiating TSB flow thru the SeriesLock® connector, the connector was submerged in the staph solution for 30 minutes. After the 30 minutes, while still submerged, the peristaltic pump transferred the TSB from the upstream reservoir, thru the SeriesLock Connector, and into the downstream reservoir. As the TSB was pumped, both upstream and downstream collection containers collected fluid samples for further analysis.

Samples for analysis were plated at 1:10, 1:100, and 1:1000 concentrations on 3M petrifilm plates and incubated for twenty-four (24) hours at 35°C. **No growth** was found on either upstream or downstream plates indicating the SeriesLock® connector did not allow bacterial ingress and that the system remained sterile throughout its use.

Sporal Suspension Submersion with Fluid Transfer Between Two Containers:

Submersion testing is considered the worst-case testing scenario to provide confidence that the connection fidelity is adequate to block bacterial intrusion during all normal use conditions. This test sequence introduces spore attacks as well as multi-directional fluid flow to the test challenge.

Prior to initiating TSB flow thru the SeriesLock® connector, the connector was submerged in a spore suspension containing *Geobacillius stearothermophilus* (ATCC 7953) in 0.1% Carboxy-Methyl Cellulose for 30 seconds. The assembly was then placed in a laminar flow cabinet to air dry at ambient

temperature for twelve (12) hours. After drying, 1L of TSB was placed in the upstream reservoir (bag1) and pumped thru the system and SeriesLock connector into the downstream reservoir (bag2). The system was then reversed to pull the solution from bag2 back into bag1. This series of forward and reverse flow pump operations occurred five (5) times to simulate normal system use with more than normal cycles applied. As a control the same sequence was completed with a circuit assembly NOT CONTAINING a SeriesLock connector.

After all five pump cycles were completed, the TSB solution was equally split between bags 1 and 2. The bags were then incubated at 55°C for fourteen (14) days. Samples where then taken from the incubated solutions and plated at 1:10, 1:100, and 1:1000 concentrations on 3M petrifilm plates and incubated for twenty-four (24) hours at 35°C. **No growth** was found on either the control, bag1, or bag2 plates indicating the SeriesLock® connector did not allow bacterial ingress and that the system remained sterile throughout its use.

RESULTS

Conservative Approach

All test sample assemblies performed as expected.

1. No Growth was observed on upstream and downstream plates with both the aerosol and submersion bacterial challenge tests.
2. No Growth was observed on bag1 and bag2 plates with the spore suspension challenge tests after multiple forward and backward fluid transfers between upstream (bag1) and downstream (bag2) collection reservoirs.
3. All control sample plates also showed No Growth.

Summary

1. Aerosolized bacterial challenge testing ***PASSED*** with no bacterial ingress detected.
2. Submersion bacterial challenge testing ***PASSED*** with no bacterial ingress detected.
3. Submersion spore suspension challenge testing ***PASSED*** with no contamination ingress detected.

Disconnection of the Fitting Validation

Additional testing was completed by Microchem Laboratory, Round Rock TX, to provide evidence that the SeriesLock connectors would preserve the sterile environment once disconnected. To prove a sterile boundary is maintained during simulated, realistic use, the system consisted of:

	In Normal Practice	Conservative Approach
Component Sterilization	Clean-room, pre-assembled kits terminally sterilized	Individually sterilized components
System Assembly	Cleanroom assembled and packaged prior to sterilization	System assembled in grey space with pre-sterile components
Bacterial / Sporal Assault During Use	Typical bacterial loading due to room bioburden and operator exposure (i.e. sneezing, coughing, touching)	Assembled circuit submerged in a heavy bacterial load slurry or Aerosol attacked with multiple direct shots per extended period of time.

Test Definition and Setup

SeriesLock® connectors were tested to ensure that the fluid line connectors remained sterile during simulated usage. The test setup utilized a closed loop system where a carboy filled with sterile Tryptic Soy broth was used as the outflow/inflow reservoir with an in-line peristaltic pump metering fluid flow at a defined rate. A gamma irradiated SeriesLock connector was placed in-line between the reservoir and the pump to simulate a suction condition thru the connector. The entire test setup was aseptically assembled within a biosafety hood maintaining no better than a class 7 environment when the doors where closed between daily test setup manipulation and sample collections.

Test samples included six (6) test replicates, two (2) positive control units and one (1) negative control unit. The positive control devices were used to confirm that turbidity is present at low levels of inoculation (*P.aeruginosa* ATCC 15442). The negative control device was not exposed to any fluid and used to confirm that the circuit/connector remained sterile at ambient temperature throughout the study.

Fluid from the reservoir was pumped thru the system for seven (7) continuous days for a defined period of time per day. Samples of the connector influent and effluent were taken once per day. Samples were subsequently plated, incubated and tested for growth. Between daily flow sampling, the system was drained, tubes pulled from the reservoir and covered with sterile cones to allow the wetted surfaces to be exposed to oxygen in an effort to accelerate any potential bacterial growth.

On day eight (8), the connector was aseptically disconnected while the system was full of fluid. The female and male connector surfaces were then swabbed, enumerated and plated to assess sterility post disconnection.

RESULTS

Disconnection of the Fitting

All test sample assemblies performed as expected.

1. All six (6) test replicates retained system sterility during the seven (7) day testing with No Growth observed.
2. All six (6) test replicates retained system sterility during the eighth day disconnection.
3. Positive control unit testing determined the inoculum level to be 300cfu of *aeruginosa*. Pure growth of *P.aeruginosa* was determined within the assemblies.
4. Negative control unit testing remained sterile with No Growth observed.

Summary

1. All test replicates assembled aseptically, remained sterile for seven (7) days in both pre-connected and connected states.
2. All test replicates demonstrated the ability to disconnect while maintaining system sterility.

NOTE: *All testing was completed replicating several exaggerated and in-use conditions. Customers are encouraged to request a sample to validate their own use environments.*