BIOTECH

Technical Position Paper

USTR 3546

An Explanation and Evaluation of Sterile Connector Soiling Tests

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Signature

1 Purpose

The following is an explanation of the tests used to evaluate the ability of a sterile connector to maintain a sterile path when activated, and an evaluation of the appropriate application of each test.

2 Background

Sterile connectors are designed to facilitate the sterile connection of tubing and other components in biopharmaceutical applications. Single-use sterile connectors allow for the dry connection of two separate fluid pathways, while maintaining the sterile integrity of both. They are comprised of two separate pieces, each covered with a peel away strip that acts as a static and temporary barrier to protect the port and maintain the sterility of the dry interior fluid pathway until the connection is made.

3 Technical Position

There are currently two general classes of tests which can be used to evaluate the ability of a sterile connector to maintain a sterile path: a liquid immersion soiling test and an aerosol soiling test. It is very important to note that although the liquid immersion soiling test and the aerosol soiling test can be used to test the ability of the connector to maintain a sterile fluid path, they do so in different ways. Whereas the purpose of the liquid immersion soiling test is to evaluate the ability of a heavily externally soiled sterile connector to maintain a sterile path when activated, the purpose of an aerosol soiling test is to test the ability of the connector to make a sterile connection in a heavily contaminated atmosphere. In the liquid immersion soiling testing, contamination of the sterile path due to surface soiling ingress is evaluated and in the aerosol soiling testing, contamination of the sterile path due to airborne soiling ingress is evaluated instead. Each of these tests is designed to be 'worst case' for its respective path of contamination ingress. For surface soiling ingress, the exterior surface of the device must be heavily soiled and for airborne soiling ingress, the surrounding air must be heavily soiled.

An important side note regarding the ASTM E3251 aerosol soiling test method (Standard Test Method for Microbial Ingress Testing on Single-Use Systems). In the ASTM standard, the aerosol test method does not test airborne soiling ingress, as no direct measure of bioaerosol content is made and the test relies on gravitational settling for soiling. In that way it is primarily a test of surface soiling, similar to the liquid immersion soiling test.

4 Liquid Immersion Soiling Testing

In the liquid immersion soiling test, the connection ends of the sterile connector device are dipped in a bacterial suspension, which contains an adherent and then allowed to dry to thoroughly soil the exterior of the device. This is to ensure a robust test of the device connection when the two halves are joined. By soiling the exterior of the device, connecting it in this soiled state, and then flushing the interior of the device post connection using an appropriate growth medium, we can demonstrate that the sterile interior of the device has not been exposed to the heavily soiled exterior surface when the connection was made, and thus proving a sterile connection.

Dipping the device in a soiling solution containing an additional adherent, such as carboxymethyl cellulose, is an effective way to ensure heavy soiling of the exterior surface of the device. To evaluate the effectiveness of external surface soiling, sterile connector devices were subjected to liquid soiling exposure for up to 24 hours and then sonicated in Tryptic Soy Broth (TSB) to recover surface bacterial contamination. Irrespective of liquid exposure time, 18/18 liquid soiled devices all had recoverable external contamination with $>10^5$ total colony forming units (CFU) per device using $>10^5$ CFU/mL inoculation fluid (Table 1 and Figure 1). Thus, exposure to liquid soiling consistently soiled the exterior of the device.

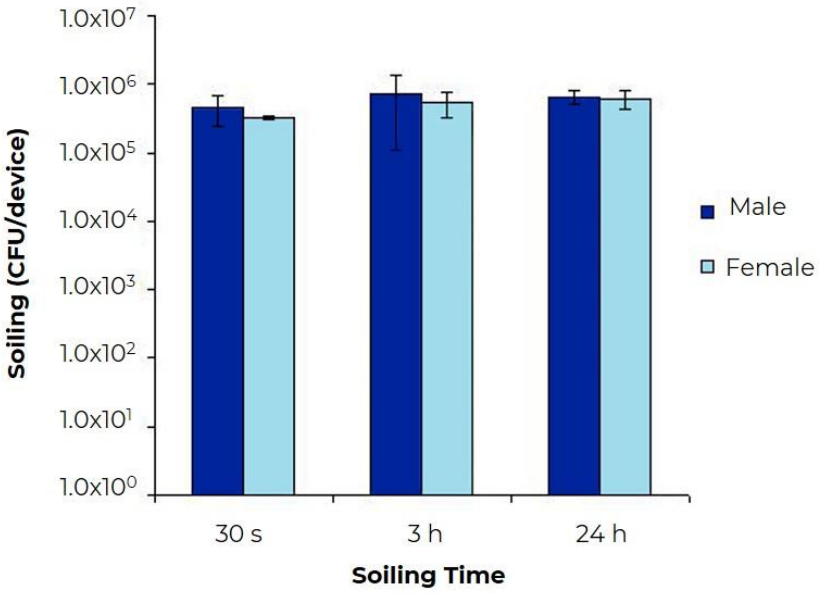
Table 1

The concentration of bacteria (*Serratia marcescens*) in the soiling solution at three time points, each value is an average of three measurements.

Time Point	Soiling Solution Titer (CFU/mL)
30 seconds	7.8×10^5
3 hours	6.4×10^5
24 hours	7.1×10^5

Figure 1

The total number of CFU recovered from the soiled devices after exposure to the soiling solution, each value represents the average CFU recovered from 3 devices. The error bars represent the standard deviation and the two halves of the sterile connector are differentiated as 'male' and 'female' halves.



5 Aerosol Soiling Testing

For the aerosol soiling test, a soiled atmosphere is generated using a nebulizer containing a liquid suspension of the soiling bacteria, aerosolized within an enclosed space. For our tests, a minimum bioaerosol concentration of 10^6 CFU/m^3 was consistently generated using at least 665 mbar (9.5 psi) air pressure to a nebulizer containing 10^9 CFU/mL of *Serratia marcescens* (Table 2). This concentration provides a rigorous test of the effectiveness of the sterile connector device to maintain a sterile fluid path in an uncontrolled atmosphere considering that this bioaerosol concentration exceeds that commonly found at an outdoor composting facility (Table 3).

Table 2

Serratia marcescens concentration in the nebulizer (CFU/mL) and the resulting bioaerosol concentration in the test chamber (CFU/m^3).

Nebulizer Pressure [mbar (psi)]	Concentration of *Serratia marcescens* in the Nebulizer (CFU/mL)	Concentration of *Serratia marcescens* in the Test Chamber (CFU/m^3)
655 (9.5)	3.5×10^9	1.9×10^6
655 (9.5)	4.7×10^9	3.4×10^6
655 (9.5)	5.6×10^9	6.8×10^6
655 (9.5)	3.8×10^9	2.9×10^6
690 (10)	4.6×10^9	2.9×10^8
690 (10)	5.7×10^9	1.4×10^8
690 (10)	5.5×10^9	2.3×10^8
690 (10)	5.5×10^9	1.3×10^8
690 (10)	3.6×10^9	7.5×10^8
690 (10)	2.8×10^8	4.2×10^6
690 (10)	3.4×10^9	3.8×10^7
690 (10)	1.2×10^9	4.5×10^7
690 (10)	2.5×10^9	8.0×10^6
690 (10)	6.2×10^9	1.2×10^8

Table 3

Some typical bioaerosol exposure levels (CFU/m^3).

Site	Bioaerosol Concentration (CFU/m^3)
Hospital clean room [1]	1-423
Hospital operating room [2]	1.67-157
Pet shop [3]	923-960
Pet clinic [3]	696-729
Flower garden [3]	938-2399
Private homes (indoors) [4]	2188-2512
Public bars (indoors) [4]	3891-4266
Outdoor composting facility [5]	10^3 to 10^4

6 Summary

In summary, for liquid immersion soiling, dipping the device in a soiling solution is an effective surface soiling method, and for aerosol soiling, a high bioaerosol concentration is appropriate. **Neither is worse nor better than the other since they each test completely different routes of microbial ingress.**

To determine which test is appropriate, it is necessary to consider how the device will be used and which route of bacterial soiling exposure is more likely to occur. For example, is the device likely to be activated while exposed to a high aerosol concentration or is it more likely to be soiled from handling? Given the intended use of the device for aseptic processing, it is not likely to be activated while exposed to high bioaerosol concentration, but it is much more likely to get soiled on the exterior surface when handled prior to connection, when transported, or otherwise handled prior to and during activation. Unless the device is likely to be connected and activated while exposed to a high bioaerosol concentration (as high as is found in a composting facility), heavy surface soiling is the test most reflective of actual use contamination risk.

7 References

[1] C.-S. Li and P.-A. Hou, "Bioaerosol Characteristics in Hospital Clean Rooms," *The Science of the Total Enviroment,* vol. 305, pp. 169-176, 2003.

[2] G. Ortíz, G. Yagüe, M. Segovia and V. Catalán, "A Study of Air Microbe Levels in Different Areas of a Hospital," *Current Microbiology,* vol. 59, no. 1, pp. 53-58, 2009.

[3] W.-K. Jo and J.-H. Kang, "Workplace Exposure to Bioaerosols in Pet Shops, Pet Clinics, and Flower Gardens," *Chemosphere,* vol. 65, no. 10, pp. 1755-1761, 2006.

[4] W.-K. Jo and Y.-J. Seo, "Indoor and Outdoor Bioaerosol Levels at Recreation Facilities, Elementary Schools, and Homes," *Chemosphere,* vol. 61, no. 11, pp. 1570-1579, 2005.

[5] M. Folmsbee and K. A. Strevett, "Bioaerosol Concentration at an Outdoor Composting Center," *Journal of Air and Waste Management Association,* vol. 49, no. 5, pp. 554-561, 2011.

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