Why Robust Microbial Ingress Testing Matters

A practical framework for evaluating connector sterile barrier performance

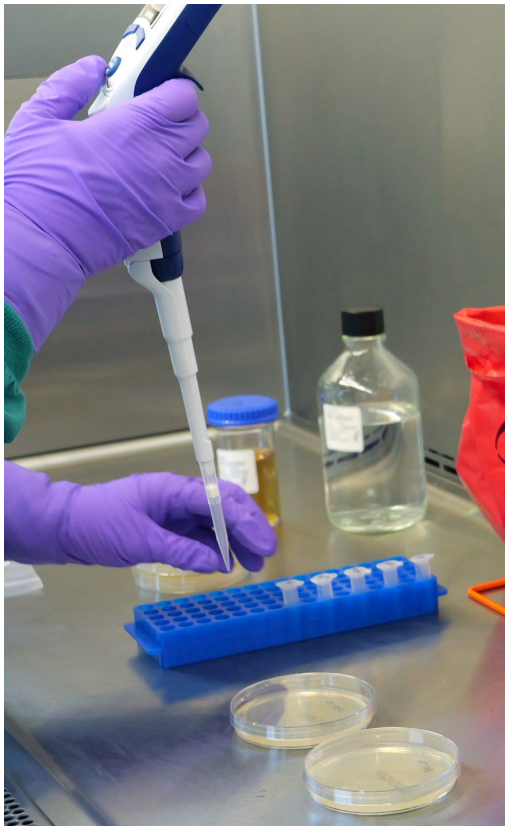
BY STACEY HAINEY, SENIOR MICROBIOLOGIST, AND KRISTINA PILOT, TEST LAB MANAGER

BACKGROUND

Bioprocessing and cell and gene therapy (CGT) manufacturing depend on maintaining an aseptic fluid pathway during processing manipulations. Single-use connector technologies used in these applications are often described as either open aseptic or intrinsically sterile. Open aseptic connections rely on controlled environmental conditions and aseptic technique to protect the fluid path during connection. Intrinsically sterile connectors are designed to maintain separation between the process fluid path and the external environment throughout connection and disconnection, subject to their validated conditions of use.

The ability of connectors such as AseptiQuik®, MicroCNX®, or RevolveSD® to maintain a validated sterile barrier is important for manufacturing flexibility and preservation of a closed process after the final sterilizing-grade filter. This is especially consequential where biological drug products are not compatible with terminal sterilization.

Manufacturers commonly support sterile barrier claims through a combination of design verification and validation activities. Microbial ingress testing provides evidence of whether a connector design can maintain isolation between a nonsterile environment and the flow path before a connection, during a connection, disconnection, and/or other use-relevant exposures.

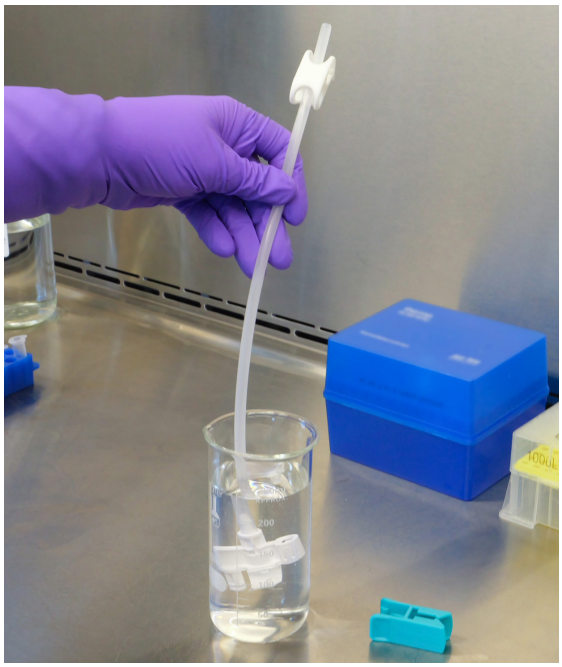


A key advantage of microbial ingress testing, relative to static physical methods such as pressure-hold or burst testing, is the ability to assess the connector under dynamic actuation conditions. Pressure-hold, burst, and other deterministic tests provide important complementary evidence of mechanical and leak integrity; however, they may not detect a transient condition during actuation that could permit microorganisms to move from the external environment into the fluid path. Even a brief loss of barrier performance during connection or disconnection may be critical.

When properly designed and executed, microbial ingress testing can identify potential vulnerabilities before a design is finalized. CPC Biotech incorporates early product testing, including microbial ingress testing, into its design process. Results from these studies inform subsequent design iterations. Before a design is locked, the product is evaluated through a risk-based battery of testing intended to ensure that it meets its defined performance requirements, including sterile-barrier performance under validated conditions of use.

WHY STUDY DESIGN MATTERS

A passing microbial ingress result is meaningful only when the study can detect a relevant failure. A recurring risk in component development is a retrospective “design-to-pass” approach: test conditions are selected after a product design is effectively locked, with parameters that favor a passing result rather than rigorously challenging credible ingress mechanisms. Late-stage failures can then create pressure to narrow the challenge, adjust test parameters, or otherwise weaken the method.



A more rigorous approach begins during early product development. Test methods should target credible worst-case ingress paths, demonstrate recovery of the challenge organism, and distinguish intact barriers from known or intentionally introduced vulnerabilities. Test results should then inform design decisions—not merely document a completed design.

Applying this perspective to microbial ingress studies means that they should be assessed not only by pass/fail outcomes, but also by whether their challenge, controls, recovery method, exposure conditions, and sample plans could detect a meaningful failure.

TYPICAL MICROBIAL INGRESS TESTS

In a typical ingress study, a device or container is sterilized, filled with sterile nutrient medium, and exposed externally to viable microorganisms by immersion, aerosolization, direct inoculation, or another justified method. For connectors, the exposure may challenge an assembled connector set or the sterile barrier of each individual connector before connection.

Following exposure and, where applicable, connection, samples are incubated under defined conditions and monitored for evidence of microbial growth. Incubation duration and conditions should be justified for the selected organism, medium, method, and study objective. If growth is detected within the sample, the result may indicate loss of sterile-barrier integrity; however, investigators should also evaluate control performance and potential procedural contamination before interpreting the outcome.

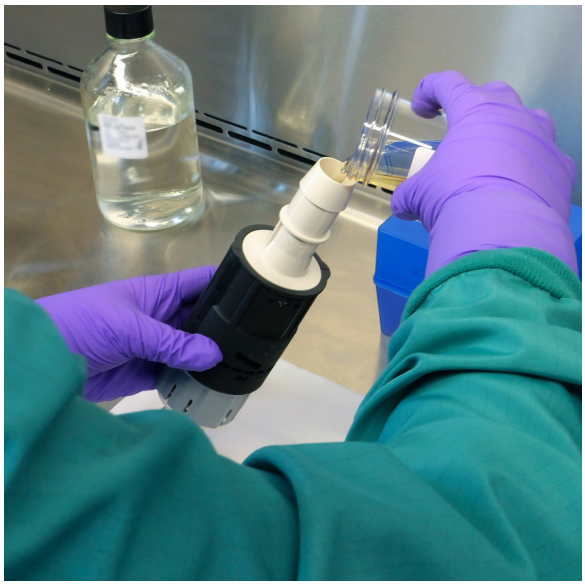


Dip test Submersion test

Static deterministic methods, including hydrostatic, bubble-leak, or helium-leak tests, can provide important complementary information.

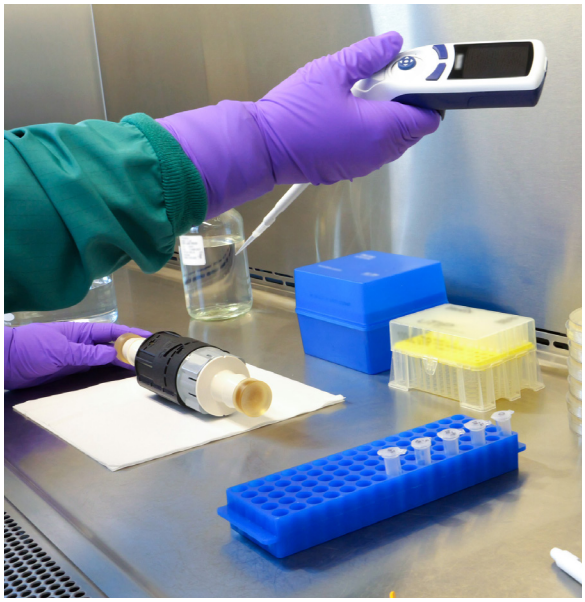
They may not, however, replicate the dynamic handling, mating interfaces, sealing behavior, or biologically relevant contamination conditions involved in connection and disconnection. Microbial ingress testing is therefore one component of a broader, risk-based verification strategy.

A RIGOROUS, RISK-BASED CHALLENGE FRAMEWORK



CPC Biotech has developed and uses a microbial ingress testing framework informed by ASTM International's E3251 *Standard Test Method for Microbial Ingress Testing on Single-Use Systems*. It is tailored to the connector design, intended use, claimed sterile-barrier function, and credible ingress vectors. Rather than applying a single generic test to every product, the test panel is selected to assess the specific risk presented by the design.

For some configurations, direct inoculation is used to target more typically vulnerable areas such as material weld seams, dynamic seals, mating interfaces, subsurface sealing O-rings, membranes, and other potential leakage paths. Immersion or aerosol challenges may also be appropriate when they reflect the intended use or the suspected risk mechanism. The method of challenge should be justified by the question the study is intended to answer; no one exposure method is universally appropriate.



During early prototyping, CPC Biotech applies microbial ingress testing as an iterative product-development tool rather than solely as a final verification activity. This is an important distinction relative to how some other suppliers test their products. CPC Biotech wants to ensure that key factors are tested and refined to optimize performance, not just to address pass/fail requirements.

Tailored challenge methods assess barrier-critical features and credible ingress paths, including weld seams, dynamic seals, mating interfaces, subsurface O-rings, and membranes. Using discriminating tests capable of revealing performance differences allows engineers to evaluate design modifications, sealing geometries, material interfaces, or molding parameters. Results can inform subsequent design iterations before the design is finalized and can also support later process-qualification activities when manufacturing variables may affect a barrier-critical feature.

Challenge organisms should be selected using a documented, risk-based rationale. Their size, motility, resistance characteristics, expected recovery, and relevance to the use environment should be considered. *Brevundimonas diminuta* is often used to challenge small physical boundary tolerances because of its small dimensions and motility; however, its suitability for a particular ingress study should be justified rather than assumed.

Where technology relies on manual surface sanitization, additional organisms may be relevant to the real-use risk assessment. For example, *Bacillus subtilis* endospores may help assess vulnerability to alcohol-resistant environmental organisms. Spores, fungi, viruses, or viral surrogates may also warrant consideration where they reflect credible contamination hazards or limitations of the overall contamination-control strategy.

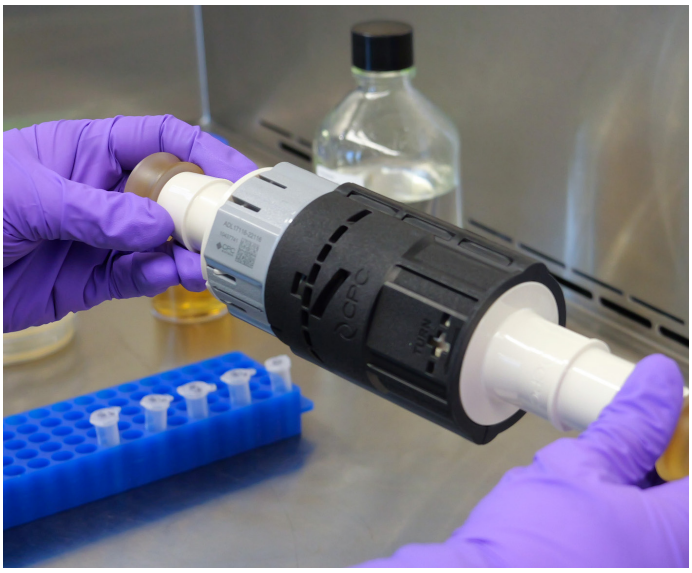
The presence of an isopropyl alcohol disinfection step does not by itself determine whether a connector is intrinsically sterile. It does, however, require a clear understanding of whether disinfection is part of the claimed contamination-control strategy and whether relevant organisms could remain after that step.

CHALLENGE VERIFICATION AND RECOVERY

Microbial concentration should be verified in the final challenging form at the point of exposure, not only before delivery through a nebulizer, spray device, or other processing step. In applicable CPC Biotech protocols, viable challenge is verified at not less than 1×10^6 CFU/mL in the final challenge preparation, accounting for potential loss of viability associated with processing, aerosolization, desiccation, or handling. The target concentration and its rationale should be defined in the study protocol.

Recovery must also be evaluated. A challenge method that damages or desiccates cells may reduce recoverability, creating a false appearance of barrier performance. Independent kinetic or extraction-efficiency or "growth promotion" studies can help demonstrate that stressed organisms can be recovered from relevant component surfaces or interfaces within the time frame used by the test. These can also include "positive control" studies where representative samples are tested with deliberately introduced defects.

WHAT TO LOOK FOR WHEN REVIEWING MICROBIAL INGRESS RESULTS



Biopharmaceutical manufacturers should assess not only whether a study passed, but whether it was designed and controlled well enough to support the intended claim.

- **Risk-based test configuration:** The protocol should address the connector design, intended use, credible leak paths, handling conditions, and relevant environmental hazards.
- **Appropriate challenge organism(s):** Organism selection should be justified by size, motility, resistance characteristics, and use-case relevance.
- **Verified challenge at exposure:** The viable concentration actually contacting the test article should be measured and reported, particularly for aerosolized or sprayed inocula.
- **Meaningful controls:** Negative media controls, positive or intentionally compromised controls, viability or recovery controls, and growth-promotion controls should demonstrate that the method can both prevent false positives and detect true failures.
- **Representative exposure conditions:** The study should challenge the sterile-barrier interface and potential leakage paths rather than external surfaces only.
- **Defined sample size and acceptance criteria:** The protocol should specify sample quantity, a statistical or risk-based rationale, criteria for invalid runs, and the approach to repeat testing.
- **Use-case connection:** Where cleaning or disinfection is required, the study should consider whether the selected microorganisms and exposure conditions remain relevant after those controls are applied.

Potential sources of bias include a test configuration selected primarily to favor the product, insufficient or poorly characterized challenge delivery, reliance on pre-delivery inoculum counts, absence of recovery verification, inadequate controls, nonrepresentative exposure sites, and acceptance criteria established after results are known. These limitations can reduce confidence in an otherwise passing test.

A COMPREHENSIVE APPROACH TO PRODUCT ASSESSMENT

Microbial ingress testing is only one component of the package that assures products serve their purpose in isolating a sterile flow path from the environment, which is itself only one part of a comprehensive product-assessment strategy. Other in process tests, validation studies, and controls supplement the ingress studies to ensure that each component released by CPC Biotech is suitable to serve its intended purpose. No standalone test establishes product suitability. Findings should be interpreted together with other functional, mechanical, material-compatibility, and biocompatibility evidence.



Depending on the product and intended application, CPC Biotech may evaluate leak integrity, burst resistance, cleanliness, flow performance, tensile and side-load performance, freeze/thaw resistance, time-temperature-pressure exposure, biocompatibility, chemical resistance, and extractables. Together, these evaluations help identify and address potential weaknesses before product release and customer use.

CONCLUSION

Microbial ingress testing should do more than document a passing result. When incorporated early in product development, a robust, risk-based challenge can reveal barrier-critical weaknesses while design changes are still possible, supporting better engineering decisions and stronger evidence of sterile-barrier performance.

The most valuable studies are those designed to detect credible failures under justified, worst-case conditions. They should use targeted challenge methods, verified viable challenge at the point of exposure, demonstrated organism recovery, meaningful controls, and predefined acceptance criteria.

CPC Biotech is helping advance industry expectations for microbial ingress testing by applying this framework as an iterative development and verification tool across its single-use technology portfolio. By combining microbial ingress testing with complementary mechanical, functional, material, and quality assessments, CPC Biotech's rigorous standard for evaluating aseptic connection and disconnection technologies helps biopharmaceutical manufacturers build confidence in the integrity of their fluid paths under validated conditions of use.

To learn more about CPC Biotech, visit cpc-bio.com.

Disclaimer: Aseptic connector integrity is a system-level outcome. CPC Biotech microbial-ingress evidence supports CPC Biotech-controlled connector features within the applicable tested scope; it must not be extrapolated to qualify the customer-assembled tubing, equipment interfaces, installation, handling, environmental conditions, or process-use conditions beyond CPC Biotech's control.

ABOUT THE AUTHORS

Stacey Hainey is the Senior Microbiologist & Kristina Pilot is the Test Lab Manager for CPC Biotech. CPC Biotech is at the forefront of bioprocessing fluid management and flow control, offering a comprehensive portfolio of critical components: connectors, pumps, flow meters and sensors. Our industry leading products permit biopharma innovators to produce next generation biologics and therapeutics with optimal purity, yield, efficiency and scalability. From lab to launch, we enable those who drive breakthroughs in medicines and biologics that transform lives and advance humanity. CPC Biotech is part of Pump Solutions Group (PSG), an operating company within Dover Corporation. This document is educational in nature and does not replace product-specific validation, risk assessment, or applicable regulatory requirements.

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