Designation: E3251 – 23

Standard Test Method for Microbial Ingress Testing on Single-Use Systems[1]

This standard is issued under the fixed designation E3251; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 The microbial test method outlined in this test method applies to microbial ingress risk assessment of a single-use system (SUS) or its individual components that require integrity testing either by the assembly supplier or the end user of the assembly based on a potential risk of a breach to the product or manufacturing process.

1.2 The aim of microbial ingress testing of sterile SUSs used in biopharmaceutical manufacturing is two-fold:

1.2.1 Firstly, it is used to evaluate the ability of a SUS fluid path to remain sterile after a SUS has been challenged by microbial exposure. Microbial exposure is achieved either by directly placing a SUS into a container of microbial challenge solution, or by delivering an aerosolized microbial challenge onto a SUS that is placed inside a test chamber designed to generate and deliver the aerosol. The choice of the test challenge organism should be justified based on a risk assessment of the SUS and conditions of use.

1.2.2 Additionally, microbial ingress testing can be used to determine the maximum allowable leakage limit (MALL) that does not allow microbial ingress under specific test conditions. The defect size that can be detected by specific physical integrity testing methods (see Test Method E3336) can be correlated to this MALL in order to claim microbial integrity. Test articles bearing calibrated defects over a range of dimensions, including up to a defect size expected to consistently allow microbial ingress as a positive control (defect-based positive control), may be tested to determine the MALL.

1.3 Both purposes for microbial ingress testing as described in 1.2.1 and 1.2.2 can either be conducted by liquid immersion or aerosol exposure. For the purpose described in 1.2.2, the type of exposure should be determined according to the SUS's use-case conditions and a risk assessment.

1.4 The method used to create a breach, hole or defect in single-use film or in a SUS test article, as well as the analytical method used to physically characterize the defect size is outside of the scope of this test method. The sampling plan for a given test article should be justified with the rationale of sampling size to obtain a statistically meaningful effect (Practice E3244). Determining the appropriate number of SUS test articles will depend on a risk assessment of the SUS and the conditions of its use and is also outside of this test method's scope.

1.5 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E3244 Practice for Integrity Assurance and Testing of Single-Use Systems

E3336 Test Method for Physical Integrity Testing of Single-Use Systems

2.2 *Other Documents:*

USP <1207> Sterile Product Packaging — Integrity Evaluation, 2016[3]

ISO 15747 Plastic Containers for Intravenous Injections[4]

3. Terminology

3.1 *Definitions:*

[1] This test method is under the jurisdiction of ASTM Committee E55 on Manufacture of Pharmaceutical and Biopharmaceutical Products and is the direct responsibility of Subcommittee E55.04 on General Biopharmaceutical Standards.

Current edition approved March 15, 2023. Published March 2023. Originally approved in 2020. Previous edition approved in 2020 as E3251 – 20. DOI: 10.1520/E3251-23.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from U.S. Pharmacopeial Convention (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

[4] Available from International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

3.1.1 *calibrated leak, n*—a hole which is characterized by its size (for example, artificially created into a SUS, a SUS's material, or component and used for creating positive controls).

3.1.1.1 *Discussion*—Often, the size is a nominal size which is equivalent to a gas flow through an idealized geometry. A commonly used idealized geometry is the "nominal diameter orifice size," corresponding to the size of a perfect circular hole of negligible length that would give the same gas flow in the calibration conditions (for example, dry air flow rate measured at 25 °C, with 1 bar_g inlet pressure and 1 atm outlet pressure).

3.1.2 *challenge solution, n*—a liquid suspension containing a selected microorganism used to generate an aerosol or used for liquid immersion.

3.1.3 *defect-based positive control, n*—a test article exposed to a challenge solution with a calibrated breach or defect. The size of the breach or defect will depend on a previous determination of the defect size that can be consistently detected under given conditions. This positive control is used as a control to ensure that the microorganism can pass through a defect and can be detected by the test method.

3.1.4 *exposed negative control, n*—a test article without defects exposed to a challenge solution. The purpose of the exposed negative control is to confirm the correct preparation and assembly of the test article.

3.1.5 *growth promotion test, n*—a test, using a negative control after the complete incubation time, by inoculating ≤100 CFU of the microbial challenge organism and incubating at the appropriate temperature until either visible growth is seen, or a maximum of 7 days is reached. The purpose of the growth promotion test is to demonstrate that the selected solution can support microbial growth.

3.1.6 *integrity test, n*—a test used to confirm the defined barrier properties of a SUS.

3.1.7 *leak, n*—a breach in a SUS's material or a gap between SUS's components through which there is a break-down of the barrier property of interest.

3.1.8 *leak test, n*—a test used to identify leaks not correlated to the defined barrier properties of a SUS.

3.1.9 *maximum allowable leakage limit (MALL), n*—the greatest leakage rate (or leak size) tolerable for a given product package to maintain its barrier properties under its use-case conditions (for example, prevent any risk to product safety, product quality, or operator and environmental safety).

3.1.9.1 *Discussion*—In this test method's context, the product package is a SUS containing a (bio)pharmaceutical product, but not a final dosage form.

3.1.10 *non-exposed negative control, n*—a test article that is not exposed to a challenge solution. The purpose of the non-exposed negative control is to validate the test system's sterility. This could be accomplished by filling a SUS control with growth medium and incubating for several days to ensure that the SUS test article was not contaminated upon filling.

3.1.11 *single-use system (SUS), n*—process equipment used in (bio)pharmaceutical manufacturing, disposed of after use and usually constructed of polymer-based materials.

3.1.12 *viability-based positive control, n*—a test article directly inoculated with a test organism. The purpose of this positive control is to validate the viability of the test organism under the test conditions, throughout the test.

3.2 *Acronyms:*

3.2.1 *CFU*—colony forming unit.

3.2.2 *IQ*—installation qualification.

3.2.3 *IT*—integrity test.

3.2.4 *MALL*—maximum allowable leakage limit.

3.2.5 *OQ*—operational qualification.

3.2.6 *PQ*—performance qualification.

3.2.7 *SUS*—single-use system.

3.2.8 *TSA*—tryptic soy agar.

3.2.9 *TSB*—tryptic soy broth.

4. Significance and Use

4.1 Single-use systems (SUSs) used for biopharmaceutical manufacturing must maintain sterility and product quality of the fluid inside. Such articles or systems should therefore be validated as providing an effective barrier against microbial ingress. The microbial barrier properties of a SUS may be demonstrated using deterministic physical tests that have been correlated to microbial integrity. Such physical test methods are described in Test Method E3336. Two microbial test methods (aerosol exposure and immersion exposure) are described in this test method that can be used to demonstrate microbial integrity of a SUS or determine the MALL, the maximum defect size that does not allow microbial ingress, into a SUS.

4.2 It is important to note that the results of microbial ingress tests are heavily dependent on the conditions under which the test is performed and are not suitable for routine checking of a SUS due to the test's destructive nature.

4.2.1 Any size defect may be forced to fail under sufficiently aggressive conditions (including a large enough sample size, high differential pressure, or high hydrostatic pressure, for example) that would not ordinarily reflect normal use conditions. Thus, it is necessary to clearly define the relevant conditions for a test through a risk assessment of both the actual SUS claims and its final use (Practice E3244). Once that is established, the size of defect that can be detected under those conditions can be determined, if required, using defined defects.

4.2.2 "Relevant conditions" refers to worse-case actual use conditions but does not mean that a SUS must be tested under theoretically absolute (extreme) "worst-case" conditions.

4.2.3 Testing may be performed on individual components or entire systems. Considerations for defining "relevant conditions" and testing design should be based on a risk assessment for the SUS intended use and should include: