Designation: E3244 – 23

Standard Practice for Integrity Assurance and Testing of Single-Use Systems[1]

This standard is issued under the fixed designation E3244; 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 This practice uses quality risk management (QRM) and life-cycle approach to establish integrity assurance of single-use systems (SUSs), such as but not limited to bag assemblies and liquid transfer sets for processing, storage, and shipping of (bio)pharmaceutical products. It gives recommendations to identify failure modes and risks associated with such systems and their use-cases and how to identify the relevant leak(s) of concern. Integrity assurance in this context is limited to the barrier properties of the SUS, linked to microbial integrity and bioburden control (product quality) and liquid product loss (operator and environmental contamination). The required level of integrity assurance will depend on how critical the application is and can be interpreted in different ways. It can also vary between processes and applications used for different modalities (for example, advanced therapies). Other package barrier properties different from that, such as but not limited to gas barrier properties for gas headspace preservation, as well as porous barrier packages are not considered. Specific aspects how to address the contamination control strategy (CCS) for SUS are also described in chapters 8.131ff of the new Revision of Annex 1 (**1**),[2] including chapter 8.137 regarding SUS integrity.

1.2 The test method overview provides descriptions that focus on the standard test setup and the identification of challenges in combination with SUSs. Details, including specific test setups, test parameter, and result interpretation, are not discussed. For more detailed information refer to Test Method E3251 for microbial test methods, and to Test Method E3336 for physical test methods.

1.3 This practice is not intended to apply to the use of single-use technology for primary containers, combination products (products composed of any combination of a drug, device, or biological product), or devices. Appropriate procedures related to these products are discussed in documents covering the integrity assurance for primary containers (**2**) or medical products (**1, 3**).

1.4 Techniques and procedures for complaint management and root cause analysis related to integrity failures are also not discussed.

1.5 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:*[3]

E3051 Guide for Specification, Design, Verification, and Application of Single-Use Systems in Pharmaceutical and Biopharmaceutical Manufacturing

E3251 Test Method for Microbial Ingress Testing on Single-Use Systems

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

2.2 *ICH Documents:*[4]

ICH Q9(R1) Quality Risk Management

3. Terminology

3.1 *Definitions:*

[1] This practice is under the jurisdiction of ASTM Committee E55 on Manufacture of Pharmaceutical and Biopharmaceutical Products and is the direct responsibility of Subcommittee E55.07 on Single Use Systems.

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[2] The boldface numbers in parentheses refer to a list of references at the end of this standard.

[3] 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.

[4] Available from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), ICH Secretariat, 9, chemin des Mines, P.O. Box 195, 1211 Geneva 20, Switzerland, http://www.ich.org.

3.1.1 *bioprocess container (biocontainer), n*—a container (bag, bottle, tank, etc.) used primarily for liquid (or frozen liquid) storage during various stages of biopharmaceutical manufacturing processing.

3.1.2 *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).

3.1.2.1 *Discussion*—Often, the size is a nominal size which is equivalent to a gas flow through an idealized geometry (**2**). 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 barg inlet pressure and 1 atm outlet pressure).

3.1.3 *destructive test method, n*—a test method that will alter the intended use of the tested SUS during the test and not allow further use (see also *non-destructive test method*).

3.1.4 *end user, n*—a company processing (bio)pharmaceutical products.

3.1.5 *integrity assurance, n*—a holistic approach of risk analysis and mitigation by means of product and process robustness, quality, and process control and integrity testing to assure that a SUS maintains its integrity prior to and during use.

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 document's context, the product package is a SUS containing a (bio)pharmaceutical product, but not a final dosage form.

3.1.10 *non-destructive test method, n*—a test method that maintains the test article in a condition for further use, without impacting its quality attributes (see also *destructive test method*).

3.1.11 *single-use components, n*—parts used in single-use systems, most commonly, but not limited to, bioprocess containers, tubing, connectors, clamps, valves, sensors, and filters.

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

3.1.13 *SUS supplier, n*—a manufacturer that produces and/or assembles single-use systems, also known as a system integrator.

3.1.14 *tracer gas, n*—a gas to be detected against the background of all other gases.

3.2 *Abbreviations:*

3.2.1 *BPOG*—Biophorum

3.2.2 *BPSA*—Bio Process Systems Alliance

3.2.3 *cGMP*—current Good Manufacturing Practice

3.2.4 *ICH*—International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use

3.2.5 *LoD*—limit of detection

3.2.6 *MALL*—maximum allowable leakage limit

3.2.7 *QbD*—quality by design

3.2.8 *QRM*—quality risk management

3.2.9 *SUS*—single-use system

3.2.10 *SUSI(T)*—single-use system integrity (testing)

3.2.11 *SUT*—single-use technologies

4. Significance and Use

4.1 This practice provides:

4.1.1 A holistic approach to evaluate risks associated with an integrity breach in a SUS, considering its life cycle from development to disposal.

4.1.2 An overview of physical and microbial test methods that could be applicable to SUS testing, for qualification and validation purposes, as well as for routine testing.

4.1.3 Information on the main challenges faced when testing SUSs for integrity.

4.2 This practice can be used by SUS suppliers and SUS end users to define an integrity assurance strategy for SUSs, with the relevant tests when appropriate.

5. Procedure

5.1 *Quality Risk Management (QRM) and Life-Cycle Approach:*

5.1.1 *Introduction of Quality Risk Management (QRM):*

5.1.1.1 QRM, as defined in ICH Q9, is a methodology to assess potential risk to product quality within a process. Potential risks are managed based on their occurrence and severity in the process/product and are reviewed throughout the life cycle of the process/product. When discussing a SUS, its integrity can be a critical attribute for maintaining product quality or protecting the operator or environment from exposure, or both. There must be necessary controls, monitoring, and testing in place to ensure that the integrity of the SUS is maintained throughout its life cycle. To accomplish this, the SUS supplier and end user can adopt a life-cycle approach, where the integrity assurance of the SUS is considered from the design and production process at the SUS supplier to its final application in the end user's manufacturing process. Within the life cycle, the risks to SUS integrity (SUSI) can be proactively identified and the necessary controls and testing put in place. These risks can be different for both the SUS supplier and end user, which can necessitate differences in the test methods, testing frequency and sensitivity utilized for ensuring SUSI.

5.1.1.2 The general approach of identifying and mitigating risks is the same regardless of the modality and the manufacturing process for which the SUS is used, but risk rating and

consequential mitigation actions can vary. As an example, a single-use bioreactor might be considered as a low risk in a classical mAb manufacturing process, while it could be highly critical for manufacturing cell or gene therapy products. It is important that the process and the associated risks are known and properly identified to implement an effective risk mitigation strategy.

5.1.1.3 The end-user's risk assessment should include the relevant aspects of the SUS life cycle related to integrity, the impact of a potential integrity failure and whether this could be acceptable or not. This is generally done by a risk rating combining severity (S), occurrence (O) and current mitigation control. One potential mitigation action can be to implement an in-process control (IPC), for example, a leak/integrity test or visual inspection, in the SUS supplier's manufacturing process and/or in the end-user's process. Such an implementation should be evaluated in detail, balancing the additional risks versus the benefits brought by this control, as well as the actual sensitivity of the control. As illustration, some elements that should be included in the risk assessment are listed below (non-exhaustive list):

(1) process step classification (low bioburden or sterile).

(2) process conditions.

(3) potential operator or environmental safety risk.

(4) risk of damages due to shipping and handling steps.

(5) market supply risks (risk of drug shortages).

5.1.2 *Life-Cycle Approach for Single-Use Systems (SUSs):*

5.1.2.1 When adopting a life-cycle approach for any SUS, both the supplier and end user will ensure it meets the necessary requirements for the end product. Fig. 1 illustrates the manufacturing and use of a typical SUS, showing the necessary steps that will be encountered at both the supplier and the end user's sites.

5.1.2.2 The supplier will identify the critical requirements for the SUS at the start of development. The supplier will then qualify a manufacturing process to meet those critical requirements of the design, identifying steps critical to the quality of the SUS according to its design and intended use. Based on these critical requirements, testing and controls of the components and the SUS will be conducted. Likewise, testing or controls, or both, will be performed on critical process steps that could impact the quality of the SUS.

5.1.2.3 User requirements will be identified during the end user's process development and shared with the supplier to determine if a SUS will adequately operate in the end user's application. These requirements will help determine critical parameters of a SUS during processing steps at the end user's site along with the end user's product requirements.

5.1.2.4 Both the supplier and end user will perform risk assessments during their respective process development to identify these critical parameters. Additionally, controls and testing will be put in place to ensure the critical quality attributes are met and quality is assured during routine manufacturing at both the supplier and end user's sites based on these risk assessments. Throughout the life cycle, the supplier and end user processes will be evaluated for any modifications to improve the quality of the SUS. The supplier and end user will need to be aware of changes in their process or SUS, or both, that have the potential to impact process parameters (**4**).

5.1.3 *Application to Integrity Assurance for a Single-Use System (SUS) within the Life Cycle:*

5.1.3.1 Integrity assurance is a critical attribute of a SUS. An end-to-end risk assessment of the entire SUS's life cycle is recommended to ensure implementation of risk management controls that are suitable for its intended use. While end users are ultimately accountable for SUS performance, they rely primarily on supplier controls to achieve the necessary level of integrity assurance. Therefore, alignment between the end user's requirements and the supplier's capabilities is critical.

5.1.3.2 The first step for an end user is to define the requirements for the SUS and communicate these to the supplier. In compiling the requirements, the end user should consider the application specific factors that may impact the tolerance for integrity risks (for example, proximity to final drug product, existence of downstream filtration steps, toxicity / exposure to the operator and environment), and key areas of the process that may impact integrity assurance (such as application details, operating conditions). When formal user requirements are necessary, utilizing the BPOG/BPSA single-use user requirements template (**3**), is recommended. This

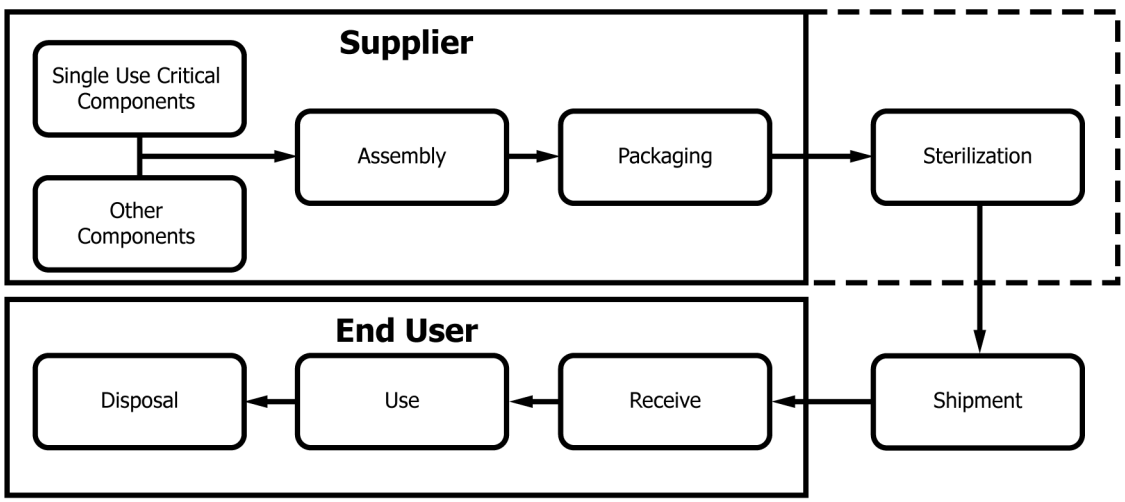

FIG. 1 SUS Life Cycle

includes a mechanism for suppliers to communicate their capabilities, enabling alignment with the end user application needs.

5.1.3.3 The end user should engage in quality audit and technical due diligence activities to evaluate how each potential supplier's controls contribute to the level of integrity assurance they can provide for the product. By understanding the basis of a supplier's qualified design space, an end user is better informed on what additional work may be required. For further discussion and recommendations around technical due diligence activities, see Guide E3051.

5.1.4 *Identifying End User Requirements That Can Impact Integrity:*

5.1.4.1 The end user will define the requirements critical to the integrity assurance of the SUS based on their processing conditions and product requirements. Additionally, the SUS supplier will determine the parameters that are critical to assure integrity of the SUS based on their processing conditions for SUS assembly and packaging/shipping, as well as the sterilization processes. The processing conditions at both the SUS supplier and end user identified as critical to integrity assurance will help to determine test requirements. Some examples of these processing conditions include the temperature, pressure, and flowrates that a SUS will experience during use at the end user's site. The SUS supplier's environment and handling conditions during assembly and packaging, as well as the temperatures and pressures the SUS will experience during shipping from the supplier to receipt at the end user along with the SUS sterilization process should also be accounted for during the risk assessment.

5.1.4.2 The constraints critical to integrity assurance during the drug manufacturing process must also be considered as part of the risk assessment when determining user requirements. These constraints will include the intended use in the end user's process, the presence of (sterile) filtration steps, and impact on chemistry/biological function, toxicity of the product to the operator or environment. All of these product constraints will be critical to determining the breach size that is acceptable for the SUS and will not impact product quality.

5.1.5 *Performing Technical Diligence:*

5.1.5.1 Suppliers may have different approaches to ensuring integrity assurance. The end user should assess a supplier's technical capabilities and controls. Depending on the composition of the SUS sourced from the supplier, the assessment may include how a supplier has qualified and implemented controls for a specific component or a combination of components (for example, the connection between tubing and hose barb, or seal between bag film layers). Understanding the scope and methods for qualification, in-process testing, and lot release testing and how these relate to integrity assurance informs the end user how to risk assess and align their application with the supplier's design space.

5.1.6 *Challenges for the Life-Cycle Approach:*

5.1.6.1 The life-cycle approach can present different challenges to supplier and the end user in reference to SUSI assurance and the test methods utilized at each stage of the life cycle. The magnitude of a significant integrity breach should be known for each stage of the life cycle where testing will occur. This can lead to differences in the testing approach during the life cycle. These differences are based on the purpose of the test (qualification versus on-going), criticality of the process step, user requirements, and nature of the test (destructive versus non-destructive).

5.1.7 *Developmental Versus On-Going Testing:*

5.1.7.1 Testing as part of the development/qualification of a process step at either the SUS supplier or end user can be performed with greater sensitivity than on-going testing. Likewise, the number of samples will have to be scientifically significant to support integrity assurance based on the potential variability present within a given process step and the SUS. The test method chosen should be able to quantify the integrity breach with a sensitivity aligned with the application needs. This is often referred to as the maximum allowable leakage limit (MALL). One of the main challenges for the supplier is often that this MALL is not fully defined given that the requirements are application driven. Because of this, additional testing of the SUS may be required by the end user prior to implementation.

5.1.8 *Stages of the Life Cycle:*

5.1.8.1 Testing performed as part of the development of the SUS and the manufacturing processes at the SUS supplier factory and the end user plant will be a factor in determining controls or testing required later in the life cycle of SUS. Understanding of components utilized within the SUS, as well as how they are connected together, is critical to determining the potential failure mode(s) that could lead to loss of integrity and the testing necessary for assurance of integrity of the SUSs. Likewise, the criticality of a step to the integrity of a SUS alongside knowledge on the type and level of an integrity breach that a supplier manufacturing step or end user operation could produce will help determine the necessary testing during on-going processing required at either the supplier or end user sites. The auditing, release, and change controls processes by the supplier and end user will also determine if testing is required as well as the specifics of the test that will be employed. Based on the auditing and release processes, the need and level of testing required could change throughout the life cycle, as alignment with expectations are demonstrated and critical parameters are met, altering the potential risks to the SUSI. Changes required within the inputs to (that is, raw materials or components) and the manufacturing process itself could require an added level of testing in order to support the change due to a lack of knowledge on the impact to integrity.

5.1.8.2 There will be a level of in-process controls and monitoring throughout the SUS's life cycle by the SUS supplier and end user to ensure its integrity. These in-process controls and monitoring will be based on critical parameters for maintenance of SUSI throughout its life cycle. The QRM process will determine at what stages within the SUS's life cycle in-process controls and monitoring are needed based on how critical it is to SUSI. By reviewing in-process controls and monitoring in place prior to and at a given stage in the life cycle, the SUS supplier or end user can then determine the acceptable level of leakage and method of integrity assurance testing that will be required. This can also help in determining the required testing frequency for assurance of SUSI.

5.2 *Challenges:*

5.2.1 The increasing uptake of SUSs in more critical current Good Manufacturing Practice (cGMP) processes and applications, especially the development of larger and complex, multi-component systems has made integrity assurance a critical attribute of the system (**5**). SUSI assurance is not easily solved as challenges exist for both groups, end users work to inform the application requirements and SUS suppliers act to meet these specifications. The challenges include practical aspects, test methodology with appropriate sensitivity, and result interpretation. Furthermore, economics of testing is a separate challenge, for example, the method cannot be cost-prohibitive to either the end user or supplier.

5.2.2 In terms of practical aspects, a consensus testing standard should ideally be applicable to all types of SUSs, regardless of components or design. Unfortunately, due to physical constraints (for example, pressure resistance, permeability) or characteristics to be tested (for example, filters versus containers), such ideal one-size-fits-all testing standard does not exist currently. More, requirements might be different depending on the application (for example, storage and shipping in non-controlled environment versus transfer made in a controlled environment like a cleanroom). For multi-component or large volume systems, or both, which can be more complex, guidance should be available allowing these systems to be divided into smaller units to accommodate the testing standard. Furthermore, the controls performed to verify SUSI are likely to differ, for practical reasons, between the design, validation or qualification, and commercial production phases. The requirements and how these are met should be phase appropriate and correlated to the application's risk level. An end user may require destructive testing of representative lot samples from the SUS supplier during design and validation or qualification phases, and potentially during manufacture of the SUS on a per sample basis. When 100 % integrity testing is required during production of the SUS, non-destructive testing must be applied. Additionally, end users may decide to perform leak/integrity testing at the point-of-use to mitigate risks associated with shipping, handling and installation during commercial production. Time, cost, and potential risks with handling the SUS during point-of-use leak/integrity testing must be balanced against the test's benefits. From a technical perspective, there may be masking effects due to contact of bag film with the supporting hardware of the SUS. Devices that prevent this masking effect should not alter the heat transfer during (bio)pharmaceutical manufacturing beyond what is acceptable to the process if these remain with the hardware.

5.2.3 Aside from practical aspects, there are numerous challenges associated with developing testing methodology for a consensus standard. The ideal consensus standard should cover the vast majority of process conditions. These process conditions can vary so much that defining conditions to cover most of them would likely lead to an over-challenge: as example, conditions to combine temperatures for frozen conditions at –80 °C up to hot conditions at +60 °C, mechanical stress from various side loading, from transfer with peristaltic pumps, diaphragm pumps, or air pressure, would be both difficult to implement but also lead to a very harsh, non-representative challenge for most of the process conditions taken separately. During design and validation or qualification phases, additional or specific tests may be performed in worst-case or failure mode conditions. These qualification tests are not in the scope of this practice.

5.2.4 A SUS is typically comprised of components which have different pressure ratings. Polymeric materials are flexible and prone to deformation under pressure, which can impact the test result (particularly upon repeat testing) and interpretation. Furthermore, pressure decay test results depend on environmental conditions; such as temperature and pressure; as discussed in later sections. Finally, the pre-treatment condition, for example, steam sterilization, gamma irradiation, or ethylene oxide, should be accounted for to ensure determination of integrity assurance is as representative as possible. In each instance, the test methodology challenges place considerable cost and time burden on the SUS supplier.

5.2.5 Finally, interpretation of test results presents challenges to both the SUS supplier and end user and must be agreed between both parties to prevent misinterpretations. SUS suppliers are generally coming with data demonstrating that their systems are passing successfully their integrity test, in their testing conditions (for example, at a defined pressure) and according their acceptance criteria. While this is valuable information, having results of tests-to-failure (for example, at what pressure the systems are failing) would be much more informative to the end users, and help them to better judge in what process conditions they can use the SUS.

5.2.6 Integrity testing is used to confirm the SUS's barrier properties; it verifies functional performance, taking into consideration the process environment and considerations (**5**). The required level of integrity assurance will depend on how critical the application is and can be interpreted in different ways, such as microbial ingress risk, operator safety, or liquid leaks.

5.2.7 Employing a quality-by-design (QbD) approach may eliminate testing in Qualification phase if different SUS designs are considered functionally equivalent under a bracketing approach, allowing to leverage previous Qualification phase results. This would require a strong dialog between the supplier and end user to get adequate understanding to justify appropriately such functional equivalent. In-depth dialog is also required when implementing point-of-use testing performed by the end user in a Commercial Production application. If planned, point-of-use testing should be incorporated in the user requirement specification (URS) with required sensitivity, in order for the SUS supplier to design the appropriate system and provide input on the test procedure. Alignment between SUS supplier and end user is crucial with point-of-use testing to ensure test results are correctly interpreted, avoiding false test failures which could lead to improper SUSs or batch discards for pre- or post-use testing, respectively.

6. Test Method Overview

6.1 The following sections are intended to give an overview about existing microbial and physical testing method to evaluate the integrity of various flexible SUS configurations. Standard test setups are shown and standard procedures briefly described. Test parameter sets and result interpretation are not discussed.

6.2 More detailed explanation for microbial test methods is provided in Test Method E3251, and for physical test methods in Test Method E3336. This includes:

6.2.1 Specific test method principles, procedures and apparatus adapted to test SUS.

6.2.2 Interference and their mitigation strategies.

6.2.3 Test method validation principles.

6.2.4 Calibration and conditions needs.

6.2.5 Calculation and interpretation of results.

MICROBIAL SINGLE-USE SYSTEM INTEGRITY TEST (SUSIT) METHODS

6.3 *Introduction:*

6.3.1 Ultimately QbD principles, leak tests, operator training, visual inspections and a thorough initial validation of the process and handling are the best steps in protecting SUSs from microbial contamination. However, in addition to the above steps, implementation of a microbial ingress test as part of a SUS's initial validation may be necessary. This test can either be done on negative test articles only to prove the microbial barrier property of the integral SUS, or on positive control test articles, intentionally compromised with calibrated defects to determine the MALL. Applying analytical validation principles from ICH Q2(R1), the detection limit of the microbial ingress test method must be determined to fulfill the requirements for test method validation. This is especially important, when using the determined MALL as a reject criterion for a non-destructive, deterministic integrity test to prove the inherent microbial integrity of an individual SUS.

6.3.2 A microbial challenge study by immersion exposure is a common microbial ingress test. Tests by aerosol exposure can also be performed. It is important to point out that, with these studies, results are dependent on the conditions under which the test is performed, and they are not suitable for routine checking of containers due to the test's destructive nature. They are also technically challenging for large systems and very labor-intensive to perform. Note that any breach larger than 0.2 µm may be forced to fail under sufficiently aggressive conditions (including sufficiently large sample size, high differential pressure, or high hydrostatic pressure, for example). Thus, one must clearly define relevant conditions for the test through a risk assessment of both the actual SUS claims and final use. "Relevant conditions" refers to the most severe use conditions but does not mean a SUS must be tested under theoretically absolute (extreme) "worst-case" conditions. Testing may be performed on individual components or entire systems. For example, a large SUS used to hold an in-process material, may be subjected to a microbial challenge test under conditions that simulate relevant pharmaceutical manufacturing conditions. However, when testing a small SUS where the primary container is intended for a sterile final drug product, more rigorous microbial test challenge conditions may be necessary to ensure system integrity during shipping, handling and use (**1**, **3**).

6.3.3 A general summary of a microbial challenge test is as follows:

6.3.3.1 *Step One*—Sterilize the SUS to be tested.

6.3.3.2 *Step Two*—Fill the SUS with sterile growth media, if possible to its limit.

6.3.3.3 *Step Three*—Submerge the SUS in a challenge solution for a specified time.

6.3.3.4 *Step Four*—Remove the SUS from the challenge solution and incubate at the appropriate temperature and length of time required for growth of the test organism.

6.3.4 Another important consideration is that microbial ingress tests are probabilistic. Even if a breach exists that is large enough for microbes to enter, it must be filled with liquid for the microbe to traverse the breach, and a viable microbe must be available to enter. Thus a breach might pass one microbial challenge test but fail under a different, and sometimes even the same, set of conditions. Consequently, a comparatively large sample size is often required for statistically relevant results.

6.3.5 Many challenges need to be overcome to develop a new testing method including the development of a consistent, reproducible, reliable test system, which provides a homogeneous suspension of the test organism. This test system should have a high concentration of viable microorganisms at the end of the test cycle, and should take into consideration purification and standardization of the test organism suspension, as well as the number of test samples required. Ultimately test conditions should be defined based on a risk assessment and validated to simulate the worst-case conditions based on expected use.

6.3.6 Given the probabilistic nature of the microbial challenge test and the dependency of pass/fail on the test conditions, test sensitivity depends on the test used. This means that sensitivity can only be determined relative to test conditions. For this reason, the test should be performed on SUS with various size breaches to determine the breach size that will be detected under specific conditions. This will define the breach size that is relevant to the application of the SUS.

6.4 *Bacterial Challenge Test by Immersion Exposure:*

6.4.1 A liquid bacterial immersion test is conducted by submerging a SUS filled with sterile growth media in a bacterial challenge suspension for a specified time. At the end of the test, the SUS is incubated under appropriate conditions for the chosen test bacteria and inspected for bacterial growth. Positive controls for the study should include a growth support control for the media, which should show viability for the test's duration. A SUS or a media sample not exposed to the bacterial solution can be used as negative controls.

6.4.2 When determining a correlation between leak size and bacterial ingress, a system suitability positive control involving the use of SUSs with leaks at sizes close to the desired sensitivity of the test should also be included in the study to determine the breach size that can be detected under the given conditions.

6.4.3 The design of a bacterial immersion test includes choice of the challenge organism, concentration of the bacterial

suspension, immersion duration, and differential pressure, if applicable, and its duration. These parameters impact the sensitivity of the bacterial immersion test. The temperature of the test must be appropriate to survival and optimal growth of the test organism.

6.4.4 Large biocontainer assemblies need not be filled to capacity but do need to be filled in a way that will wet all walls and ports when submerged. Tubing and rigid fittings should be filled. If the test organism is aerobic, sufficient oxygen containing headspace is necessary but can be added after exposure to the challenge fluid.

6.5 *Bacterial Challenge Test by Aerosol Exposure:*

6.5.1 An aerosol bacterial challenge test is conducted by exposing the SUS filled with sterile growth media in a bacterial aerosol suspension for a specified time. The SUS must be a closed system and all open ports must be closed or vented. As in the challenge test by immersion exposure, at the end of the test, the SUS is incubated under appropriate conditions and inspected for bacterial growth.

6.5.2 The positive controls for the study must include a growth support control for the media and titer of the aerosol to document the viability of the organism for the duration of the test. SUSs not exposed to the bacterial aerosol are negative controls.

6.5.3 A system suitability positive control involving the use of a SUS with leaks at sizes close to the sensitivity of the test may be included in the study when necessary to determine the size of leak which may lead to bacterial ingress.

6.5.4 The critical parameters for implementing an aerosol bacterial challenge test method are the desiccation resistance of the microorganisms, challenge exposure time, differential pressure (if applied), and titer of the test organism at the end of exposure to the exposed sample surface or titer of the organism in the aerosol.

6.6 *Manufacturing/Quality Control (QC) Testing:*

6.6.1 Quality control (QC) testing should be performed to demonstrate the consistent quality of a SUS and that it meets both its claims and criteria for its intended use. This could be generally standardized across companies so long as sufficient flexibility is built in, to support testing of widely different sized test systems. The criteria that could be standardized include the test organism, test fluid (matched to the test organism), test exposure concentration, and test exposure time.

6.7 *Process Specific Testing:*

6.7.1 Process specific microbial ingress tests can be performed to aid in risk assessment and validation of a specific process utilizing a SUS. In these cases, the microbial ingress test should model or mimic the actual use process as closely as possible. With that in mind, an aerosol microbial ingress test would be a more realistic choice over a liquid immersion test as this mimics actual use conditions more closely and an aerosol challenge level is several orders of magnitude higher (typically greater than 10^3 CFU/L of air) than would typically be seen in a pharmaceutical manufacturing space (typically less than 10 CFU/L).