BioProcess International
SPECIAL REPORT

SARTORIUS

Ensuring Single-Use Systems Integrity in Aseptic or Closed-Process Applications

Ensuring Single-Use Systems Integrity in Aseptic or Closed-Process Applications

Marc Hogreve, Inga Reinert, and Nathalie Pathier

Single-use systems (SUS) have brought numerous benefits to biopharmaceutical processes. Originally used in applications for preparation and storage of buffers and media, SUS are being implemented increasingly in commercial production of biopharmaceuticals. Today, biopharmaceutical manufacturers apply SU technology in critical drug-substance and drug-product process steps such as formulation, bulk storage, bulk transport, and final filling. By using such technologies, manufacturers can reduce costs while increasing flexibility and options for closed processing. Especially through the COVID-19 pandemic, we learned that single-use solutions can enhance speed and flexibility. Nevertheless, despite all their advantages, SUS still present risks, such as potential leakages during use.

In biopharmaceutical processes, leakage is reported as one of the top three constraints for further implementation of single-use technologies (**1**). It has been responsible for several million dollars of product losses per year at some biopharmaceutical manufacturers. Leaks can create production and planning disruptions, product shortages, and risk of exposure for operators if a product is hazardous (e.g., in work with viruses or antibody–drug conjugates, ADCs). When SUS are used in biopharmaceutical processes, implementing consistent robustness and a risk-based integrity testing strategy will enhance process efficiency, improve product quality, and help ensure product availability.

Therefore, SUS integrity (SUSI) is a critical quality attribute (CQA) for both end users and suppliers of SUS. Whereas with stainless-steel systems end users hold the main responsibility and control over system design, construction, and integrity, in SUS that responsibility is shared by end users and suppliers, requiring close collaboration and strong alignment of all parties.

Evolution of Guidance on Assurance of SUSI

SUSI was quickly identified early on as a key topic by several industry associations. The Bio-Process Systems Alliance (BPSA) reacted to this concern by initiating a work group to define best practices on the assurance of SUS integrity. That task force, together with SUS end users and suppliers, issued a white paper titled *Design, Control, and Monitoring of Single-Use Systems for Integrity Assurance* in 2017 (**2**).

To assure SUSI, the white paper recommends conducting a risk assessment that considers each step of the SUS lifecycle, from components used in manufacturing to SUS implementation by an end user. A mandatory step is to ensure the appropriate and safe implementation of SU manufacturing processes. The white paper was especially important because the industry had no previous guidance; unlike processes for sterilizing-grade filter-integrity

Figure 1: Typical biotechnology process and main critical applications for SUS integrity

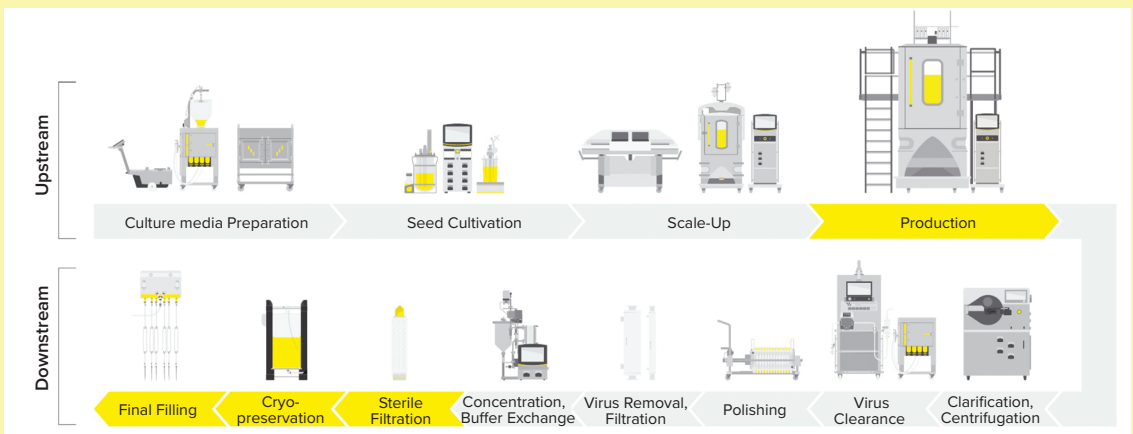
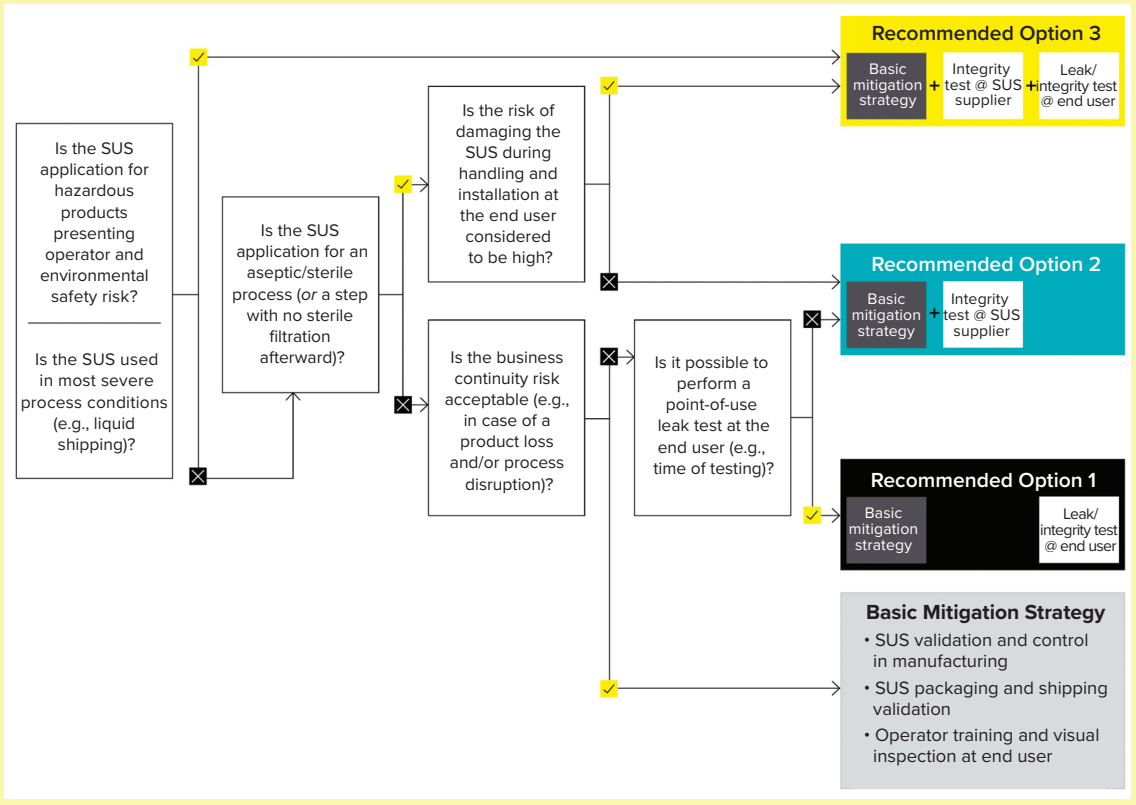


Figure 2: Decision tree for risk assessment



testing, no recommendation for SUS integrity assurance had yet been developed.

The groups working on that white paper stated that a "scientific understanding of the critical defect size for a risk of liquid leakages and/or microbial contamination is a prerequisite for integrity control strategies to correlate maximum allowable leakage limits (MALL) with the detection limits of physical testing that may be applied to ensure the microbial integrity" (**3**).

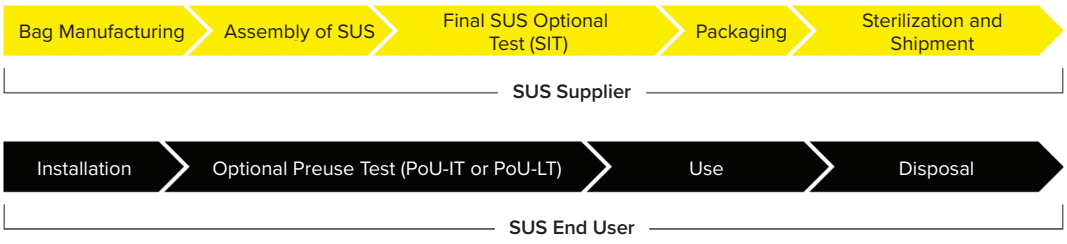
In USP ‹1207› *Package Integrity Evaluation — Sterile Products*, the MALL is related to drug-product safety and quality (sterility) of sterile drugs in their final packaging (**4**). Different package configurations with different barrier properties are defined. However, all such configurations have the common aim to "preserve sterility" because all contain final drug product. The MALL for a sterile pharmaceutical dosage-form package will ensure the content's sterility, preserve product contents, and prevent entry by detrimental gases or other substances, thus ensuring that the product meets relevant physicochemical and microbiological specifications through use and expiry. For multiple-dose product packages, the in-use MALL is defined as the degree of protection demanded by a closure to limit microbial ingress and product formulation leakage between and during dosage access (**4**).

American Society for Testing and Materials (ASTM) E3244, issued in 2020, transfers the integral/holistic concept from USP ‹1207› to SUS and describes a *Standard Practice for Integrity Assurance and Testing of Single-Use Systems* (**5**). In this document, the definition of MALL for SUS is adapted from that given in USP ‹1207› as "the greatest leakage rate (or leak size) tolerable for a given product package (SUS) to maintain its barrier properties under its use-case conditions" (e.g., to prevent risk to product quality or avoid exposure of an operator or a manufacturing environment to processed fluid).

ASTM E3251 (**6**) describes microbial test methods for SUS in detail, and ASTM E3336 (**7**) describes methods for physical integrity testing of SUS. *PDA Technical Report 86* from 2021 describes industry challenges and current technologies for the pharmaceutical-package integrity testing of sterile drugs and biologics packaging systems (**8**).

In addition to these main recommendations and ASTM standards related to SUSI assurance, the regulatory landscape has evolved. Now, recommendations do exist, especially on the use of SUS in sterile processing. The European Medicines

Figure 3: SUS lifecycle example (SIT = supplier integrity test, PoU-IT = point-of-use integrity test, PoU-LT = point-of-use leak test)



Agency (EMA) Annex 1 provides a guidance for manufacturing sterile products, applying quality risk management (QRM) principles to develop a relevant contamination control strategy (CCS) while considering several key areas such as equipment and process, personnel, and monitoring systems (**9**). Specifically for SUS used in sterile-product manufacturing processes, risks include loss of SUS integrity due to holes and leaks. Based on the CCS risk assessment, if there is a risk to product sterility, then appropriate SUSI tests are recommended.

SUS Applications and Consequences of Integrity Loss

The impact of an integrity loss or a leak in an SUS depends on the step in which it occurs in a manufacturing process (Figure 1). In applications using cell culture media and buffer preparation and handling, the main impact is economical. For cell culture steps in single-use bioreactors, such as for a monoclonal antibody (MAb) process, the impacts will not only be on costs, but also will include significant delays from production disruption, especially if contamination or leakage went unnoticed until after several weeks of culture in the main bioreactor.

For downstream processing operations, the impact is mainly economical (although there could be some operator or environment exposure concerns when dealing with hazardous components, such as ADCs). From a drug-product quality perspective, the highest impact of an integrity loss occurs toward the end of a drug manufacturing process during final formulation, filtration, and filling steps. An integrity breach during bulk drug-substance storage and shipping, whether occurring in a liquid or frozen state, also would have a dramatic economic impact because of the value of the product in the SUS.

In a pandemic context or when the drug supply is strained, batch losses from leaks can lead to drug shortages and related public health concerns. For personalized medicines, the impact of product loss or contamination could be devastating for patients.

Each SUS application and use case has its own requirements and specific considerations on which to base evaluation of the impact of an integrity breach. The previous examples do not reflect all possible cases.

Risk Assessment of SUS Integrity

A risk assessment highlights decision criteria to mitigate the risk of an integrity breach. The criteria on which SUSI strategy is based factor in process type (aseptic or not) and evaluation of the SUS design. They account for operator exposure risks, shipping and handling risks, and risks of damage at an end-user site. Figure 2 offers an example of a decision tree for risk assessment.

Ultimately, SUSI is enabled by quality by design, process validation, process controls, and integrity testing along an entire SUS life cycle (Figure 3). QRM, bag making, and SUS assembly expertise form the foundations of SUSI assurance.

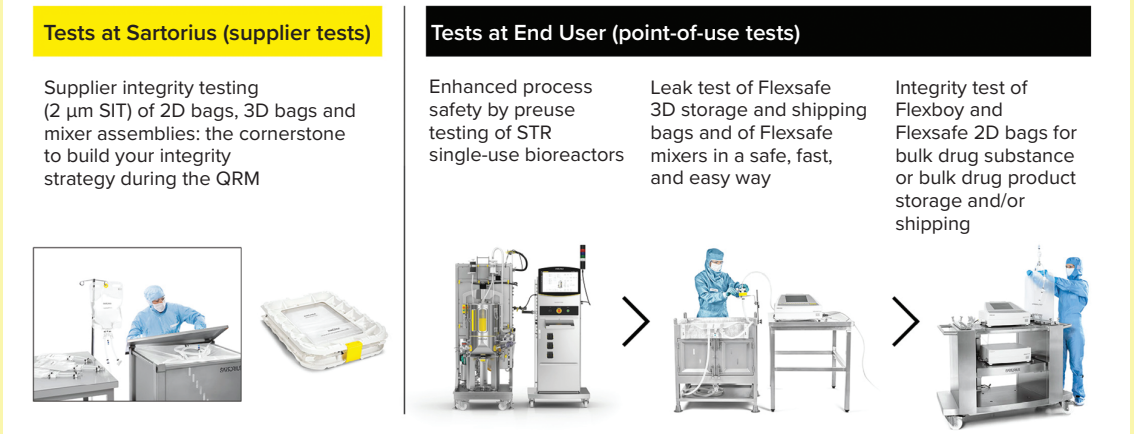
A final supplier test confirms integrity after an SUS has been assembled. In addition to comprehensive packaging and shipping validation, visual inspections, handling practices, and operator training, a point-of-use integrity/leak test confirms SUSI after shipping and handling of the SUS before its use at an end-user site. Such a multilevel approach to controls increases the level of assurance of SUSI and process integrity.

Before implementation of an SUS leak or integrity test, the value and benefits of such a test must be assessed in relation to the risks inherent to the test itself, because each test itself can introduce additional risks (e.g., damage and compromised sterility). Therefore, a comprehensive analysis should be performed. Once that is finalized, a recommendation for each application/customer case can be issued.

Implementation of Mitigation Strategies and SUSI Testing Solutions

The Sartorius SUSI strategy to mitigate the risk of product leakage or microbial ingress during bioprocesses is based on three pillars: product

Figure 4: Sartorius's testing solutions (SIT = supplier integrity test, QRM = quality risk management)



robustness, system integrity science studies, and integrity testing technologies and solutions.

For SUS testing solutions (Figure 4), supplier integrity tests (SIT), point-of-use leak-tests (PoU-LTs), and point-of-use integrity tests (PoU-ITs) are available for SUS designs used in applications such as cell culture, storage, shipping, freezing/thawing, and formulation/mixing. A SIT is based on detection of helium as a tracer gas. An SUS is placed into a vacuum chamber. The vacuum is drawn into the chamber and the SUS. Helium is then filled into the test sample up to a predefined test pressure. Helium escaping through a defect is detected with a mass spectrometer. To differentiate defective from conforming products reliably, validation tests use positive controls to detect artificially created and calibrated defects against negative controls. The leak-rate reject limit is defined by a six-sigma confidence interval.

PoU-LTs and PoU-ITs are based on pressure-decay measurements. After an SUS reaches a predefined test pressure, that pressure is stabilized for a defined time to compensate for temperature and effects linked to the elasticity of the polymeric material. After that stabilization phase, the pressure drop is measured over a defined test time. For defining the reject criteria, the same principles are used as previously described for an SIT.

With such a strong validation of the physical test methods, testing can contribute significantly to SUSI assurance. However, that is only one tool to mitigate the risk of an integrity failure. It is worth emphasizing that, for most SUS applications, supplier assembly process controls, packaging, and shipping validation, complemented by end user practices for storage, installation, and inspections, are sufficient to ensure the appropriate level of assurance of SUS integrity.

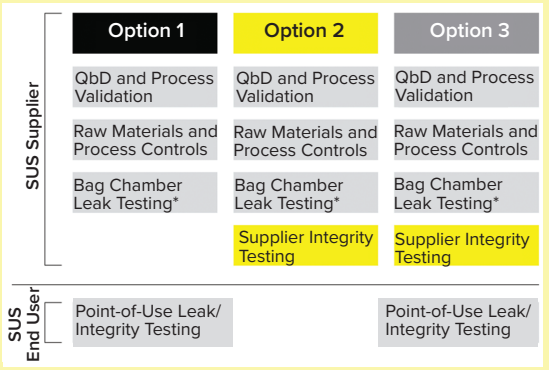
Figure 5 summarizes three main testing combinations that can mitigate the risk of an integrity breach, as dictated by an SUS application's criticality. As of today, there are limitations on the volumes and types of SUS designs that can be tested. Collaboration between an end user and Sartorius is key to defining a relevant strategy. The following use cases illustrate those different options.

For an mRNA vaccine process, option 1 from Figure 5 was implemented by performing a PoU-LT on a single-use bioreactor using a Sartocheck 4 Plus bag tester to confirm that the bag was installed without damage. That demonstrated that there was little risk of batch loss or production downtime from potential damage to the SUS, thereby providing a high assurance level of COVID vaccine market availability.

For shipment of a flu vaccine sterile bulk substance, a global biopharmaceutical company chose option 2 with Sartorius's SIT at 2 µm of its 200-L Flexsafe storage bags to ensure sterility of the bulk during shipment. For aseptic processing steps, that SIT option was adopted as the most relevant SUSI assurance strategy for all 2D, 3D, and mixing-bag assemblies because it met both quality and regulatory expectations posed by a global vaccines manufacturer.

Overall, option 2 can be part of a risk-mitigation strategy for process steps for high-value products (drug substances and drug products). For example, it can be applied to freezing containers (manifolded or not) that will be filled with drug substances before being frozen and possibly shipped to a manufacturing site. When an SIT is applied, the containers arrive with a high integrity assurance level. That comes in addition to the intrinsic robustness of those solutions guaranteed by their design (tailor-made for the application) and their extensive qualification according to their life cycle.

Figure 5: Testing options to meet different needs for risk mitigation (*bag chamber leak testing applies only to 2D bags).



One typical use case depicting option 3 is for **applications involving hazardous substances,** such as ADCs, for which the level of containment should be the highest. The 2-µm SIT shows that the SUS is integral after its manufacturing at Sartorius, and the PoU-LT at the drug manufacturer shows that no damages have occurred during subsequent transportation, handling, and installation steps before its use.

Of course, none of the SUSI testing options would be relevant without adoption of a quality by design (QbD) approach, process controls, and packaging as well as shipping validation, visual inspection, and operator training — all of which should be minimum requirements for assurance of SUSI.

The Science Behind SUSI

As described above, several strategies for risk mitigation can be derived from the outcome of a risk assessment. However, their benefits and potential disadvantages are not always well understood. Some frequent questions include the following:

- What does the test mean for a given bioprocess application?
- Is there a correlation between liquid leaks and the risk of microbial contamination of a product within an SUS?
- What is the hole-size threshold for an actual liquid leak?
- How are the test methods validated?

It is helpful to know the difference between leak tests and integrity tests. *Leak tests* identify leaks of any size in an SUS whereas *integrity tests* confirm the barrier properties of an SUS. To maintain certain barrier properties, the associated MALL needs to be identified.

It is important to establish MALLs relevant to SUS under use-case conditions. That means performing a microbial challenge test by aerosolization instead of immersions because doing so is a more accurate reproduction of environmental conditions in which SUS typically are used. Figure 6 shows that the pressure levels used for that test should represent and account for the most severe use-case conditions. Typical use cases for SUS are liquid storage, mixing, and shipping. Although the hydrostatic pressure of a liquid column is relevant for storage applications, dynamic pressure pulses must be considered for mixing and shipping applications. Pressure levels in the former are identified by computational fluid dynamics (CFD) simulation; in the latter, by recording shocks and accelerations during real-life shipping validation trials.

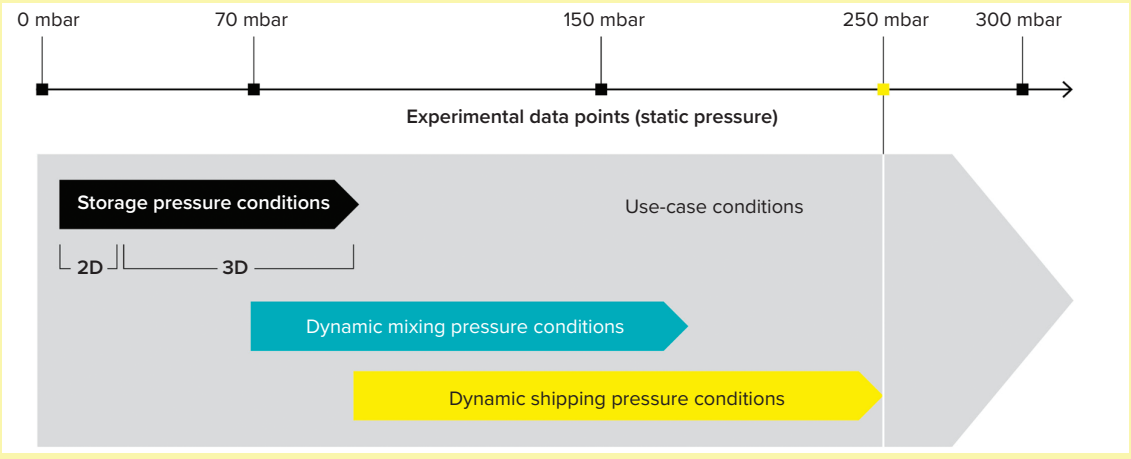
Barrier properties are divided into two main categories. In SUS used for processing or storing sterile products, the main barrier property aims to maintain a product's sterility and prevent entry of contaminants into the SUS. If processing of hazardous products can pose risks to operators and the environment, the main barrier property is to prevent any liquid loss from the SUS.

Sartorius performed three comprehensive scientific studies to achieve greater understanding of the mechanisms of liquid leaks and bacterial ingress in SUS. The primary objective was to correlate integrity-test detection limits to liquid leaks and microbial ingress under process and use-case conditions in different single-use bag systems (2D, 3D and mixing assemblies). Results demonstrate the relation between microbial ingress and liquid leaks. They also show when microbial ingress occurs and at which corresponding leak size. The secondary objective of those scientific studies was to establish predictive models for determining the MALL under all process conditions. Those models allowed us to develop and validate physical test methods with detection limits that guarantee the absence of liquid leaks and microbial ingress in SUS.

Sartorius's scientific work encompassed the following three main studies:

- a liquid leak study to determine the MALL by using different defect sizes of film patches with different model solutions at different pressures (**10**).
- a microbial ingress study to identify MALLs under real process conditions; for that purpose, a robust microbial aerosol method was developed for material used in our SUS at various pressures. The goals were to understand the microbial ingress mechanism in SUS material and correlate MALLs for microbial ingress to detection limits of physical integrity testing methods (**11**).

Figure 6: Pressure ranges for integrity science studies



• a gas-flow study to determine the relation between gas-flow rate and differential pressure for different defect sizes, leading to different flow regimes (**12**).

For the liquid leak study, liquid-filled test samples prepared with artificial defects of different sizes were pressurized and stored for up to 30 days. Different model solutions were used to evaluate the impact of surface tension. Only if no single droplet was observed during that storage period was a test sample considered to be free from leakage.

The second study on microbial ingress showed that testing by aerosol challenge can be as stringent as testing by immersion challenge as long as the most severe testing conditions are used. A high challenge concentration of 10^6 CFU/cm^2, derived from ISO7 cleanroom specifications with 6-log augmentation was used for challenging the test samples.

In addition to defect size, pressure is the main factor behind microbial contamination. Contamination occurs against the direction of pressure differential when microorganisms can enter a test sample though the liquid-filled defect channel. That was confirmed by the strong correlation with liquid leak test results, as shown in Figure 7. Because of the probabilistic nature of the microorganisms, the MALL for microbial integrity is reported based on a probability of microbial ingress. Based on all relevant use-case conditions, the MALL for microbial integrity is 2 µm. Mathematical models, established by power regression, predicted the MALL for all use-case conditions.

The gas-flow-rate study is the third part of the scientific body of work and examines the relation between gas-flow rate and leak size as a function of differential pressure. This study had two major objectives. The first objective was to check whether the relation between flow rate and leak size for flexible materials was consistent with the relation known for other materials from the literature (e.g., Table 1) (**4**). And the second objective was to establish theoretical formulas to convert leak sizes precisely into gas-flow rates. That is important because the concept of MALL in SUS is commonly related to leak size (leak diameter), usually expressed in micrometers. By comparison, gas-flow rate is the important parameter for validation of physical-integrity and leak-test methods. As a result, different combinations of differential pressures and defect sizes led to different flow regimes. The effects of differential pressure, leak size, and leak geometry were different for each of the flow regimes and reflected in the empirical formulas.

Experimental data from liquid-leak and microbial-ingress testing confirmed the correlation between microbial ingress and liquid leaks. Based on experimental data, predictive models were created to determine MALL under any process conditions (mainly pressure). With identification of 2 µm as MALL for liquid leakages and microbial ingress under most severe use-case conditions, a correlation was established between liquid leaks/microbial ingress and Sartorius's physical SIT. Artificial defects in film materials have been widely characterized, which enabled development and reliable validation of physical test methods with detection limits that guarantee the absence of liquid leaks and microbial ingress in SUS.

Integrity Assurance in SUS

Proven integrity of SUS in biotechnology processes prevents the loss of high-value product at commercial phases and meets regulatory

Figure 7: Correlation between microbial ingress and liquid leakage

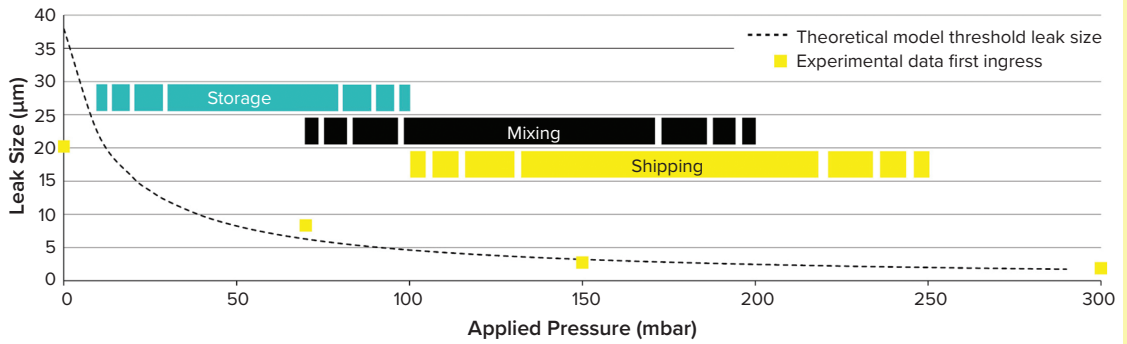


Figure 8: Summary of integrity assurance strategy

expectations for CCSs. In addition, it reduces the risk of exposing operators and environments to processed fluids. Eliminating complex SUS designs; limiting the number of manual manipulations; and performing qualifications, controls, and visual inspections along with operator training are the key measures toward increasing the level of assurance of SUSI. When justified by an SUS application and based on an end user's risk assessment, specific leak or integrity tests for SUS should be considered to enhance that level of integrity assurance further (Figure 8).

To implement relevant physical integrity and/or leak testing, it is important to understand failure modes that can occur along an SUS lifecycle. The MALL to maintain an SUS's barrier properties under its use-case conditions also should be known. In Sartorius's comprehensive scientific studies, the MALL for preventing microbial ingress and liquid leakages under most severe use-case conditions was determined to be 2 µm. By offering a supplier integrity test with a detection limit of 2 µm for SU bag assemblies up to 500 L and mixers up to 650 L, Sartorius can deliver SUS used in critical process steps, providing the required level of integrity assurance.

References

1 *18th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production*. BioPlan Associates, 2021; https://www.bioplanassociates.com/18th.

2 *Design, Control, and Monitoring of Single-Use Systems for Integrity*. Bio-Process Systems Alliance, 2017; bpsalliance.org/technical-guides.

3 Bio-Process Systems Alliance. Recommended Practices for Assuring Integrity of Single-Use Systems. *BioProcess Int.* 17(3) 2019: 30–36.

4 USP ‹1207› *Sterile Product Packaging: Integrity Evaluation*. US Pharmacopeia: Rockville, MD, 2016; https://doi.org/10.31003/USPNF_M99926_01_01.

5 *Standard Practice for Integrity Assurance and Testing of Single-Use Systems*, E3244-20. American Society for Testing and Materials (ASTM) International, 2020; https://www.astm.org/e3244-20.html.

6 *Standard Test Method for Microbial Ingress Testing on Single-Use Systems*, E3251-20. American Society for Testing and Materials (ASTM) International, 2020; https://www.astm.org/e3251-20.html.

7 *Standard Test Method for Physical Integrity Testing of Single-Use Systems*, E3336-22. American Society for Testing and Materials (ASTM) International, 2022; https://www.astm.org/e3336-22.html.

8 *Technical Report No. 86: Industry Challenges and Current Technologies for Pharmaceutical Package Integrity Testing*. Parenteral Drug Association, 2021; https://www.pda.org/bookstore/product-detail/6132-tr-86-pharmaceutical-package.

9 *Manufacture of Sterile Medicinal Products*. European Commission, Annex 1, 2020; https://www.gmp-compliance.org/guidelines/gmp-guideline/eu-gmp-annex-1-revision-manufacture-of-sterile-medicinal-products-draft.

10 M. Hogreve S, et al. Single-Use System Integrity II: Characterization of Liquid Leakage Mechanisms. *PDA J. Pharm. Sci. Tech.* 75(3) 2021: 258–272.

11 Hogreve S, et al. Single-Use System Integrity I: Using a Microbial Ingress Test Method to Determine the Maximum Allowable Leakage Limit (MALL). *PDA J. Pharm. Sci. Tech.* 73(5) 2019: 459–469.

12 Hogreve M, Aliaskarisohi S, Urbanik T. Single-Use System Integrity III: Gas Flow Rate Through Laser-Drilled Microchannels in Polymeric Film Material. *PDA J. Pharm. Sci. Tech.* 76(1) 2022: 9–18.

Corresponding author ***Marc Hogreve*** *is principal engineer, integrity testing, marc.hogreve@sartorius.com; and* ***Inga Reinert*** *is market entry strategy specialist at Sartorius Stedim Biotech GmbH.* ***Nathalie Pathier*** *is a product manager, applied services, at Sartorius Stedim FMT SAS.*