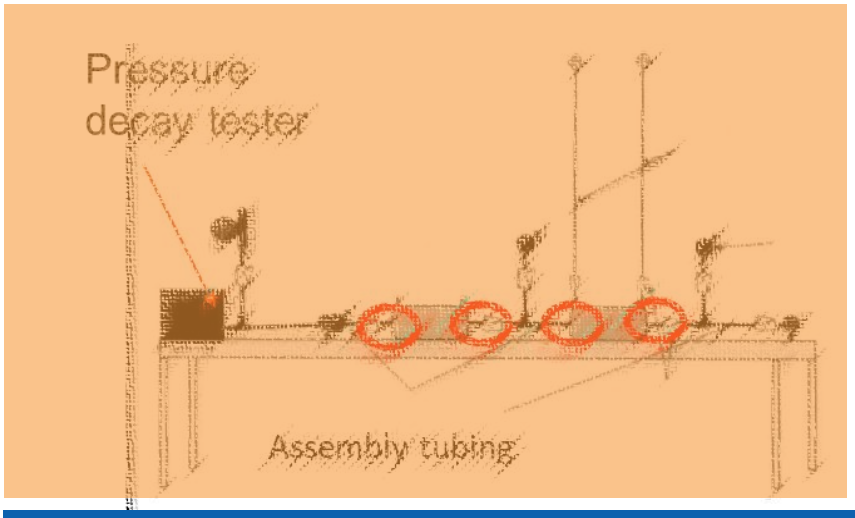
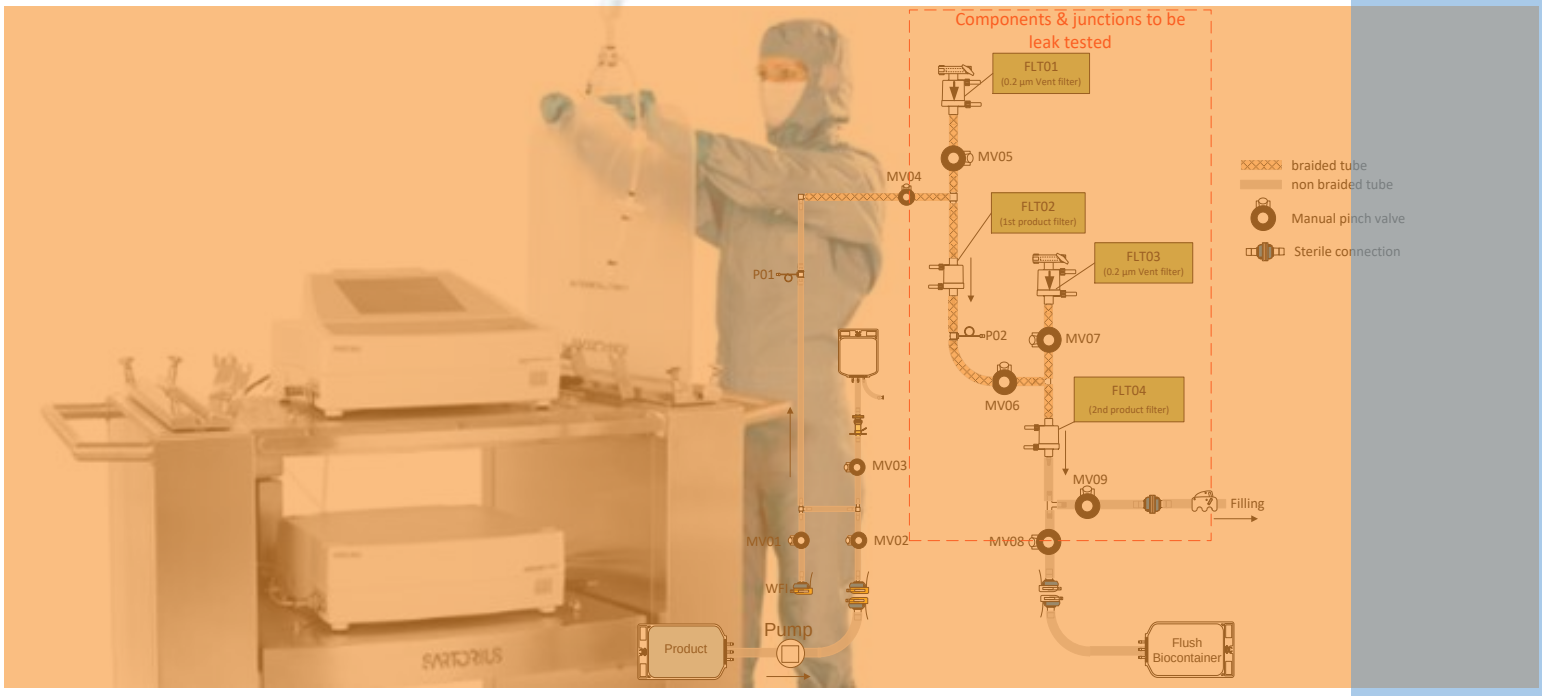


2023

DESIGN, CONTROL AND MONITORING OF SUS FOR INTEGRITY ASSURANCE

VOLUME 2



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Common Acronyms

API(s)	Active Product Ingredient(s)
APS	Aseptic Process Simulation
ASME-BPE	The American Society of Mechanical Engineers - Bioprocessing Equipment Standard
ASTM	Formerly The American Society for Testing and Materials, now ASTM International
ATMPs	Advanced Therapy Medicinal Products
BCT	Bacterial Challenge Test
BDS	Bulk Drug Substance
BPSA	Bio-Process Systems Alliance
BPOG	Formerly BioPhorum Operations Group, now BioPhorum
cGMP	Current Good Manufacturing Practices
CQA	Critical Quality Attribute(s)
DoE	Design of Experiments
DS	Drug Substance
DSP	Downstream Processing
EMA	European Medicines Agency
EU cGMP	European current Good Manufacturing Practices (or Eudralex)
FDA	U.S. Food and Drug Administration
GMP	Good Manufacturing Practices
He	Helium
ICS	Integrity Control Strategy
IPC	In-Process Control
ISO	International Standards Organization
ISPE SMEPAC	Former name of "Guidelines for Assessing Particulate Containment Performance of Pharmaceutical Equipment"
LoD	Limit of Detection
MALL	Maximum Allowable Leak Limit(s) (as defined in ASTM 3336-22)
(m)barg	(milli)bar gauge (relative pressure)
MoC	Material of Construction
MSAT	Manufacturing Science and Technology
NDOS	Nominal Diameter Orifice Size (as defined in USP <1207.1>)
OEB	Occupational Exposure Band
OEL	Occupational Exposure Limit
PDA	Parenteral Drug Association, Inc.
PoU	Point of Use
PPE	Personal Protective Equipment
PUPSIT	Pre-Use Post Sterilization Integrity Testing
QbD	Quality by Design
QC	Quality Control
QRM	Quality Risk Management
SME	Subject Matter Expert
SOP	Standard Operating Procedure
SUS	Single-Use System(s) (or Single-Use Assembl[y/ies])
URS	User Requirement Specifications

1 Intent and Scope

This document is the second volume of the BPSA guide on integrity assurance of SUS, initially published in 2017. Volume 1 summarizes the state-of-the-art industry practices and experience to ensure SUS integrity, aiming to help suppliers and end users develop their integrity risk assessment and subsequently implement an appropriate integrity control strategy (ICS).

The second volume provides supplemental information including:

- Case studies illustrating the principles developed in Volume 1
- Updates to key technologies used by the industry
- Updates to the regulatory landscape and industry guidance
- Case studies covering specific applications such as elevated pressure situations and containment of powders.

2 Introduction

Integrity is one of the most critical quality attributes of SUS and is consequently given special attention by all industry stakeholders. This attention has intensified as SUS have increasingly been used in critical cGMP applications. In 2017, the BPSA published the "Design, Control, and Monitoring of Single-Use Systems for Integrity Assurance" [1] guide to address a guidance gap. The guide explains key principles to consider when building a robust ICS and includes detailed information on leak and integrity testing technologies. A risk-based approach, based on the full SUS life cycle, and relying heavily on QbD and QRM principles, is recommended.

The document significantly influenced the industry and was used as key input to two ASTM standards:

- ASTM E3244-20, Standard Practice for Integrity Assurance and Testing of Single-Use Systems [2]
- ASTM E3251-20, Standard Test Method for Microbial Ingress Testing on Single-Use Systems [3]

It is very important to repeat that a "one size fits all" approach for integrity assurance and integrity control of SUS is neither feasible nor desirable. It would lead to unnecessary increases in complexity and cost of SUS. The requirements must be adapted to the intended application and defined further a sound risk assessment. The current level of control of SUS is adequate for most of the applications, without having to use expensive and complex control methods. Many examples included in this document are exemplary of the most requiring applications.

Soon after the publication of the 2017 guide, it became apparent to the BPSA Integrity Task Force that Volume 1 could be usefully complemented by a second volume, focused more on case studies and concrete elements, illustrating the principles presented in the first document.

The SUS industry has significantly improved leak and integrity testing technologies over the last few years, enabling application to a broader range of SUS designs and, in some cases, providing enhanced sensitivity. An update on the industry's pressure-based and tracer gas-based testing (namely helium integrity testing) capability is therefore provided in this second volume.

Moreover, the regulatory landscape and industry guidance and standards have evolved considerably in recent years, with the publication of a series of key reference documents. An update on this was then needed, and it is included in Volume 2.

Finally, the Task Force decided to include some information covering specific applications, rarely presented in industry papers:

- Applications using SUS at elevated pressure
- Applications using SUS for powder handling

The BPSA SUS Integrity Task Force strongly believes that this document will be a valuable in-depth resource for SUS suppliers and end users, enabling them to build robust and reliable manufacturing ICSs.

3 Case Study - Design, Qualification and Validation of a Single-Use System for a Critical Application Using QbD Principles

For the application considered in this case study, integrity of the SUS is of utmost importance as the SUS is used after the last sterile filtration step.

3.1 QbD/QRM Approach Applied to SUS Development and Implementation

A structured QbD/QRM approach based on risk analysis should be adopted when developing and implementing SUS in a critical application.

As general considerations, this starts by mapping the application, obtaining a detailed process flow diagram. Risks are then best mapped and addressed following this process flow as a breadcrumb trail. Risk assessment is advised to be ideally performed by a multidisciplinary team including SMEs from MSAT, operations, engineering, quality, and regulatory teams. The elements of the risk analysis are used to define key requirements of the URS. For cost reasons, URS are adapted to the criticality of the application. For example, the end-user of this case study accepts generally that a tubing to hose barb connection is secured by cable ties. For applications with high integrity assurance requirements, more expensive solutions like stainless steel crimped collars or over-molding are adopted by this end-user. Note that this approach is not universal: other end-users have other approaches to guarantee integrity of tubing to hose barb connections.

The case study that will be discussed in the following paragraphs focuses on the actions to be performed to provide a robust integrity assurance for the SUS used after final sterile filtration and is an example of how the principles covered in Volume 1 of this guide can be applied.

Two risk categories are associated with this case study: patient safety risk and business risk.

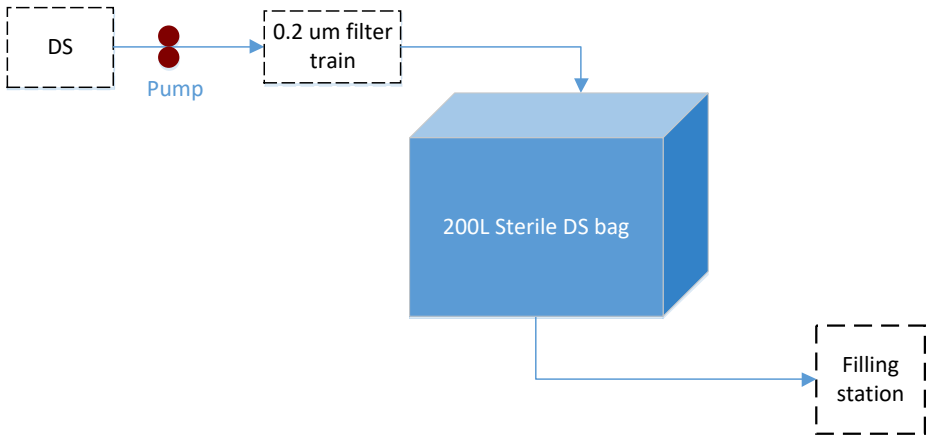
- Patient safety risk is essentially associated with risks of contamination, due to:
  - Breach of sterility, requiring therefore the highest integrity assurance possible
  - Endotoxins, to be addressed by adequate manufacturing process and controls
  - Leachables, to be addressed by a specific extractables and leachables assessment
- Business risk includes:
  - Risk of product losses due to leakage, which will cause a loss of part of the batch (if the leak is upstream of final sterile filtration) or of the full batch (if the leak is downstream of final sterile filtration)
  - Risk of product losses due to product degradation in contact with the SUS, to be addressed by a product stability validation
  - Risk of SUS supply shortage, to be addressed by adequate supply chain continuity programs (potentially including the development of alternative backup SUS)

3.2 Process Mapping

The case study that will be discussed is a downstream process where the drug substance (DS) is filtered through a 0.2 µm sterilizing filter and stored in the cold room prior to filling. The DS is sampled multiple times during the storage period of 3 to 12 weeks. The DS in the bag needs to be re-homogenized (mixed) each time before sampling and before filling.

As shown in Figure 1, the DS is transferred using a peristaltic pump through the 0.2 µm sterilizing filter train into a 200 L bag. The sterile DS is then stored in a bag placed in a tote, and the whole system is moved to a cold room for storage.

Figure 1: Illustration of the process flow with the use of 200 L sterile DS bag in tote



Vaccine DS ingredients and CQAs related to the SUS are identified, according QbD principles:

- DS ingredients: protein + buffer saline + low levels of additives and impurities (surfactants, chemical residues of inactivation process step)
- CQAs related to the SUS are DS sterility, potency, API concentration and excipients concentration

3.3 SUS Risk Assessment and Remediation

Risk assessment and remediation actions are summarized in Table 1.

Table 1: Risk Assessment and Remediation Actions

Risks	Criticality (H, M, L)	Design Consideration for Risk Mitigation
Bag integrity	H	1) Select SUS suppliers with a proven reliability track record associated with the bag type included in the single-use system 2) Select SUS suppliers with a validation package covering the integrity of the system (junctions, bag chamber, etc.) 3) Select a bag holder (also referred to as tote) design that matches the 3D bag design 4) Use a light table for visual inspection pre-use (criteria based on the "Single-Use Visual Observation Library" (see [12]) provided by SUS suppliers) 5) Consider simplifying the design by reducing the number of connections on the bag (lowering the risks of failure and having a system easier to handle) 6) Use SUS from different batches for the initial and periodic APS 7) Store the double-bagged SUS package in a specially designed shelf without stacking to minimize load on the SUS during storage 8) Perform a thorough chemical compatibility assessment (either based on literature information or lab testing) 9) Inspect the tote for the bag - smooth inner surface is a design requirement and it is good practice to visually check the smoothness of the tote before each use, to prevent risk of damage to the bag
Tubing assembly integrity	H	1) Consider using either stainless steel crimped collar as securing device or over-molding for tubing to hose barb connections, the user having a higher confidence in their reliability *Note: this does not reflect the opinion of all end-users* 2) Do not use thin-walled tubing 3) Create a tote design with a robust bottom opening and locking (latching) system to secure the bag bottom port fitment and associated bottom outlet tube, as well as a foam and a top compression system to keep the bag in place and have the top tubing assembly separated from the bag by the foam and top lid. This helps to minimize vibrations during transportation and prevents the risk of damaging the top of the bag by rigid parts 4) Store the bottom bag tubing assembly in the lower chamber of the tote 5) Perform a thorough chemical compatibility assessment (either based on literature information or lab testing) 6) Use two pinch clamps (of specific validated design) to close the tubing before the aseptic connector. The second pinch clamp is used to provide redundancy
Aseptic connection	H	1) Use aseptic connectors with a proven reliable track record 2) Conduct annual training on aseptic connector use, including visual checks and practical requalification of the engagement operation. This training can be coupled with APS 3) Simplify the design with minimized number of aseptic connectors in the bag assembly as any component that is not needed is adding a small potential

		integrity risk. A typical example is the use of a cross manifold for the connection extension if needed in the process. See Figure 2 below
Aseptic sampling	H	1) Use a pre-irradiated aseptic sampler 2) Use an aseptic connector to connect to the bag assembly before sampling 3) Disconnect the sample with crimped-seal technology to maintain the sterility when disconnecting the sample bags. Cover the sharp edges to prevent damages
Recirculation loop durability	M	1) Use pump grade tubing as a segment of bag assembly 2) Pre-test the tubing durability (lab testing) and select the best tubing
Intra-factory transportation (transportation inside a building or between buildings of the same site)	M	1) Use a bag without impeller (loop recirculation for agitation). No mixing bag with impeller is currently validated by suppliers for transportation 2) Choose a tote and bag design covered by ISTA or ASTM shipping validation. Note: this is an "over-design" approach (road shipping validation to cover intra-site transportation), providing a large safety margin and adopted for high integrity assurance 3) Perform lab and shop floor worst-case shipping/handling challenge tests with the bag in the tote, filled with surrogate fluid 4) Select a tote especially designed for the dimensions to the DS-filled bag 5) Ensure the bag in the tote is covered with foam. The top tubing assembly sits on the foam and does not contact the top of the bag 6) Sanitize tote materials repeatedly with bleach (to minimize risk of chemical attack)
Operation-caused leaks (ergonomics)	M	1) Avoid long tubing lines on the bag assembly to minimize risks of mishandling and mechanical stresses due to pulling on the tubing 2) Store unopened SUS on specific shelves and do not stack several systems on top of each other (avoid unvalidated load during storage) 3) Use a tote at the correct height for ergonomic operation, so that the bag can easily be deployed in it 4) Open the bag package without using scissors or a knife (to avoid cutting a hole in the bag and causing leaks). Scissors and knives are a common cause of bag damage when not removed from the manufacturing area 5) Transparent bag packing and protection material is highly desirable to enable a first visual inspection step before opening the packing. It is highly recommended to perform a more efficient visual inspection after removing the packaging. The visual check when the product is packed and folded, remains quite superficial.

Note: the DS considered is not sensitive to light, not denatured by polymer contact, or absorbable/adsorbable on specific polymer materials. Such considerations must be systematically part of the risk assessment.

3.4 URS, SUS Design and Associated Sketch

An adequate URS generally contains a functional section, a regulatory compliance section, and a sketch with design details to provide additional information about components. It provides clear requirements to the supplier and significantly reduces the prototyping phase. The design considerations issued from the risk analysis are used to define key requirements of the URS. Setting up a pertinent URS requires the end users to understand material science, engineering, and SUS manufacturing technology to a certain degree. On the other hand, responding to the URS requires

an understanding of bioprocessing from the supplier. For reference, a URS template has been published by BPSA and BioPhorum [4].

In this case study, URS information included the following elements:

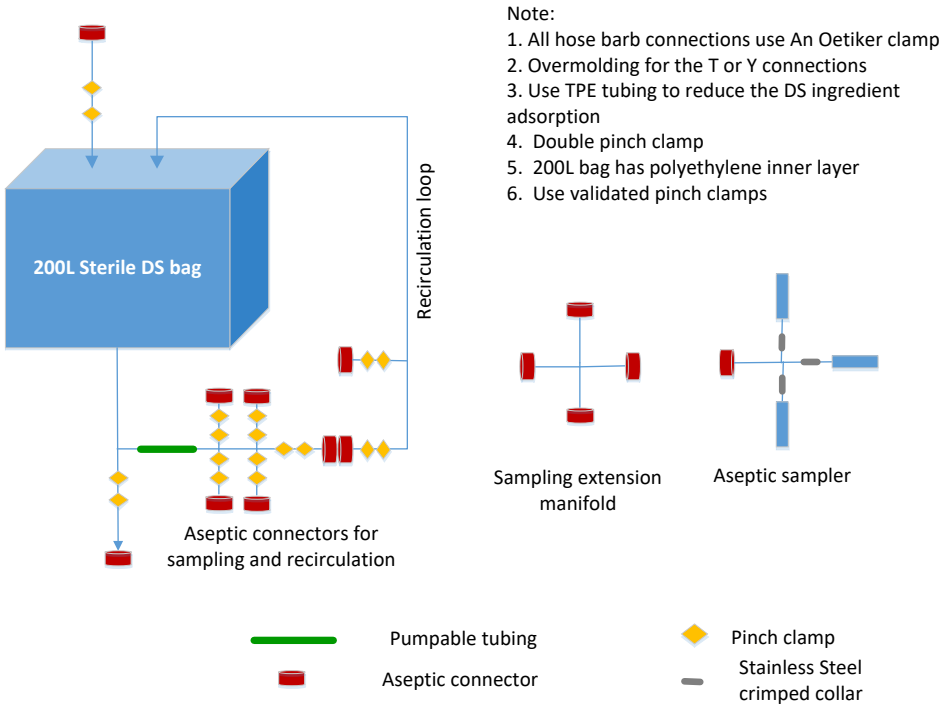
- Bag volume: 200 L
- Bag inlet and outlet tubes
- Material of construction (MoC) for each component
- Compatible pump tubing type, durability requirements and peristaltic pump model
- Tube connectors and disconnectors
- Aseptic connector technology
- Pinch valve type and quantity
- Packaging allowing visual inspection (with light table) prior to opening
- Design allowing to perform an aseptic sampling several times
- Agitation type (recirculation)
- Design to minimize risk of foaming when mixing
- Bag and tote system to be validated for truck transportation

Note: no point of use (PoU) leak test was required in the URS because, at the time of the project:

- A complete validation package of the PoU test instrument was not available
- Quality systems were not in place at the end user site to manage such PoU systems
- The risk analysis concluded it was not needed

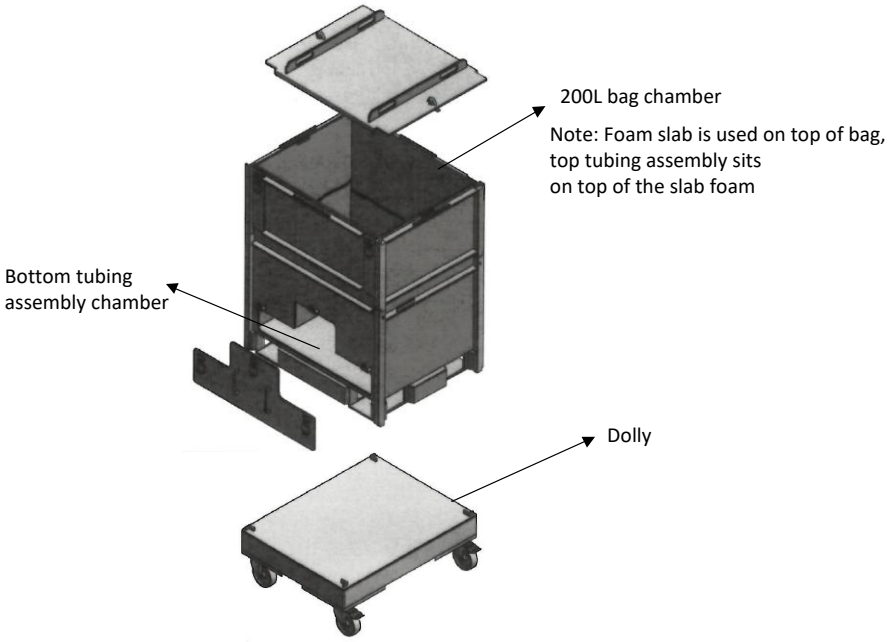
A PoU leak test could be introduced later, as a continuous improvement initiative, if the risk assessment (associated with the evolution of the technology) would conclude it is needed. This would require the SUS to be adapted, by adding a sterile vent filter on the upper side of the bag chamber.

Figure 2: Sketch of the 200 L sterile DS bag, extension manifold and the aseptic sampler sub-system



Additionally, the tote design was clarified and specified in detail after communication with the supplier (Figure 3). Some requirements to reduce the leak occurrence were added to the drawing. Note that it is recommended to issue a detailed URS for the tote. This is not described in the current case study.

Figure 3: 200 L tote design with considerations for optimum assurance of integrity



A specific storage shelving was built to prevent risk of damage during storage, as illustrated in Figure 4. The small bags are stored vertically on the racks, and the large bags are stored horizontally. None of the bag assemblies contact each other during storage or during transfer from the storage area to the process area.

Figure 4: Specific storage shelf design

3.5 Prototype, Qualification and Validation Testing

Prototype testing was performed to verify proper performance and operability of the SUS, and identify necessary improvements as soon as possible, before engaging in qualification and validation activities.

SUS coming from three different lots were used for qualification and validation steps.

The qualification included specific functional tests associated with the application. They were performed to demonstrate that identified risks associated with the SUS modus operandi were properly mitigated. They included:

- Pressure decay leak test
- Pump tube durability test: the tube was tested for a 24-hour run and visually inspected for the wear-off level
- A truck shipment study in worst-case conditions was performed with the bag filled with surrogate fluid. Absence of leaks was verified post transport simulation, letting the bag filled for three days (the three days giving enough time for the liquid to go through small leaks in a quantity that is easily detectable by visual inspection).
  Note that there are many other potential ways to check the absence of leaks, some requiring specific equipment and/or additional handling of the SUS. This includes (and is not limited to):
  - performing ink penetration test in the areas of concern
  - performing tracer gas sniffer test in the areas of concern
  - covering the areas of concern with colored absorbent paper to enhance detectability
  - wiping the areas of concern (e.g., all joints) with absorbent paper to enhance detectability

The validation tests demonstrated that the DS's CQA were properly maintained when processed with the SUS. They included:

- Aseptic process simulations
- Media hold time validation
- DS stability validation
- Recirculation mixing validation

The first two items provided key supporting information on the SUS integrity.

3.6 Routine Integrity Control Strategy

From a regulatory perspective, the end user is responsible for the assurance of integrity associated with their manufacturing operations. However, a well-designed integrity assurance program requires close communication and collaboration between the supplier and end user on their shared tasks. This is essential to address the different aspects associated with SUS as efficiently and timely as possible. This includes, but is not limited to, proper design definition, supply lead times during development (for prototypes and qualification SUS), and adequate routine quality management including change management.

Changes initiated by suppliers can have a myriad of goals, such as supply chain adjustment, e.g., replacing a component due to supply shortage or adding a new manufacturing plant to meet the market supply needs. Some changes are made to improve assurance of integrity, but others might impact it negatively. Changes must therefore be carefully assessed with the information included in the supplier's validation package. Implementation of changes bringing an improvement to the process stability, product quality, or supply chain are logically prioritized.

Single use technologies are in constant innovation. New developments are continually made, proposing new components or assembly options, or developing new processes, that allow us to mitigate risks or to improve the product yield and quality. Changes initiated by end users are often seeking quality or reliability improvements based on experience gained after use of the SUS. In most cases, only a limited quantity of SUS is tested during the qualification and validation phases. SUS are consumables, and slight variations, occurring between lots or over time, might not be fully captured during the qualification and validation tests, while some of them may cause failures during use.

For the end user implementing the SUS of the case study, topics that have been required to set up an adequate routine ICS were:

- Rigorous operator training on the storage (use of specific storage shelf), handling and deployment of the SUS, as well as on the use of the aseptic connector is needed to prevent operation errors
- Visual inspection using a light table pre- and post-opening of the SUS packaging
- Technical visit of the supplier manufacturing site, auditing manufacturing process, qualifications and validations, quality controls and quality system

For the reasons mentioned in section 3.4, implementation of a PoU leak test for the SUS was evaluated and was not implemented. There is no formal regulatory mandate nor internal quality mandate to implement such a test. Moreover, the test sensitivity is limited (see section 5.1 for the distinction between leak and integrity test). Due to this limitation, end-users have different opinions about the risk/benefit balance of such PoU leak testing. Implementation of a point of use leak test is defined case by case, depending on the end-user risk analysis. End-users decide the most relevant control strategy for their application.

For the supplier, the program had to include, at minimum:

- Qualification and validation of the manufacturing process, e.g., welding steps, assembly and securing of tubing lines and equipment calibration (for the tubing securing device crimping tool).
  Note that in this case study, cable ties as tubing securing devices were excluded. In cases where such systems are used, it is critical to have the cable tie guns frequently recalibrated
- In-process controls associated with the manufacturing process steps, including leak tests, visual inspection and other destructive or non-destructive testing
- Preventive maintenance schedule for the welding equipment, tubing securing tools and any other tools related to the SUS integrity

- Use of visual inspection stations (preferably light tables) at different manufacturing steps. Operators should be specifically trained for visual inspection

Suppliers may offer the option to perform enhanced IPC on the SUS, that can be considered as integrity tests. This was not implemented in this case study as the development team was not aware of such an option.

The supplier plays an important role in the integrity assurance program, as a significant part of the quality chain associated with the SUS integrity is under its direct control.

4 Case Studies - Integrity Issues with Various Components

4.1 Using Integrity Testing Results as Design Verification Tool

Helium and pressure decay testing can be used to detect small defects on components, including design defects. For adequate detectability, the method applied should be highly sensitive, typically able to detect 10 µm NDOS defects or better. As described in section 5, depending on the application, typical limits considered to provide a robust assurance of integrity are 2 µm NDOS and 10 µm NDOS, depending on the use-case. Gross leak tests such as the one currently proposed as pre-use leak test (sensitivity higher than 10 µm NDOS) are not able to detect the type of defect presented below.

Using these high sensitivity limits both challenges and verifies the integrity performance associated to the design of a SUS or a single component. Tiny leaks detected by helium testing in the 2 µm NDOS range represent a risk to microbial integrity (see [1], [5], [6], [7]). Helium testing can detect small pinholes on bags, but such high sensitivity testing may also detect very tiny leaks associated with design issues, such as:

- A junction showing a variable leak level depending on handling (such as the movement of a tubing on a hose barb)
- The presence of a hole close to the tube's sealing on a 2D bag chamber. Such leak occurs at low frequency because there are multiple factors inducing it.

Helium testing is usually performed at low to medium pressures while pressure decay needs to be done at elevated pressure to reach the desired high sensitivity levels. Tiny leaks detected by helium testing at low pressure, in the 2 µm NDOS range, do not correspond to liquid leaks at low pressure but can correspond to liquid leaks at elevated pressure[1]. Due to the deformability of many components in SUS, it is theoretically best to use a test pressure close to the operating pressure to prevent unrepresentative leaks from being created by unrepresentative deformations or representative leaks from being plugged by unrepresentative deformations. It is important to note that such issues have not been encountered up until now: in the cases where SUS were detected leaking by helium testing and subsequently tested at high pressure, the leak was systematically confirmed. Helium testing is therefore considered to be a very sensitive and relevant test method.

The use of these tests and the subsequent improvements in design will limit loss of drug product (in case of leaks) and scrap of SUS (in case of rejects).

[1] Theoretical calculation, applying Hagen-Poiseuille equation for a 3 µm diameter orifice of 1 mm in length, for a 2 bar differential pressure, indicates that a water droplet of 1 mm in diameter is created in less than 20 minutes.

Different examples of leaks detected with integrity test methods are described below, including actions taken based on this experience.

4.1.1 Quick Connectors and Repeated Opening and Closure

Some components with o-rings (like quick connectors) showed leaks above the acceptance criteria after repeated opening and closure. The test method used was helium testing with a 2 µm NDOS acceptance criteria. The leak was observed during preliminary tests to set up a helium integrity testing system using a quick connector to connect the test article in the vacuum chamber. The issue was resolved by using another kind of connection (hose barb connection). This leak could be due to compression set[2] of the o-ring, causing imperfect sealing after repeated use, and leading to a very fine leak (2 µm NDOS). The end-user that experienced this issue is now systematically checking that, in critical applications, quick connectors are not opened and closed several times in cGMP conditions and, more generally, is avoiding the use of such a connector type in sterile processes when not operating under grade A.

4.1.2 Mold Parting Line on Hose Barb End

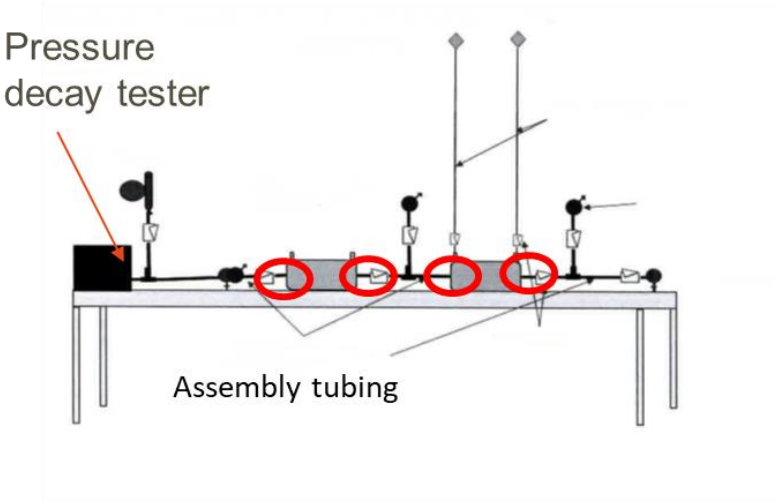
With the implementation of 100% integrity testing by helium as routine control, a SUS supplier was able to identify an improper connector that was responsible for a high number of rejects, due to a mold parting line along the hose barb connection that led to leaking SUS. The same principles as the ones described in more detail in section 4.1.6 were used to identify the leaking component.

In a second case, a mold parting line on the hose barb end of a filter capsule was the cause of leaks (Figure 5). Pressure decay testing, performed with a dye solution, put this defect in evidence during pre-validation tests (Figure 6). A properly designed hose barb end should be free of any parting line on its "mushroom" end. The mold of the capsule was reworked to remove the parting line in this area. High sensitivity pressure decay testing performed by the supplier (in validation and/or as routine 100% control) can be used to identify such issues.

Figure 5: Example of mold defect on hose barb of a filter capsule (parting line over full length of hose barb)

[2] The compression set is the permanent deformation remaining after removal of a force that was applied to a given material sample.

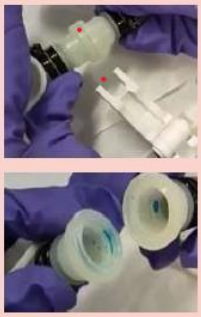
Figure 6: Pressure testing of a filter capsule assembly



4.1.3 Tri-clamp Connection

An end-user found a tri-clamp connection (often found on filter capsules) showing some inconsistent integrity performance with pressure decay testing. Based on some failures observed with pressure decay, further testing was done using dye ingress (Figure 7). Engineering tests concluded that the minimum torque required to have a tight tri-clamp connection was 1.5 Nm. Four assemblies with tri-clamp were closed at 2.5 Nm and subjected to a simulation of the use process. Despite control of the closing torque and a 70% safety margin, one out of four assemblies leaked at the tri-clamp. This leak was found to be due to some small variations in dimensions of the different parts of the assembly. The number of parts (four parts are used: the two tri-clamp fittings, the seal, and the tri-clamp collar) and potential variations at the level of the screw thread of the collar make this connection very difficult to model and can lead to inconsistent performance. Moreover, life cycle conditions, with vibrations or with conditions promoting creeping, can further impact the tightness of the connection. Considering this experience, aligned with several other similar cases, the tri-clamp connection is not considered to be reliable enough by the end-user and their strategy is to avoid this type of connection in aseptic processing steps wherever possible.

Figure 7: Tri-clamp integrity tested by pressure decay test, using a dye solution

Tri- clamp 1	Tri- clamp 2	Tri- clamp 3	Tri- clamp 4
2.59Nm	2.58Nm	2.57Nm	2.58Nm
No leak on exterior and union	No leak on exterior and union	Leak (droplets) in union	No leak on exterior and union
			

4.1.4 Leaks Due to Molding Defects

In this case, a leak was observed during pressure decay testing performed by the supplier of an in-line sensor. The leak was caused by a molding defect on the hose barb end. A follow-up investigation, using the dye test method, confirmed the leak, and further investigation with an appropriate visualization tool identified the location of the defect (Figure 8).

Figure 8: Example of defect found on hose barb end

4.1.5 Leaks Due to Operator-Dependent Clamp Closure

Helium leak detection testing may allow one to understand a failure mode during process validation. Figure 9 below shows a failure that was observed during a bacterial challenge test on a bag. A study on the failed bag showed the location of the leak and confirmed its magnitude (6 µm NDOS). In this case, the failure was associated with a component that allows some variation in the closure position (multistep clamp). Based on this study, the component allowing variable operation has been removed and replaced by a more robust design (one closure position only), not operator-dependent, before implementing the system in production.

This example also gives an illustration of the accuracy that can be obtained using helium leak testing under full vacuum.

Figure 9: Bacterial challenge failure of a bag

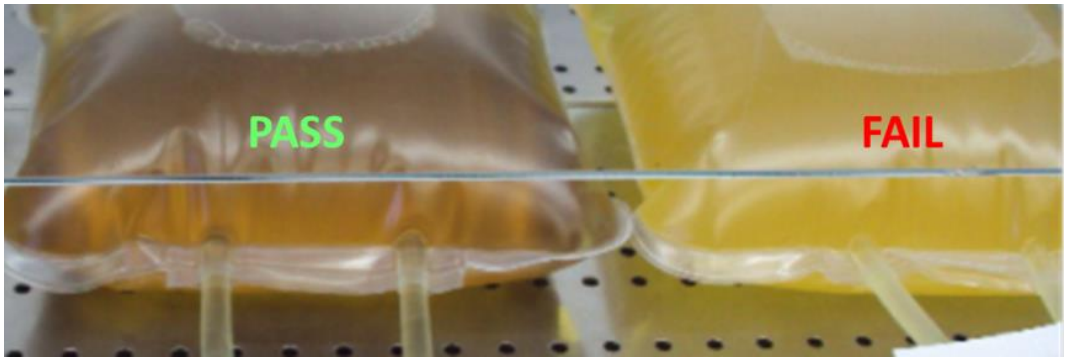
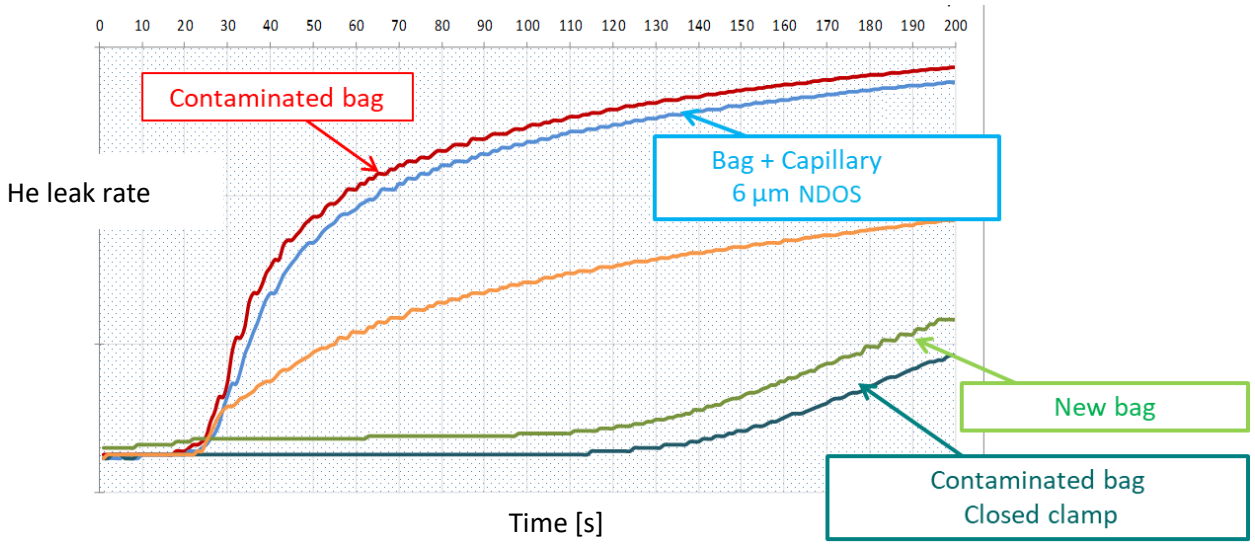


Figure 10: Helium leak test of bag with BCT failure



1. *The red curve shows the helium leak rate versus time for the bag contaminated by the BCT.*
2. *The light green curve corresponds to the helium leak rate vs. time for a new non-leaking bag (negative control).*
3. *The blue curve corresponds to the same bag as the light green curve but equipped with a reference leak made with a capillary, corresponding to a 6 µm NDOS (positive control).*
4. *The orange curve corresponds to the same bag but equipped with a capillary size corresponding a 2 µm NDOS (second positive control).*
5. *The dark green curve corresponds to the bag of the red curve, but with the clamp fully closed, eliminating the leak that caused the contamination.*

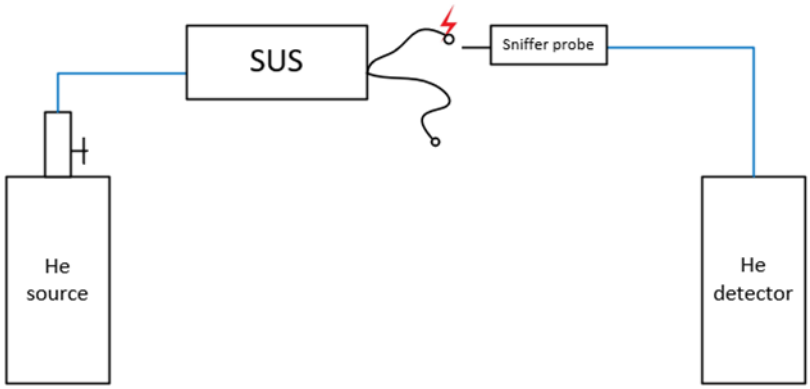
4.1.6 Leaks on Luer Lock Screw Cap

One SUS supplier who implemented 100% integrity testing by helium leak detection method on systems used for the critical application of sterile DS storage was able to identify a luer lock screw cap as an improper component to reliably maintain the microbial integrity of the SUS.

During routine testing, it was observed that the SUS design using this luer lock was accountable for about 50% of total rejects. When comparing the bill of material of this specific design to others that did not show such a high reject rate, this luer lock component was identified as potential root cause. That led to further investigation of the rejected SUS. Multiple helium testing in the vacuum chamber, by sequentially clamping different parts of the product until it became tight, confirmed this component as a root cause for the leakage. This simple investigation and the change of the component led to a reduction of rejects for this SUS of more than 60%.

To further reduce the rate of non-integral products, an additional investigation was initiated. As the screw cap is subject to manual manipulation in the manufacturing process (cap is screwed manually, and the luer lock is fitted manually on the tubing), the impact of the torque being applied to the screw cap was analyzed. As helium testing in vacuum chamber mode cannot be used to target a specific location, rejected products were connected to a helium source and a helium sniffer was used to identify the location of the leak (Figure 11). Approaching the screw cap connector, the measured helium leak rate increased significantly, which confirmed the identified component as the root cause for the leaks.

Figure 11: Helium testing setup in sniffer mode (inside-out test)



As helium permeation through the tubing material could have an impact on the measured helium leak rate by sniffing the surface, additional tests in spray mode (Figure 12) were done to confirm the leak location. For that, the products were connected to the helium leak detector, and a spray nozzle was used to spray helium on the identified area of leak.

Figure 12: Helium testing setup in spray mode (outside-in test)

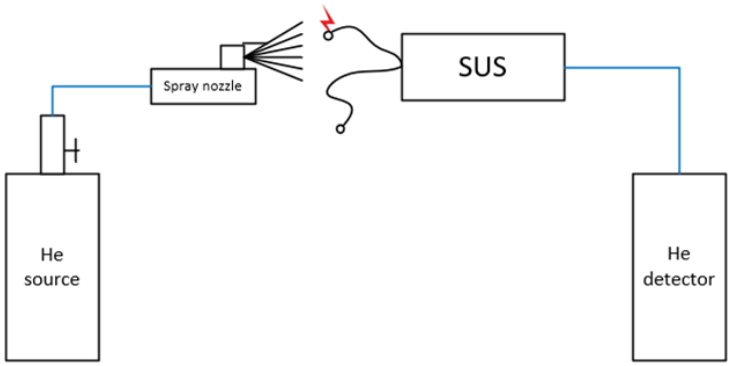
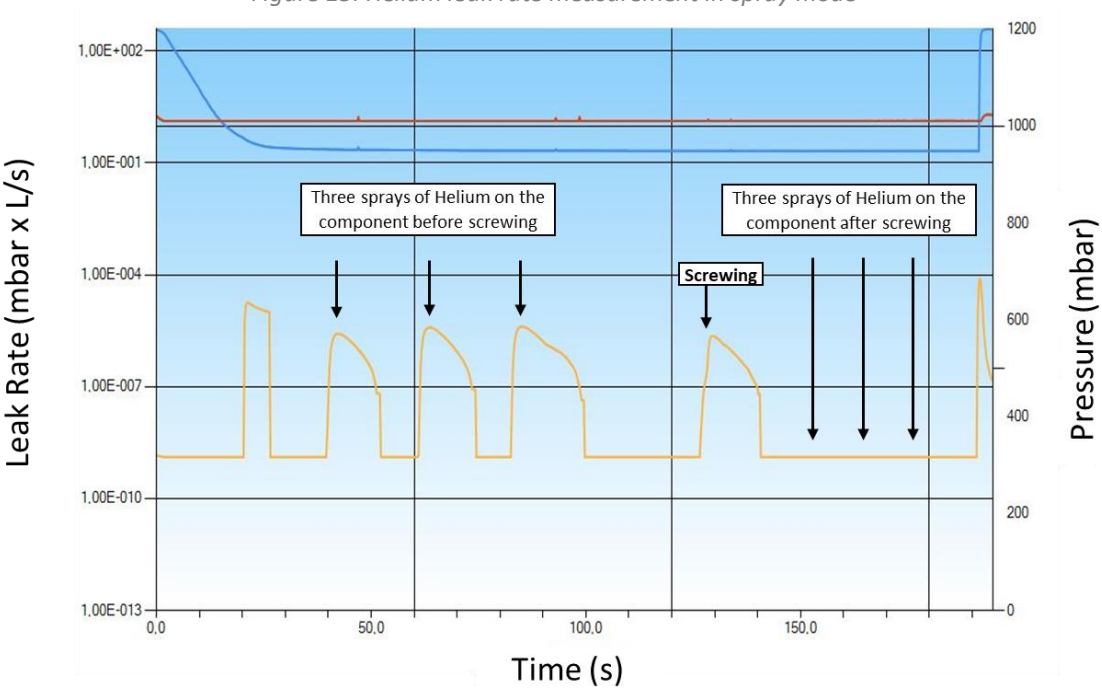


Figure 13 shows the results of spraying helium on the connector before and after the screw cap was tightened. This testing finally confirmed the identified component as the root cause for the leak.

Figure 13: Helium leak rate measurement in spray mode



1. *The blue curve on top of Figure 13 corresponds to the pressure inside the test article (in vacuum when tested by spray mode).*
2. *The red curve corresponds to the pressure in the vacuum chamber (not relevant for spray mode).*
3. *The orange curve corresponds to the reading at the helium tracer gas detector.*

An additional leak rate peak was observed during the screwing operation, likely due to the release of helium that was caught in the thread of the part.

Torque measurement techniques were evaluated but not considered as a reliable solution. Therefore, based on this investigation and following QbD principles, this specific luer component should not be considered for future designs to further reduce the risk of non-integral SUS.

4.2 Other Causes of Integrity Failures

4.2.1 Leaks Due to Improper Chemical Resistance

An incorrectly selected aseptic connector was not exhibiting a leak during validation, but did leak occasionally as it was in contact with high pH fluid over a long period of time. This risk had not been well captured during the initial risk analysis. The connector was replaced by another connector made from a different material of construction (MoC) and was resistant to these conditions.

4.2.2 Training in Accordance with Instructions for Use - Case of Aseptic Connectors with Peel Strips

When using an aseptic connector with peel strips, residual pieces or debris between sealing gaskets can sometimes be generated due to incorrect manipulation, typically by pulling the strips in an incorrect direction.

This failure mode is caused by improper handling and must be addressed by proper operator training, in accordance with the instructions for use provided by the supplier. This will ensure that the strips are pulled together in the correct direction, and that a proper visual check is performed after this step, to verify that the peel strips are intact.

Each technology presents potential risks associated with its design and mode of operation. This example illustrates the importance of proper training to ensure adequate use of any single-use technology and maximize its reliability.

4.2.3 Impact of Exposure to Elevated Temperature

Nylon cable ties can creep further and relax due to exposure to the autoclave process. This can lower the compressive force around the tubing, which, in some cases, may lead to leaks. On the other hand, nylon cable ties, if tightened with too high a force, can sometimes break after the autoclave process. These two examples illustrate how important it is to use calibrated cable tie guns to ensure that an adequate, validated tightening force is used.

5 Integrity Testing Technologies - Updates

Several integrity testing methods were described in Volume 1 (Table VIII), with the helium tracer gas method in vacuum mode as the most sensitive technology for testing SUS, followed by pressure-based tests with a significant gap in sensitivity. The helium tracer gas method has been implemented for a limited range of single-use designs, mostly restricted in size and complexity. Several constraints still existed at that time to adapt this technology to the specificities of SUS, including permeation of He through polymeric materials and the high configurability of SUS in volume, size, and complexity. They have been mostly resolved, with systems being able to test volumes from a few milliliters to a few hundreds of liters.

In recent years, this technology has evolved, as has its penetration into the biopharmaceutical industry. Many SUS suppliers now offer 100% integrity testing by helium tracer gas for SUS used in critical applications, upon customer request. In addition, the test method has expanded to include larger volumes and more complex designs.

As pressure-based tests were already quite mature, there has been no significant improvement in the technology itself. However, the range of products for which this technology is offered by the leading SUS suppliers has widened, partially because of the demand coming from the (bio)pharmaceutical industry.

5.1 Leak vs. Integrity Testing and Establishing a MALL

Physical testing of SUS can only provide a full integrity guarantee when the detection limit can be correlated to the barrier properties of the SUS. According to the common definitions in ASTM E3244 [2], E3251 [3] and E3336 [10], only a test method with a LoD good enough to confirm the barrier properties of a SUS is an integrity test. A test method with inferior (less performant) LoD is only a leak test. As explained in section 5.2, the limit to be considered for integrity testing can differ, depending on the use of the SUS.

In case the barrier property requirement is to maintain microbial integrity, the physical integrity test should be able to find any defect that may lead to microbial contamination during use of the SUS. For that reason, it is important to understand microbial ingress mechanisms through defects into SUS under use-case conditions. The same applies accordingly for liquid leaks, e.g., if the content is toxic and could compromise the operator or environmental safety.

A study conducted on material used for the assembly of SUS has shown that for most severe use-case conditions (e.g., liquid shipping applications), the defect size for which no microbial ingress and/or liquid leak can occur is around 2 µm NDOS. For rather gentle pressure conditions, this defect size is more in the range of 10 µm NDOS. These defect sizes are called the Maximum Allowable Leakage Limit (MALL) and are linked to specific use cases. For the first part of the study [5], polymeric film patches with different artificially created and calibrated defects were assembled on a polypropylene cup, that was filled with growth medium and subjected to a microbial aerosol exposure for several hours. During that exposure, the test articles were pressurized to represent typical use-case conditions. All samples were afterwards incubated and visually checked for growth. Based on the definition for the MALL in USP <1207>, for each pressure condition, the defect size having a probability of microbial ingress of less than 10% was defined as the MALL. In the second part of the study [6], the same kind of film patches were assembled onto a glass cylinder filled with different model solutions. Test articles were pressurized from the top for several days, and indicator paper below each glass cylinder was used to visually indicate a leak. Considering the intrinsic differences in test method (probabilistic for microbial ingress and deterministic for liquid leak), the results for microbial ingress testing and liquid leak testing have shown a close correlation to each other as well as to the theory for each individual pressure condition.

Another study (covered in [7]), whose results were already described in Volume 1, sections 3.2.1. and 3.2.2., was performed to identify the MALL in classical bacterial challenge validation conditions, without applying any pressure differential: the flexible character of the container automatically equilibrating the pressure. Positive and negative controls were included, as described in Volume 1. Test articles consisting of 2D bags of 1L size, equipped with artificial defect of a known size, made by laser drilling, were filled with growth medium. Test articles were then either subjected to a microbial challenge by liquid immersion or by aerosol. This study led to MALLs of 2.6 µm NDOS and 12 µm NDOS, respectively, for the liquid immersion and the aerosol challenge.

Table 2: Overview of Microbial Ingress Testing Studies

	Study 1		**Study 2**	
BCT Method	**Aerosolization without overpressure**	**Immersion**	**Aerosolization with overpressure**	
Container	Flexible (bag)		Rigid cup closed by film coupon	
	Deformability of the container minimizes pressure at leak level		Pressure at leak level is stable and according to values below	
Pressure	Ambient (no overpressure)	Ambient (pressure only given by immersion height)	70 mbarg	300 mbarg
MALL	~12 µm	~2 µm	~10 µm	~2 µm

5.2 Risk Assessments and Experiences with 100% Testing

An end user performed a risk analysis that led to the conclusion that they needed to implement highly sensitive test methods, but with different detection limits for the storage and the transfer part of the SUS. This outcome is based on the following:

- Based on review of deviations, the company identified that there is a remaining risk in the manufacturing of bag chambers. Some manual operations, made during critical steps of bag chamber manufacturing such as welding around tubing on 2D bags end port seal[3] or making a K-weld[3] in 3D bags, remain significantly operator

[3] See 'SUS Bag Assembly Anatomy' in BioPhorum's 'Disposables: Single-use systems bag assembly leakage and defect toolkit' (reference [12]) for further details

dependent. For this reason, despite validation of the welding process, there is a remaining risk that should be covered with helium test performed on 100% of the production, having a detection limit of 2-3 µm NDOS

- The subpart of the SUS associated with storage will have long contact time with the process fluid; the risk of contamination is therefore higher. For this reason, a BCT by immersion was requested by some authorities to ensure that the risk is covered. Since the MALL for BCT by immersion is in the range of 2-3 µm NDOS, as described in the previous section, the 100% in-process manufacturing release test should be of same sensitivity, if possible
- The subpart of the SUS associated with transfer will have a short contact time; the risk of microbial ingress is therefore lower. 10 µm NDOS is a detection limit below the most stringent value found in the literature for non-pressurized BCT by aerosolization. See previous section, plus references [8] and [9]

Integrity assurance of SUS is a joint responsibility between SUS suppliers and end users, where several measures can be implemented along the SUS's life cycle to mitigate the risk of having an integrity loss (see Volume 1). For several reasons, testing the integrity of SUS using helium tracer gas (currently the most sensitive technology) is meant to be done at the end of the supplier manufacturing process to confirm the inherent integrity of the SUS. One reason is that the technology is best adapted when testing a non-sterile SUS. It is important to understand that such technology is quite complex to handle, requiring a high degree of technical expertise and a close follow-up of the equipment. Without rigorous and demanding preventive maintenance, it can quickly become out of order and generate supply continuity issues. However, strictly adhering to certain rules can make this technology fully reliable:

- Performing frequent controls like self-tests and calibrations significantly contribute to a smooth operation and reliable detection of defects
- Performing rigorous preventive maintenance to help avoid machine downtimes
- Designing properly and robustly the test equipment and the test method to obtain a reasonable testing cycle time, compatible with manufacturing capacities
- Applying systematically a QbD approach, starting with choosing components proven to stand the helium test

The high degree of design variability of SUS challenges SUS suppliers to develop several product-specific test systems or to create a flexible, modular setup. Modular setups have limitations when considering that test article volumes may span several milliliters up to several hundred liters.

Even though using this technology adds operational constraints on SUS suppliers, after having implemented 100% integrity testing for several years now in routine production, it is perceived by the end user as a valuable measure for SUS used in critical-to-integrity applications. It allows for detecting and rejecting non-integral SUS, but it is also a powerful tool for continuous improvement of SUS design and quality. As described in section 4.1.6, 100% testing is also used to monitor scrap rates and investigate rejected items, in case of abnormal scrap rates. Conducting this investigation to identify the root cause and recommending design change in collaboration with the end user can help to consistently improve the quality of SUS.

5.3 Updates on Helium Integrity Testing Technology

5.3.1 Integrity Testing at 10 µm NDOS Detection Limit for Final Fill Transfer Sets

Extending the range of SUS tested with the helium testing technology, some suppliers are now offering the possibility to test filling sets equipped with a 2D surge bag. These filling sets are characterized by the presence of long tubing lines, more permeable to helium than the bag. The sensitivity has therefore been limited to a 10 µm NDOS defect size. This is

aligned with the logic that such systems are used in a transfer application, which can be covered by an aerosol microbial challenge.

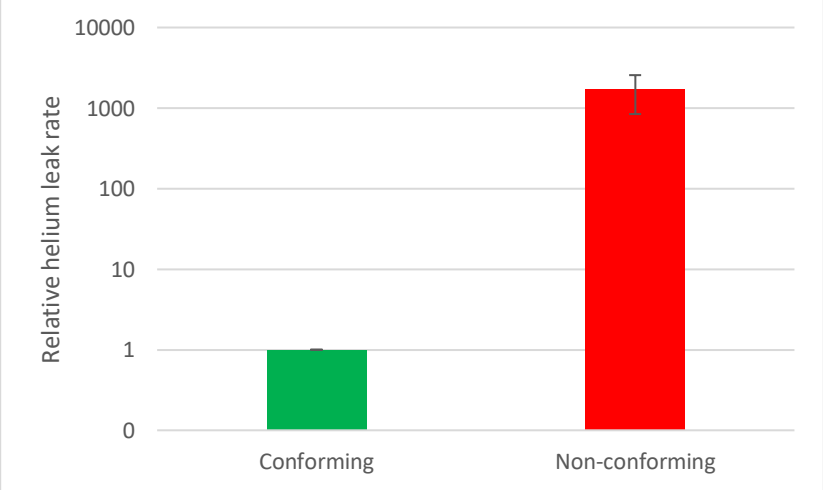
Such 100% supplier integrity testing can be used as a key in-process manufacturing release test for filling sets (or part of them) implemented outside of the core sterile grade A environment.

5.3.2 Integrity Testing at 2 µm NDOS Detection Limit for SUS up to 650 L

A SUS supplier has successfully validated integrity testing using helium tracer gas in vacuum mode on 3D storage and shipping bag assemblies up to 500 L volume and mixing bag assemblies up to 650 L, with a limit of detection at 2 µm NDOS. In this case, the two different maximum volumes are not due to technical limitations but are based on the product range offering.

As SUS are often composed of several polymeric materials with different gas barrier characteristics and vary a lot in terms of design (e.g., internal volume, length and dimension of tubing, number, and type of components), it is important to carefully define families of products for the validation of the test method [10]. The first step is the identification of extremes for each family, based on used material and design. This was done using a DoE for testing different combinations of components and materials to evaluate the impact of helium permeation, as well as absorption and desorption characteristics during the test. The same test method was used with identical parameters (vacuum level, helium background level, helium test pressure) to be used for routine testing. The time from which helium permeation starts and the amount of permeation per material quantity (per tubing length) were evaluated. While the helium permeation is important for the sensitivity of the test method, helium absorption and desorption characteristics were evaluated to assess the general testability related to manufacturing output requirements. The second step is the determination of the pass/fail criteria for each individual family by using positive and negative controls (non-defective SUS and those with an artificially created and calibrated leak attached).

Figure 14: Helium leak rate of conforming and non-conforming SUS



As shown in Figure 14 above, in this case the SUS supplier was able to validate the entire design space as one family, meaning with only one set of test parameters and acceptance criteria. Using multiple test articles, the test was validated as being able to distinguish between positive and negative controls with a confidence interval of six times the standard deviation (6 sigma). In this case, the average value of positive controls (non-conforming test articles) shows a distance of about six sigma to the acceptance criteria. Due to the measurement principle and with the pass/fail evaluation being

done before permeation takes place, the helium leak rate for negative controls (conforming test articles) is very repeatable at a low level, therefore resulting in a very small standard deviation. The average value of negative controls shows a distance of about 440 sigma to the acceptance criteria. In addition, two types of pinhole defects were used, in film material (Figure 15a) and capillaries (Figure 15b), to represent different failure modes in the SUS.

Figure 15: Artificial defects used for integrity test method validation

a: *Laser-drilled patches welded into the bag* b: *Capillaries attached to a tubing end*

5.3.3 Validation of Physical Test Methods

To ensure integrity testing performed on SUS is effective and accurate, the validation of the test method should consider the SUS properties (pressure capabilities, volume, material properties, etc.). As described in reference [10], the sample quantity for a method validation is chosen to permit an adequate determination of representative performance. Positive and negative controls should be used to define the acceptance criteria with a confidence interval that ensures a reliable differentiation between defective and non-defective SUS. For non-destructive testing, the absence of impact of the test on the CQA of the SUS must be validated.

Identical SUS design should be used for method validation as later in the routine testing. If the validation is designed to cover a range of designs of SUS, a family approach is possible with testing the extremes of each family. Reference [10] describes in more details how to define families and identify the respective extreme designs.

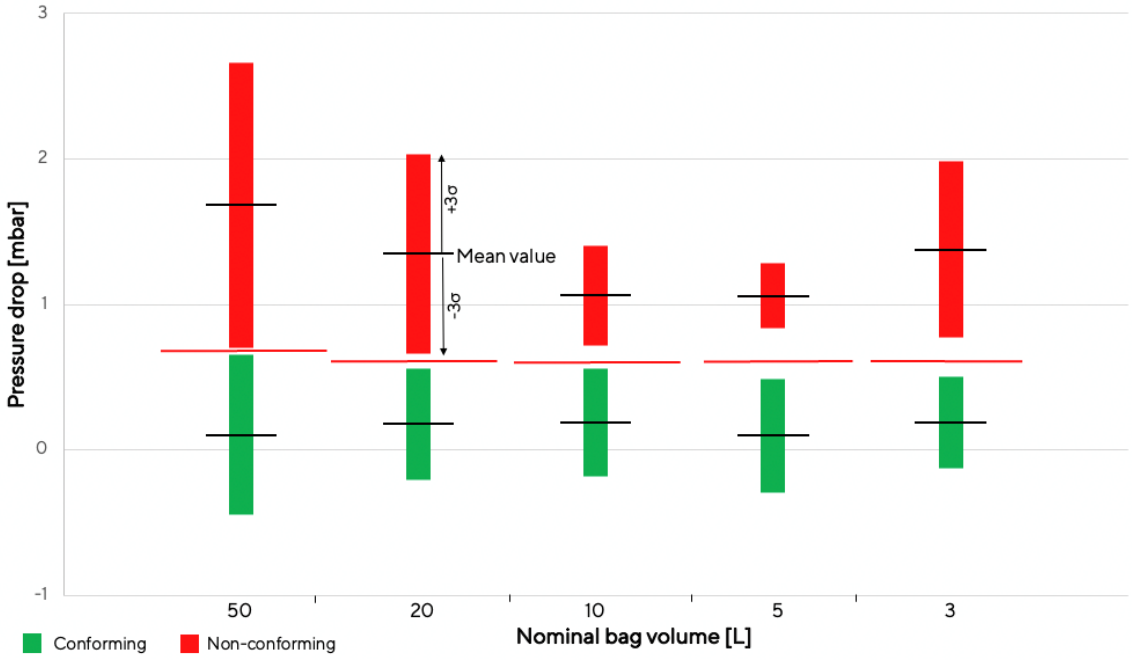
5.4 Updates on Pressure-Based Technologies

5.4.1 Pressure Decay Testing with 10 µm NDOS Sensitivity on 2D Bag Assemblies

As mentioned earlier, for small volume 2D storage applications, the pressure conditions are rather gentle compared to shipping conditions. The MALL for microbial integrity associated with such applications is in the range of 10 µm NDOS. By reducing the internal volume of 2D bag assemblies using a pair of rigid restraining plates, a SUS supplier was able to successfully validate a pressure decay integrity test with a detection limit of 10 µm NDOS. As these restraining plates provide mechanical support, it is also possible to apply a higher test pressure, which also increases the sensitivity significantly. As shown in Figure 16b, average values of negative and positive control test articles of each SUS family show a distance of at least three sigma to the acceptance criteria. The positive controls were intentionally compromised using artificially created 10 µm NDOS defects.

Figure 16a: Test set-up for pressure decay testing of 2D bag assemblies

Figure 16b: Pressure drop values of conforming and non-conforming SUS



Red lines in the figure above indicate the acceptance criteria defined further to the validation of the system.

5.4.2 Point-of-Use (PoU) Leak Testing of Single-Use Systems with the Gas Flow Methodology

Introduction

In very critical applications, such as formulation and filling, the drug product is typically of high value and/or highly potent. In addition to employing the QbD approach and performing an IPC test during SUS manufacturing, end users may want to mitigate the risk of product loss or operator exposure due to a SUS being damaged during shipping, storage, unpacking and installation. When performed at elevated pressure, typically close to the operating pressure, such a test can also reveal leaks that would be observed only during use at the operating pressure.

As part of a risk assessment and mitigation strategy, a robust approach for the end user is to perform an in-line PoU leak test of a SUS with the objective of improving the confidence that the SUS will not leak during use. Test instruments using the gas flow measurement methodology have been designed and used for such PoU leak tests since 2015.

Scope and Targeted Test Sensitivity

Two types of PoU tests are on the market: in-line and off-line tests. The key difference is that the in-line PoU test is performed after the installation of the SUS in its supportive hardware, avoiding any handling of the SUS and associated risks after completion of the test. The off-line test allows to adopt a specific testing configuration, such as the use of restraining plates (minimizing the volume of the tested SUS and allowing to test at higher pressure), to reach a higher sensitivity, but requires additional handling steps after the test. The current section presents the case of an in-line PoU test.

Unlike a manufacturing helium integrity test that offers adequate sensitivity for the detection of small pinhole defects with the risk of sterility breach, the in-line PoU leak test was developed with the more modest goal of improving the detectability of a damage that can result from shipment, storage, installation, and use. Such defects are typically larger than 200 µm in size but may not easily be detected by visual inspection after installation of the SUS in the supportive hardware. When applied to bag chambers with volumes up to 200 L, this gas flow leak test has a leak size detection limit well below 200 µm (see Table 3) and is therefore suitable for the detection of such defects in bag sizes up to 200 L, in less than 15 minutes.

Table 3: Threshold Values and Approximate Test Sensitivity for 2D and 3D Bag Chambers

Bag Chamber Size	Threshold Value @ 50 mbarg	Approximate Leak Size Detection*
5 L (2D)	3 mL/min	≥ 30 µm
10 L (2D)	6.5 mL/min	≥ 40 µm
20 L (2D)	10 mL/min	≥ 50 µm
50 L (2D)	48 mL/min	≥ 100 µm
100 L (3D)	55 mL/min	≥ 100 µm
200 L (3D)	85 mL/min	≥ 125 µm

** Assuming the leak is a perfect orifice (geometrical dimensions - not correlated to NDOS)*
Leak sizes are approximate values, based on a few tests with positive controls. They have not been validated with extensive testing of positive controls.

Test Instrument Working Principle

The test instrument applies a test gas (air or nitrogen) to the SUS. The instrument measures the gas flow to be injected into the SUS to compensate for the deformation of the SUS, plus the eventual leak coming out of the tested SUS. The final flow measurement is compared to a pre-set threshold to determine if a leak is present.

Considerations for the Test

It is necessary to have a stable environment where temperature fluctuations during the test (around 15-20 minutes duration) will remain within ±1 °C to minimize the impact on the test gas pressure in the closed volume of the tested SUS.

Figure 17: 3D and 2D SUS tested in their container

Selection of the Test Pressure

The test pressure for a SUS must be selected by balancing two competing considerations. The test pressure must be high enough to reach targeted sensitivity, but below a level that might induce permanent deformation in the film and potentially compromise the SUS integrity. In testing performed by a specific supplier, a test pressure of 50 mbarg was found to be optimal for bag chambers up to 200 L.

Determination of Threshold Values

The threshold flow values (or acceptance criteria) as shown in Table 3 can be defined by testing a set of new non-leaking bag chambers in their respective bag holder and choosing a value that covers three standard deviations above the mean flow value obtained for these negative control samples (non-leaking samples). This is repeated for each size. The threshold values for the different sizes are shown in Table 3. These threshold values are valid for tests performed at a room temperature ranging from 15 to 25 °C with a stable temperature (within ±1 °C during the test).

Conclusion

The gas flow measurement technology can be used for in-line PoU SUS leak testing, to detect damage that could have occurred between the time the SUS was assembled and tested in manufacturing and the time it is used. When performed at elevated pressure, leaks that would only be observed during use are put in evidence. Test instruments are available for running in-line PoU gas flow leak tests. With such instruments, no handling of the SUS is required between the pre-use test and the subsequent processing step. The sensitivity of the test on SUS of volumes up to 200 L is adequate for improved detectability of damage that can result from shipment, storage, unpacking and installation of the SUS. Performing an in-line PoU leak test on SUS is an option available to the end user to reinforce the assurance of integrity, which can be of high interest for high-value products in critical processing steps conducted outside of the grade A area or with highly potent drugs. This approach has been designed to be easily implemented in a cGMP manufacturing environment and has been successfully implemented in the industry for several years, especially in formulation and filling steps. Potential use of such a test method should be considered by the end user when performing the risk assessment covering the use of SUS in their processes.

5.4.3 Pressure Decay Integrity Testing on Filter Assembly

Introduction

Some SUS suppliers offer an optional high-sensitivity air-flow manufacturing-release integrity test on each unit of a lot (a "100%" release test). This test is available upon end user request based on their internal risk assessment. The applicability of the test to a specific SUS configuration needs to be discussed and agreed upon with the SUS supplier.

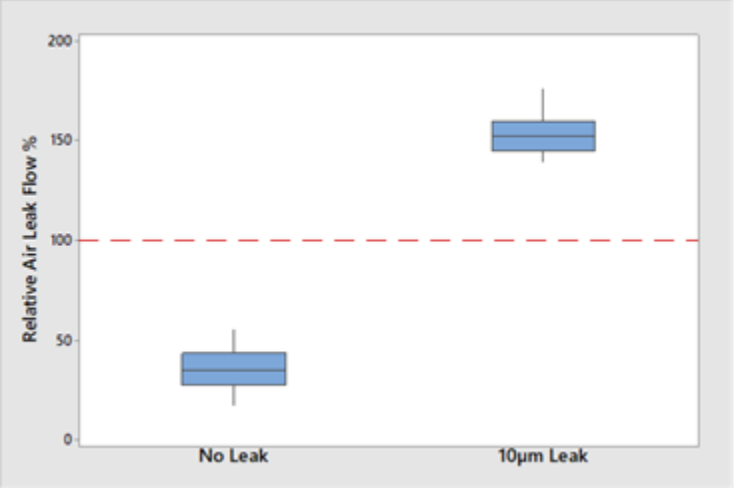
Once the request is accepted, a feasibility study is performed to determine if the required sensitivity can be achieved. Depending on the outcome of the feasibility study, the supplier can proceed with the validation of the test.

Case Study

An integrity test with a detection limit of 10 µm NDOS for a SUS including a 10" filter and braided tubing using the pressure decay test method was successfully validated. The acceptance criterion was set at 10 µm NDOS, corresponding to the MALL for microbial integrity associated with the use conditions, as described in sections 5.1 and 5.2.

The pressure drop, time of measurement and volume of the tested assembly are used by the equipment to give an air leak flow value, in mL/min. Positive controls were obtained by attaching a known reference leak of 10 µm to standard, non-leaking assemblies. This reference 10 µm leak was calibrated by comparing it to a 10 µm NDOS leak under the test conditions (same pressures at both sides of the reference leak, same test gas). The result of this calibration showed a similar leak rate (deviation of the leak rate values in mL/min < 2%). As shown in Figure 18, presenting the outcome of the validation of the test, negative controls and positive controls could reliably be differentiated from the acceptance criteria (red dashed line). The distance of the negative controls mean leak rate value to the acceptance criteria is larger than six sigma (of the negative controls values) and one of the positive controls mean leak rate value to the acceptance criteria is larger than five sigma (of the positive controls values).

Figure 18: Air leak flow values of negative and positive controls of the filter assembly test



NB: the "boxplots" indicated in the figure give some information on the statistical distribution of the population (1st, 2nd, 3rd and 4th quartiles).

Conclusion

Based on the data shown above, a SUS including tubing and filters can be subjected to a high sensitivity air leak flow integrity test with a sensitivity as high as 10 µm NDOS, used as a manufacturing release test.

6 Handling Practices and Training

6.1 Introduction

Good handling practices and an exhaustive training approach are two elements that are paramount in supporting assurance of integrity of SUS. Such systems require a lot of manual operations, and operators must therefore be aware of the SUS specific characteristics and of all precautions to be taken that prevent damaging the SUS during all steps of their life cycle in the end user factory [11]. A specific and detailed visual observation of the systems to detect signs of potential critical damage is key to complement the approach to support the assurance of integrity.

As mentioned by BioPhorum in the introduction of its "Single-Use Systems Bag Assembly Leakage and Defect Toolkit", improper handling of bags is a significant contributor to leaks in single-use systems [12]. This BioPhorum toolkit is described in more detail in section 7.2.4. Improper handling may cause significant, often unreported, failures of systems due to tearing and punctures, and subsequent product losses. Associated costs (including investigations, lost time, and product losses) can represent several millions of dollars and cause market supply problems.

6.2 Handling Practices

Implementation of single-use systems should therefore come with a comprehensive program of rigorous and detailed operating procedures and trainings. This must encompass the full life cycle of the SUS, in both supplier and end user facilities. For the end user, it starts with visual inspection of the SUS packages as the boxes are received. This and all subsequent steps to support integrity assurance should be well defined. For example, precautions taken during intermediate storage of SUS when taken out of their boxes are sometimes underestimated. A carefully designed, specific handling and installation procedure can significantly simplify the manipulations of the operators and minimize risks of damage to the SUS.

When designing the SUS, specific attention must be given to the handling and installation of complex SUS, to make these steps as easy as possible for the operator(s) and to prevent damage. For example, different solutions should be developed to properly handle large single-use bioreactors, especially when the bioreactor requires handling by more than one operator. Another classic example is associated with the removal of the overwrapping packaging. This step should be done with enough space in the cleanroom, on a flat surface large enough to support the entire SUS and prevent bending of parts of the SUS (when, for example, they hang over the borders of the table). Similarly, automation should be considered when the SUS operation is complex, such as PUPSIT systems.

Applying a holistic approach corresponds exactly to the expectations of the authorities, as described in detail in the new revision of the EU cGMP Annex 1 [13]. See section 7.4 for more details.

6.3 Training

SUS training programs must leverage the best learning methodologies. Information should be accessible and useful, taking into consideration employees with limited or no experience in biopharmaceutical manufacturing. It is equally as important that SUS operators know the dos and don’ts of proper handling as it is to know how to properly maintain the cleanroom environment and performing tasks without causing damage.

Learning style is critical to the good assimilation of a training content. Studies have shown that most adults prefer to learn by doing and will retain more knowledge when participating in the learning process, such as in group discussions, practicing, and teaching others [14]. Of various passive methods, audio-visual and demonstration approaches provide the highest retention levels.

Modern training approaches use a blend of face-to-face, e-learning, virtual classrooms, virtual reality and mixed reality training, to optimise the training efficiency. Activities are focused on achieving rapid SUT knowledge transfer in a scalable manner to ensure the workforce can successfully and reliably operate cGMP-compliant, critical, single-use bioprocesses. Self-directed programs (in several languages) like on-demand solutions are useful for all levels of learning and understanding. Modules can be complemented with easy-to-follow guidance and knowledge check on the rules and procedures for maintaining adequate conditions, taking into consideration that access to actual installed SUS and cleanroom suites is limited. Virtual reality (VR) is an exceptional complement to in person and e-learning methods, as it provides a more real feeling and tactile feedback, which are important for SUS.

Typical training required to support the use of SUS and which must be translated to SOPs by the end-user include:

Table 4: List of Typical Tools Supporting the Use of SUS on the Shop Floor

Supplier Documents and Trainings		**End-User SOP and Tools**
Handling practices - do's and don'ts		SOP for SUS handling and installation in hardware
PoU testing (when applied)		SOP for PoU testing
IFU - Instructions for Use	⇨	SOP for operating the SUS, including controls during use SOP for closing, disconnection and disposal of the SUS
Single-Use Visual Observation Library (SUVOL) [12]		SOP for visual checks at different steps of the process
BioPhorum Recommendations		
Single-Use Systems Bag Assembly Leakage and Defect Toolkit [12]		SUS event response checklist, as listed in section 7.2.4
		SUS leak root cause analysis guidance, as listed in section 7.2.4

Such rigorous training methodology is the best guarantee to get consistent and reproducible practices that reliably support the assurance of SUS integrity and full control of the process.

7 Industry Interest Group Initiatives and Regulatory Updates

7.1 BPSA Reliability Data - Review of Integrity Issues and Comments

As the importance of SUS grows, so does the impact to manufacturing associated with the unintended use of a defective system.

Drug manufacturers' risk management of SUS can be greatly facilitated by the availability of consolidated, accurate industry-wide data on defect rates. Without such data, drug manufacturers are forced to rely on internal data and are likely to be influenced by anecdotal reports or feelings of high failure rates.

To counter this, a professional, data-driven industry-wide measure of quality enables suppliers and end users to benchmark not only against their own internal quality and trending data, but also against the industry. This is an important promoter of quality improvement within the whole SUS industry.

Dating back to 2017, the BPSA leadership team envisioned a pilot program, to collect and share pertinent industry data. This led to the release in November 2019 of the BPSA benchmarking report "A Data-Driven Assessment of Single-Use System Reliability: 2016-2018". An update of this report [15] was released in June 2021, covering the 2016-2019 period.

This report utilizes data collected from main systems integrators of SUS, presenting complaint rates for nine complaint categories around five SUS product categories. All information has been aggregated and anonymized to prevent any identification of any specific company information.

Some of these nine complaint categories are directly related to integrity, as illustrated in the example below, from the 2020 BPSA report, as updated:

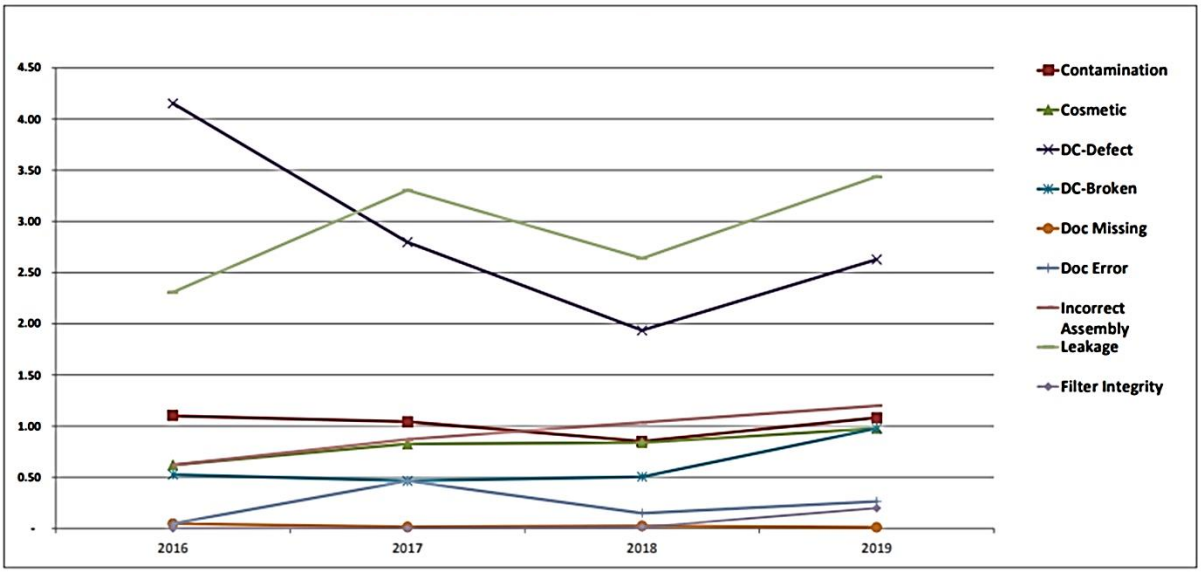
Figure 19: Complaint data associated with "2D & 3D bioreactors" - 2020 update

2020 BENCHMARKING REPORT

	2016	2017	2018	2019
Contamination	1.10	1.04	0.85	1.08
Cosmetic	0.62	0.83	0.84	0.98
Defective Component-Defect	4.15	2.80	1.94	2.63
Defective Component-Broken	0.53	0.46	0.51	0.98
Doc Missing	0.05	0.01	0.02	0.01
Doc Error	0.05	0.46	0.15	0.26
Incorrect Assembly	0.62	0.87	1.04	1.20
Leakage	2.31	3.30	2.64	3.44
Filter Integrity	-	-	0.01	0.20
Overall	9.42	9.78	7.99	10.77

("0" interpreted as statistically insignificant)

2D & 3D BIOREACTORS



This report is a key tool to enable risk assessment based on reliable, industry-wide facts and data. The data can be shared with BPSA members upon request, after completing the inquiry form on BPSA's website.

7.2 Updates on Guidance and Standards Issued by Industry Interest Groups

An overview of industry guidance and standards on integrity was presented in Volume 1, Part 2, Table I. Significant effort has been made over the last few years to provide more specific recommendations on SUS integrity and SUS integrity assurance, resulting in the release of key reference documents. The following sections detail the most important documents published since the release of Volume 1.

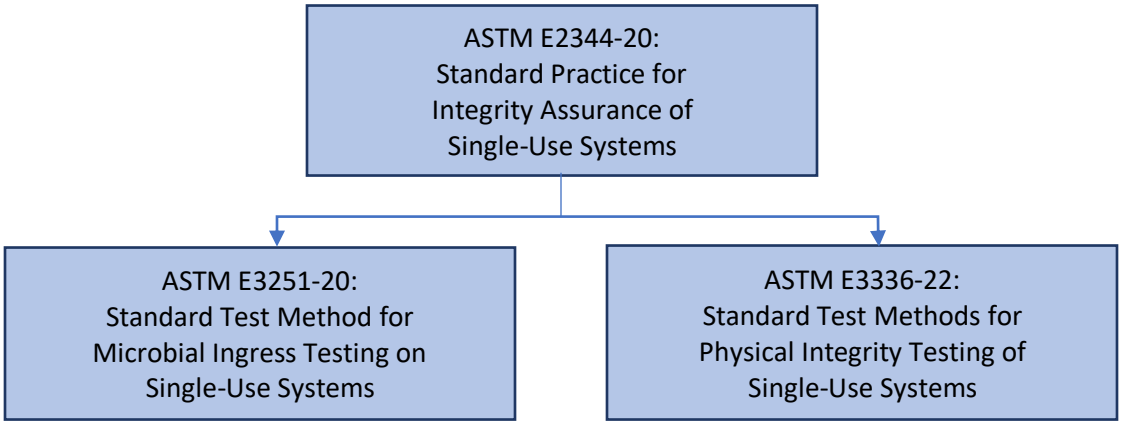
7.2.1 ASTM International Standards

The ASTM International E55 Committee on the "Manufacture of Pharmaceutical and Biopharmaceutical Products" has been particularly active in issuing standards on the integrity of SUS. Two standards were published in 2020, and a third was published in 2022:

- ASTM E3244-20: Standard Practice for Integrity Assurance and Testing of Single-Use Systems [2]
- ASTM E3251-20: Standard Test Method for Microbial Ingress Testing on Single-Use Systems [3]
- ASTM E3336-22: Standard Test Methods for Physical Integrity Testing of Single-Use Systems [10]

ASTM E3244-20 is an overarching standard, providing up-to-date standard practice for performing a robust risk assessment to support SUS integrity assurance. The two other standards provide detailed information to perform integrity testing on SUS.

Figure 20: Hierarchy between the ASTM standards on SUS integrity



ASTM E3244-20 is well aligned with the BPSA Technical Guide Volume 1 and many elements included in USP <1207> [16]. It usefully closes the gap left by USP <1207>, which explicitly excludes the SUS from its scope.

7.2.2 ASME BPE, 2022 Edition

This edition of the ASME BPE [18] contains an updated section on SUS integrity controls, including a non-mandatory table on commonly used leak test methods. It provides a useful mapping of the main methods used by the industry.

7.2.3 PDA Technical Report 86

PDA Technical Report 86, "Industry Challenges and Current Technologies for Pharmaceutical Package Integrity Testing" [19], published in May 2021, focuses on advanced issues related to pharmaceutical package integrity, covering challenges encountered with complex packaging systems, such as large volume bulk packages and prefilled syringes systems. It provides updated information not addressed in PDA TR 27 and USP <1207>, covering final packaging containers and SUS.

As other state-of-the-art reference documents, it recommends applying a QRM approach based on the SUS life cycle and distinguishes a "design life cycle" and a "routine, manufacturing and use life cycle".

A case study illustrating the approach in some detail is provided as a useful reference. This case study covers a final packaging system, but it is easily transferrable to a SUS used in a critical, sterile application.

7.2.4 BioPhorum Single-Use Systems Bag Assembly Leakage and Defect Toolkit

In January 2021, BioPhorum released an interesting package focused on SUS titled, "Disposables: Single-Use Systems Bag Assembly Leakage and Defect Toolkit" [12]. This package provides a set of tools to be included in trainings or utilized in responding to issues. This toolkit is intended to reduce product losses, investigations, and manufacturing disruptions.

The package is divided into five modules:

- **SUS bag assembly anatomy**, providing commonly used names for components included in SUS, useful for standardizing the vocabulary and improving the communications involving SUS.
- **SUS visual observation library**, providing clear terminology to help create detailed, precise descriptions of observations made during visual inspection, promoting better understanding of what constitute "critical

defects" and what count as "minor cosmetic items". It provides the foundation for single-use suppliers to issue their own "visual observation library," an essential reference document for end users to set up robust and adequate visual controls at each step of the SUS use life cycle.

- **SU event response checklist**, ensuring consistent and complete recording of key information in case of issue, essential for proper communication both internally and between the end user and the supplier.
- **SU leak root cause analysis guidance**, providing guidance for investigations, with a list of questions specific to the use and handling of SUS.
- **Guidance for developing effective bag handling training**, providing elements to be included in SUS handling training for manufacturing operators to minimize the risk of mistakes. It is important that the training is adapted to the specific SUS case.

7.3 Findings from the Development of ASTM Standards on Single-Use Systems Integrity

During the development of the listed ASTM standards, some questions arose repeatedly. It was also realized that even within the task groups that were composed of dedicated SMEs, some terms were used interchangeably, and definitions were not aligned. Three topics were discussed intensively:

- Clear definition and differentiation between leak and integrity testing in the scope of SUS. As explained in section 4.1, there is a clear difference between leak and integrity testing. The main criterion to name a test as an integrity test is the correlation of its detection limit to the barrier properties of the SUS
- Introduction of the concept of "Maximum Allowable Leakage Limit" (MALL) for SUS. To be maintained under the intended use-case conditions, each barrier property is associated with a MALL. Different cases of barrier properties, their corresponding MALLs and how the correlation can be done, are described in more details in section 5.1 and 5.2, as well as in the mentioned ASTM standards [2, 3, 10]
- Quantity of statistically and scientifically significant number of test articles, both for microbial and physical testing. There is no final, definite answer to this question. Specifying the quantity of samples in general terms is not possible as this number depends on several specific parameters. Individual decisions should be taken based on internal SOPs and quality guidelines, as well as the level of confidence targeted

7.4 Revision of EU GMP Annex 1

"Annex 1: Manufacture of Sterile Products" complements key chapters 3 and 5 of EU cGMP and was first published in 1971 to ensure sterility of medicinal products. A profound revision started a few years ago, and the update of this fundamental regulatory document was issued in August 2022 [13]. This document requires application of principles of Quality Risk Management, and to document a "Contamination Control Strategy" (CCS). It requires a transversal, holistic approach from the end user to ensure that contamination is prevented in the final product. All elements of the end user manufacturing process must be considered, like APIs, excipients, primary product components, consumables, SUS, etc., until the final packaging. Contamination is considered under its different forms: microbial, particulates, pyrogens, chemical.

The last draft of Annex 1 includes sections of specific interest for SUS:

- Filter sterilization (sections 8.79 to 8.95), with the main topic of debate being the PUPSIT
- Closed systems (sections 8.127 to 8.130)
- Single use systems (SUS) (sections 8.131 to 8.139)

As a strong emphasis is put on the microbial contamination risk, the integrity of closed systems and SUS is expected to be carefully risk assessed, and appropriate measures should be in place to ensure integrity. Supplier assessment must include the collation of data associated with risk of loss of sterility.

Specific risks associated with SUS must be documented in the CCS. Here again, the risk of integrity failure is mentioned several times in the section on SUS. It is clearly the most important risk identified by the authorities. Visual inspection must be performed by the end user at different steps of the use cycle of the SUS, reinforcing the need for a visual observation library. Risks associated with the handling of the SUS are also identified, implying proper training. Finally, appropriate system integrity tests should be considered when there is a risk of compromising product sterility.

It is important to note that despite the title of the document refers to sterile products, some of the principles and guidance, such as contamination control strategy, design of premises, cleanroom classification, qualification, validation, monitoring and personnel gowning, is expected to be used to support the manufacture of other products that are not intended to be sterile such as low bioburden biological intermediates, but where the control and reduction of microbial, particulate and endotoxin/pyrogen contamination is considered important.

All these elements highlight the importance of a reference document like the present guide.

8 Specific Case Studies

8.1 Single-Use Systems Used at Elevated Pressure: Applying a Gas Flow Test Method for the Confirmation of the Integrity of SUS Assemblies in Critical Final Sterile Filtration Applications

This section examines how a gas flow leak test can be conducted with test pressures up to 3.5 barg at the point of use on a redundant filtration assembly to increase the confidence that the assembly will not leak when exposed to high pressures during PUPSIT or during filtration. The pressure for the leak test on this specific SUS design (Figure 21) was limited to 0.4 barg, which is the maximum allowed operating pressure for the filtrated tubing.

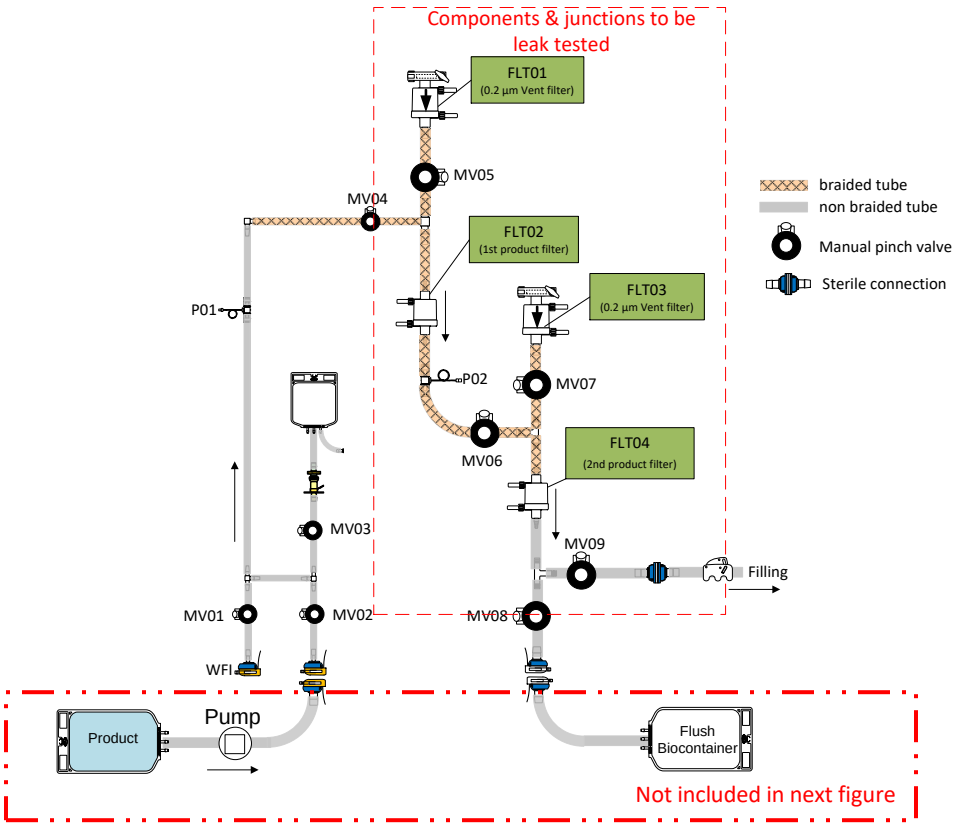
8.1.1 Identification of Risks for Leaks with High Pressures

SUS assemblies can be exposed to high pressures before or during use. This can lead to increased risks for leaks from the pressurized SUS components and/or the pressurized junctions between the SUS components [20]. One typical example is the test pressure being applied during PUPSIT on filters being used for the final sterile filtration of drug products. Although these SUS are designed to withstand such high operating pressure, they are not individually tested at such a high pressure during SUS manufacturing in-process control. Risk assessments and experience suggest there is a potential risk for leaks at junctions between pressurized components if the junction is not properly secured during SUS manufacturing (assembly error), or if the integrity of the junction is jeopardized during SUS handling (shipping, storage, installation).

An end user risk assessment was conducted for the redundant filtration assembly presented in Figure 21. This risk assessment identified the risk for leaks upstream of the filters due to the high pressure applied on these components and SUS junctions during in-situ PUPSIT operations. Such leaks upstream of the filters would typically be detected by failed PUPSIT test(s). The risk assessment also identified the risk for leaks downstream of the filters due to the SUS handling before use or due to the pressure being applied during filtration. Such leaks downstream of the filters represent a risk for the sterility of the filtered product combined with a risk for operator exposure and would not be detected by a failed PUPSIT test because the filtrate line is kept at ambient pressure (no overpressure) during PUPSIT.

The integrity of the SUS components and SUS junctions downstream of the sterilizing filters is highly critical, and even more so when the redundant filtration assembly is to be used outside a grade A area and the filtered product cannot be terminally sterilized in the filled primary containers. Based on their own risk assessment and associated risk mitigation strategy, the end user requested a point of use, pre-use leak test be developed and implemented for all components identified in Figure 21. The scope for this test was to cover all components that would be pressurized during PUPSIT and all SUS components with product contact downstream of the second filter including the sterile connection to the filling equipment.

Figure 21: Scope for the leak test on redundant filtration assembly



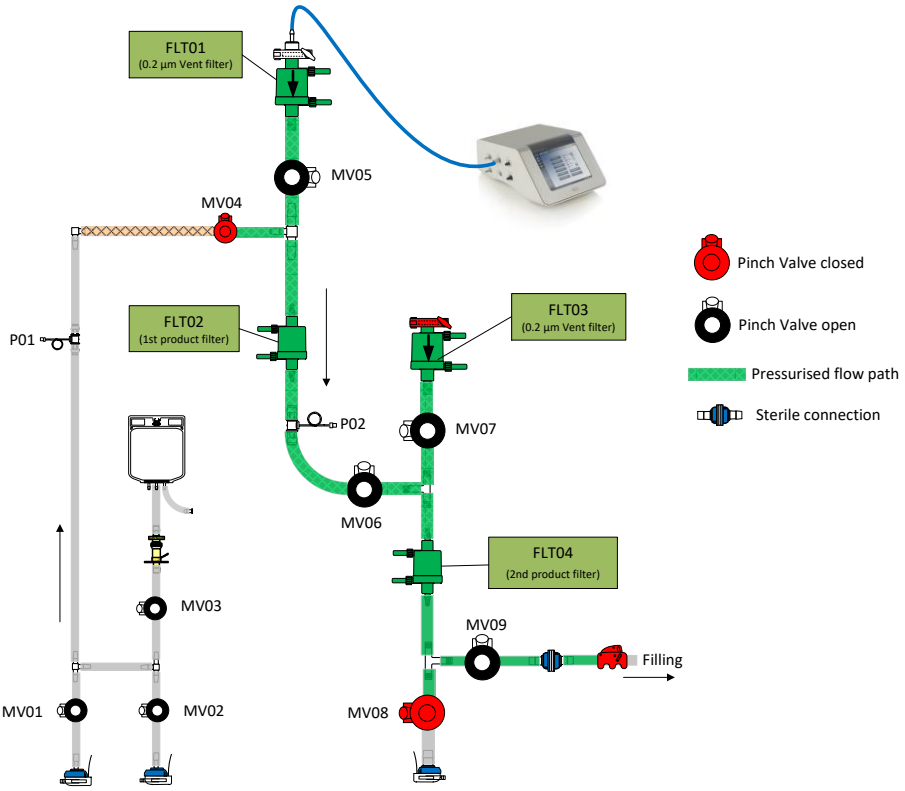
8.1.2 Leak Test Methodology

The test is performed aseptically pre-use after connection of the test instrument via the 0.2 µm gas filter FLT01 at the top of the dry assembly. See Figure 21.

Before starting the test, the pinch valves are closed on the SUS assembly to achieve a closed volume in the required test area. See Figure 22.

When the leak test is started, the instrument pressurizes the dry assembly at the required test pressure and measures the injected gas flow that is required to keep the test pressure constant in the closed volume of the tested assembly. See Figure 22.

Figure 22: Leak test on redundant filtration assembly - pressurized flow path highlighted in green



8.1.3 Test Parameters

As done generally, the test parameters were selected according to the design of the SUS, as

- The test pressure was limited by the component of lowest pressure rating. For the design presented in Figure 22, the test pressure was limited to the maximum operating pressure of the tube on the outlet of the second product filter
- The maximum flow limit (acceptance criteria) was impacted by the combined effect of the gas flow due to the air diffusion (permeation) through the wall of the pressurized tubes and the gas flow to compensate to the tubing expansion at the applied test pressure

As is typical, the maximum allowed flow for a non-leaking assembly was set at three standard deviations above the average leak flow measured on a series of negative controls (non-leaking assemblies). Any flow measurement above this acceptance level was considered as a "FAIL" result. Test parameters for the given SUS design were recommended by the SUS supplier based on their knowledge of the background flow for typical SUS assemblies at different test pressures.

A test time of 10 minutes was used to achieve a stabilized flow reading.

8.1.4 Sensitivity of the Test

The sensitivity of the leak test can be estimated by adding a calibrated orifice to non-leaking assemblies to obtain positive controls (leaking assemblies of known leak level). The smallest orifice leading to systematic rejection of the positive controls (e.g., with average minus three standard deviations being above the acceptance level) corresponds to the sensitivity of the test.

For typical redundant filtration assemblies as illustrated in Figures 21 and 22, a sensitivity of 30 µm NDOS can typically be achieved. A sensitivity down to 10 µm NDOS can be reached for smaller, optimized assemblies when running the test at high test pressures.

The sensitivity of the leak test is dependent on

- The test pressure: increasing the pressure will increase the leak flow through the calibrated orifice but will also proportionally increase the background flow due to gas permeation through the wall of the tubing
- The size of the tested SUS: shorter tubing length will lower the background flow due to permeation, hence improving the test sensitivity
- The tubing type: thermoplastic elastomer (TPE) tubing exhibits a significantly lower gas permeation rate than silicone tubing. TPE tubing can therefore be expected to give a lower background flow and a corresponding higher signal to noise ratio, enabling better sensitivity to be achieved with the test

8.1.5 General SUS Design Recommendations

For mitigation of the risks for SUS leaks, it is recommended that the complexity of the SUS designs be minimized by limiting the number of SUS components and junctions to a minimum and getting all junctions validated for the intended pressures during use and integrity/leak testing.

8.2 Containment of Powders

The need for special handling of powders has not traditionally been a major area of concern in the bioprocess industry; rather, the focus has been on liquid handling, sterility, and production yields. There is, however, a growing appreciation that improved powder handling and the use of containment technology can provide significant benefits.

This section discusses the risks associated with both toxic and non-toxic powders, and the potential benefits of deploying containment solutions. The implementation of reusable and single-use containment technologies is reviewed, and the relative merits addressed.

8.2.1 Risk Evaluation

Concerns related to powder containment typically fall into two categories:

- Operating cost concerns
- Environmental, Health & Safety (EH&S) concerns

The overwhelming majority of powders used in bioprocessing contexts are not considered especially toxic or dangerous. Media powders, buffer salts, acids, and bases, while potentially harmful in cases of excessive contact or ingestion, do not present an unusual hazard to the health of operators handling them. Rather they are considered “nuisance” powders requiring only ordinary precautions and personal protective equipment (PPE). The frequent handling of these materials can even result in a degree of complacency.

Despite a perception of less severe EH&S consequences, containment and protection of these materials remain important due to the considerable operating cost implications of open handling:

- The loss, adulteration or contamination of valuable materials is costly and, when supply chains are constrained, such losses may not always be quickly or easily replaced
- Clean-up and internal reporting on spills impose significant manpower costs and downtime
- Concerns about material migration can lead to inefficient asset utilization (e.g., only running one process in a room at a time due to fear of cross-contamination), particularly in multi-use facilities

In addition to cleanliness concerns, spills of non-toxic powders can create a significant safety hazard that may not be obvious to most users: dust ignition. The U.S. National Fire Protection Association reports that the U.S. alone experienced over 200 dust fires or explosions in 2019. Such incidents present obvious dangers to personnel and plant resources, potentially shutting down whole facilities for extended periods of time while investigation, cleanup and repair are underway.

Despite representing a minority of powders handled in bioprocessing, toxic materials present a danger wholly out of proportion to their quantity. Amongst the concerns are:

- Harm to the health of personnel, and potentially the public
- Disproportionately high clean-up costs
- Intense EH&S and regulatory scrutiny

8.2.2 Characterizing Toxicity

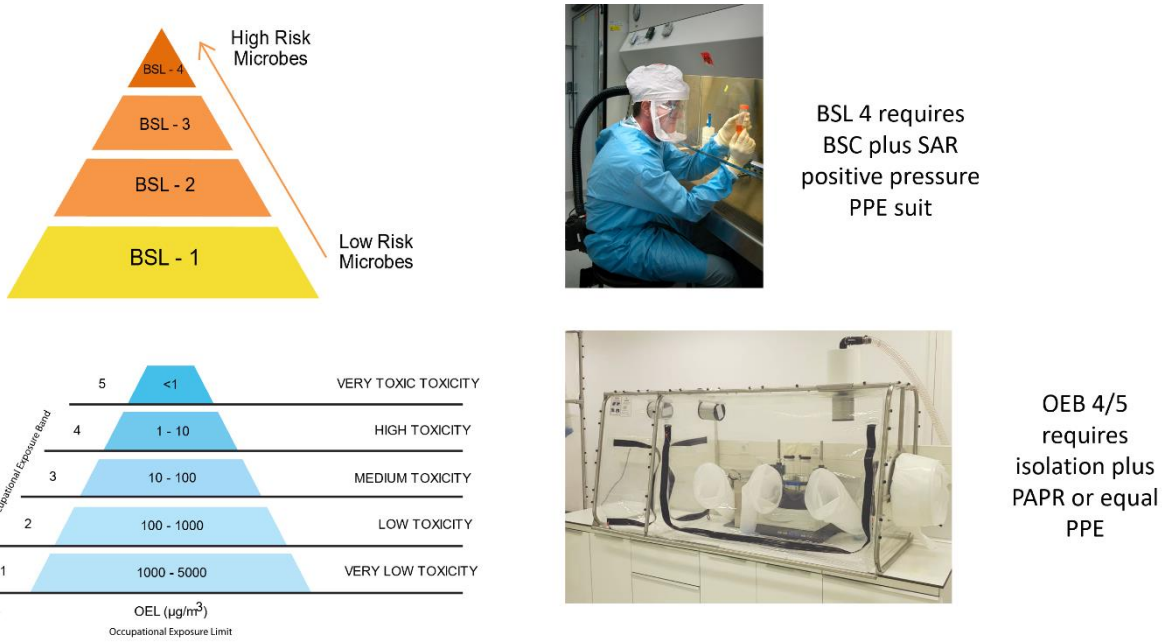
Toxic materials and high-potency APIs can cause severe health effects in humans and, depending on their toxicity, can occur at very low airborne concentrations.

Such materials are typically classified based on their toxicity level and consequent occupational exposure limit (OEL). While OEL value is derived from the maximum airborne concentration of the material that an operator can be exposed to over a working lifetime, the compounding effect of potential exposure to multiple materials should also be considered.

The need to assess risk when handling toxic materials is clear, but the methods for doing so are not necessarily well understood in the bioprocess industry. One common approach is to evaluate the exposure potential (EP) of a process and align that with toxicology work that places an airborne particulate material into an occupational exposure band (OEB).

The OEB system is a banding mechanism analogous to the biosafety level (BSL) approach developed by the U.S. Centers for Disease Control (CDC). Just as the BSL system has a four-tier ranking for microorganisms in ascending order of pathogenicity and virulence with corresponding guidelines for engineering controls, the OEB system classifies toxic compounds into predefined categories or bands depending on their respective toxicity (Figure 23).

Figure 23: Similarity of the biosafety level (BSL) and occupational exposure level (OEL) banding approaches



OEB categorization can vary from one company to another and, despite having a common designation, the OEL limits for the same banding category could be significantly different.

Lastly, OEL and OEB categorization of toxic compounds should not be confused with the containment performance target (CPT). CPT is an airborne concentration limit, typically measured in µg/m³, set for the engineering control being used to contain the material and typically verified via a containment performance test (normally following the ISPE SMEPAC - guidelines for Assessing Particulate Containment Performance of Pharmaceutical Equipment [22]) to confirm the device is fit for purpose.

8.2.3 Containment Technologies

When handling known toxic materials, regulations typically mandate protection of operators from exposure. The use of PPE is not an acceptable solution when an engineering control is possible. Fortunately, the chemical and pharmaceutical industries are well served by a diverse range of proven engineering controls designed to maintain appropriate levels of toxic material containment, and the resulting benefits from installing those engineering controls to protect the product, personnel, equipment, and facility are realized when handling both toxic and non-toxic components.

The selection and application of a suitable containment device should take into consideration the complete process requirements, including the number of potential exposure points throughout the process, the scale or volume of material being handled, and the properties of the material at the different stages of the process.

Isolator or glovebox technologies offer the highest levels of containment. These enclosures are typically bespoke, reusable systems designed to meet specific process requirements, although single-use versions have become more available in recent years.

Further downstream, streamlined technologies may be more practical. Examples include rapid transfer ports, split butterfly valves and flexible liner systems. During the selection process, it is important to bear in mind that many such devices can be proprietary in nature; the question of supply security should factor into the decision.

Where powder transfers must take place from one stage to the next, this should be performed with as few transfer operations as possible. The adopted engineering controls should not be limited to PPE but instead must include safeguarding layers designed to protect against the associated material toxicity levels. These engineering controls must provide sufficient and proven levels of containment performance, as demonstrated through qualified containment test methods.

The positioning and layout of the device can influence the effectiveness of the engineering control; for example, locating the device close to the operator's breathing zone will increase the risk of exposure to airborne particulates. Where possible, automation can reduce the need for operator intervention (and thus exposure). Any SOPs necessary to maintain equipment performance, such as wiping or decontamination, should be carried out away from the operator's breathing zone while taking into consideration ergonomic requirements.

Many containment technologies are available in reusable, single-use, and hybrid formats (Figure 24). The relative merits of reusable and single-use systems are now well understood by the bioprocess industry. While the toxicity of the material being handled may change the calculus in some applications, the risk assessment approach remains the same. Both reusable and single-use technologies have established track records for satisfactory performance in a diverse range of containment applications.

Figure 24: Examples of the hybrid approach to contained powder transfer via active/passive split butterfly valves using single-use bag/single-use passive half-valve (left) and single-use bag/reusable passive half-valve (right) systems. Both require a reusable active half of the split valve on the process equipment side.

8.2.4 Implementing Single-Use Containment

Assuring the integrity of single-use systems is particularly important in toxic material handling applications. Several publications, including from BPSA [21], address the issue of leak testing, both in manufacture and at the point of use.

A less well-known challenge of powder handling in single-use systems is the buildup of static electricity via a phenomenon called triboelectrification, which presents two potential concerns:

- Static charge causes powders to flow less freely and cling to single-use system surfaces, which inhibits efficient transfer leading to process delay and/or material losses
- Sudden discharge of un-dissipated static electricity can cause sparking, potentially leading to dust ignition – a major concern as previously discussed

Static electricity buildup can be mitigated by the use of static-dissipative materials or additives. Cost, cleanliness, suitability, and shelf-life of such additives require careful consideration. As these additives are surface-active, the risk of product contamination via dry shedding (in the case of dry powders) or leaching (in the case of slurries) should factor into the overall risk assessment. The subject of leachable risk assessment has been extensively discussed [23].

An alternate strategy is to perform a water wash-down of the powder container. Wetting the powder can reduce risk of dust ignition, dissipate static charge, and assist with more complete material transfer, but can prove less convenient. A suitable adjacent water supply is required, and the added water will need to be accounted for in the batch record.

8.2.5 Cleanliness

While the bioprocess industry is conversant with the challenges of working with liquid materials, the containment of potentially harmful powders is less familiar.

While gravity causes escaped liquids to fall and pool, thus becoming visually apparent, the same cannot be said of powders. In most cases, some proportion of a leaking powder will become airborne and thereby prone to traveling over a wider area. In the case of less harmful powders, this creates extra cleanup work. However, if the powder is toxic, it can cause considerably greater harm.

A hybrid case is obtained when powders are put in solution and a leak arises. This can result in powder crystallizing out of the evaporating pool and/or becoming aerosolized. Both situations can create an airborne powder safety hazard as well as a cleanliness risk.

Such liquid leaks should be mitigated by creating procedures for identifying and containing leaks based on a risk assessment. Containment measures for these leaks typically include:

- Ready availability of suitable PPE and spill containment resources
- Measures to detect, identify and stop leaks quickly
- Capture of small/chronic liquid leaks immediately with suitable absorbent materials, and containment of larger/acute liquid leaks with a dam or catch basin
- Management of contaminated materials and use of suitable PPE (e.g., double gloving) to avoid the spread of contamination
- Safe, legal, ethical disposal of waste
- Reporting to ensure the causes and consequences of leaks are handled in a transparent way to avoid recurrence

9 Conclusions

In the last five years, the bioprocessing industry has made tremendous progress on leak and integrity testing of SUS. The compilation of experiences of suppliers and end users included in this document is an obvious illustration of these developments and associated learnings. The examples in section 8 provide useful references for cases not often addressed in the literature and will provide some guidance on how to approach these uncommon cases.

The industry is only at the beginning of the learning path, as most of the existing components and many newly created ones are not yet incorporating the requirements associated with some of the advanced integrity control technologies discussed in this Volume 2. This gap is likely due different reasons:

- These characteristics are a "step change," and knowledge and understanding need to spread more broadly throughout the different actors of the industry
- The associated technologies are expensive and require specific skills
- The test equipment that meets the requirements for large SUS requires full custom development. No off-the-shelf testing equipment is currently proposed on the market to integrity test SUS

Case studies above clearly show that some components are meeting these characteristics, but that others, by design, cannot currently meet them. It is the responsibility of suppliers and end users to decide on what component can be used and what is required or nice to have as validation data and controls. On top of long-standing classical elements such as SUS handling practices or operator training, some details, often neglected in the past, must now be carefully taken into consideration at any step of the product life cycle to prevent very small leaks that may be present from the start or appear due to manufacturing deviations.

The interest in this matter continues to grow as it corresponds to an obvious need for new market segments such as ATMPs. Moreover, new regulatory requirements requiring a more explicit, holistic control strategy are expected to further increase the attention devoted to the integrity of SUS. More and more stringent requirements will be included in the URS of SUS. And consequently, new components and SUS will be developed, or existing components will have their manufacturing adapted (especially the manufacturing control strategy) to meet these requirements.

The case studies included here show once again the fundamental role played by QbD and the detailed characterization of the life cycle of SUS to perform a relevant risk assessment and define the adequate risk mitigation strategy across the whole life cycle of the SUS, including defining the proper integrity requirements and the test method to be chosen.

New developments are expected to emerge in the future, enriching the portfolio of technologies available to SUS suppliers and end users, which will certainly justify an update of the Technical Guide or the publication of Volume 3.

References

[1] BPSA Design, Control and Monitoring of Single Use Systems for Integrity Assurance, Bio-Process Systems Alliance, 2017, accessible on https://www2.bpsalliance.org/forms/store/ProductFormPublic/design-control-and-monitoring-of-single-use-systems-for-integrity-assurance/
[2] ASTM E3244-20: Standard Practice for Integrity Assurance and Testing of Single-Use Systems
[3] ASTM E3251-20: Standard Test Method for Microbial Ingress Testing on Single-Use Systems
[4] BPSA/BPOG Single Use User Requirement Toolkit, accessible on https://www2.bpsalliance.org/forms/store/ProductFormPublic/an-industry-proposal-for-change-notification-practices-for-single-use-biomanufacturing-systems
[5] Single-use System Integrity I: Using a Microbial Ingress Test Method to Determine the Maximum Allowable Leakage Limit (MALL) - https://journal.pda.org/content/73/5/459
[6] Single-use System Integrity II: Characterization of Liquid Leakage Mechanisms - https://journal.pda.org/content/early/2020/11/16/pdajpst.2020.012088
[7] Integrity Assurance for Single-Use Systems: End-User Perspective, C. Masy and P. Evrard, webinar accessible on https://biopharmawebinars.com/webinars/integrity-assurance-for-single-use-ststems-end-user-perspective/ - 1:10:50 to 1:14:00
[8] Single-Use Technology: Application of single-use technology in a parenteral facility for syringe filling, Dr. Andrea Detroy, Dr. Christian Matz, Mark Leykin, Ernest Jenness, and Ross W. Acucena, Supplement to BioPharm International, March 2014
[9] Assessing of Flexible Packaging Integrity: Using the Aerosolization Bacteria, Nastaran Moghimi, Sun-Jong Kim and Su-il Park, Packaging Technology and Science, March 2016, DOI: 10.1002/pts.2191
[10] ASTM E3336-22: Standard Test Methods for Physical Integrity Testing of Single-Use Systems
[11] Choosing Between Single-Use and Multiuse Technologies: An Interphex 2015 Roundtable Discussion, Cheryl Scott, BioProcess International, accessed on https://bioprocessintl.com/2015/choosing-between-single-use-and-multiuse-technologies-an-interphex-2015-roundtable-discussion/
[12] Disposables: Single-use systems bag assembly leakage and defect toolkit, Biophorum, accessed on https://www.biophorum.com/download/disposables-supply-base-reliability-single-use-systems-bag-assembly-leakage-and-defect-toolkit/
[13] Revision of Annex 1, on manufacturing of sterile medicinal products, of Eudralex volume 4, draft version 12, accessible on https://ec.europa.eu/health/system/files/2020-02/2020_annex1ps_sterile_medicinal_products_en_0.pdf
[14] Addressing the Training Gap for Single-Use Technologies, Helene Pora, accessed on https://www.bioprocessonline.com/doc/addressing-the-training-gap-for-single-use-technologies-0001
[15] A Data-Driven Assessment of Single-Use System Reliability: 2016-2019, BPSA, June 2021; enquiry form on https://bpsalliance.org/2020-bpsa-benchmarking-report/
[16] USP <1207> - Package Integrity Evaluation - Sterile Products, USP 44-NF 39 (2021)
[17] USP <1207.1> - Package Integrity Testing in the Product Life Cycle - Test Method Selection and Validation, USP 44-NF 39 (2021)
[18] ASME Bioprocessing Equipment (BPE), 2022 Edition
[19] PDA Technical Report No. 86, Industry Challenges and Current Technologies for Pharmaceutical Package Integrity Testing, Parenteral Drug Association, Inc., 2021
[20] https://bioprocessintl.com/manufacturing/single-use/pressure-rating-for-bioprocess-single-use-assemblies/
[21] https://bpsalliance.ftlbcdn.net/wp-content/uploads/2021/10/BPSA-BPI-Integrity-Paper_093021.pdf
[22] Good Practice Guide: Assessing the Particulate Containment Performance of Pharmaceutical Equipment 2nd Edition, ISPE, May 2012
[23] https://www.biophorum.com/download/best-practices-guide-for-evaluating-leachables-risk-from-polymeric-single-use-systems/

Photographs of certain products are included in this document:

1. Figure 16a, courtesy of Sartorius
2. Figure 17, courtesy of Cytiva
3. Figure 24, courtesy of ChargePoint

They have been included for illustration purposes and do not constitute any recommendation or endorsement by BPSA.

About BPSA

The Bio-Process Systems Alliance (BPSA) was formed in 2005 as an industry-led corporate member trade association dedicated to encouraging and accelerating the adoption of single-use manufacturing technologies used in the production of biopharmaceuticals and vaccines. BPSA facilitates education, sharing of best practices, development of consensus guides and business-to-business networking opportunities among its member company employees.

For more information about BPSA, visit www.bpsalliance.org.

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