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Application Note

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Supplier Integrity Testing (SIT) Based on Helium Tracer Gas Detection for Flexsafe® 3D for Storage | Shipping and Flexsafe® for Mixing

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Abstract

This technical report describes the validation of our helium tracer gas test method used for the supplier integrity test of Flexsafe® 3D for Storage | Shipping and Flexsafe® for Mixing. This test is performed by Sartorius upon request on single-use assemblies.

With its 2 µm detection limit, the test provides integrity assurance correlated to microbial ingress and liquid leaks under worst-case process conditions of these single-use assemblies.

For more information, visit
www.sartorius.com/en/products/fluid-management/proven-integrity

Introduction

Executive Summary

Sartorius Stedim Biotech has validated an integrity test method to detect potential leaks in Flexsafe® 3D for Storage | Shipping and Flexsafe® for Mixing using the helium tracer gas method.

The study is aimed at validating a leak size detection of 2 µm, over the range of Flexsafe® 3D for Storage | Shipping from 100 L to 500 L, and from 50 to 650 L for Flexsafe® for Mixing.

The validation has been carried out over several steps:
a) A Design of Experiment (DOE) to determine the impact of leak position on the single-use system (SUS),
b) Researching the range of helium filling parameters suitable for the covered range of SUS
c) The determination of reject threshold following recommendation of ASTM E3244, using negative and calibrated positive controls representing two different kinds of potential defects:
- Patches with a puncture in the film
- Capillaries with a channel on tubing lines

The test reproducibility, accuracy and sensitivity for detecting a 2 µm leak has been validated during this study.

The supplier integrity test with this sensitivity is correlated to the Maximum Allowable Leakage Limit (MALL) for single-use systems under use-case conditions.

This report summarizes the method and the results to demonstrate the robustness of the validation study and the reliability of the helium tracer gas test method for Flexsafe® 3D for Storage | Shipping and Flexsafe® for Mixing.

Materials & Methods

Helium Integrity Testing Machine for SUS

Test principle

The test method is derived from ASTM E3244.

The helium test machine consists of two test compartments, that can be operated alternately. A test compartment can receive a SUS up to 650 L with tubing, fittings and components attached to it. Each test compartment is equipped with restraining plates to limit the inflation volume and mechanically support the SUS under test pressure. The area for the SUS bag chamber is equipped with stainless steel spacers to avoid masking effects and a dedicated space for tubing and components. The principle of the method is to measure the helium leak rate caused by the leak inside the tested SUS.

Due to the thin film thicknesses of only a few hundred microns, it is mandatory to differentiate between the natural leak rate of an integral SUS caused by gas permeation through the film, and the leak rate caused by a real leak.

The SUS is placed inside a well-sealed, rigid vacuum compartment and connected through a valve to a helium source. Restraining plates are used to reduce stress on the SUS film while increasing the allowable test pressure. Porous spacers are inserted between the SUS film surface and the restraining plates to avoid masking any leaks and allow testing of the entire film surface (see figure 1).

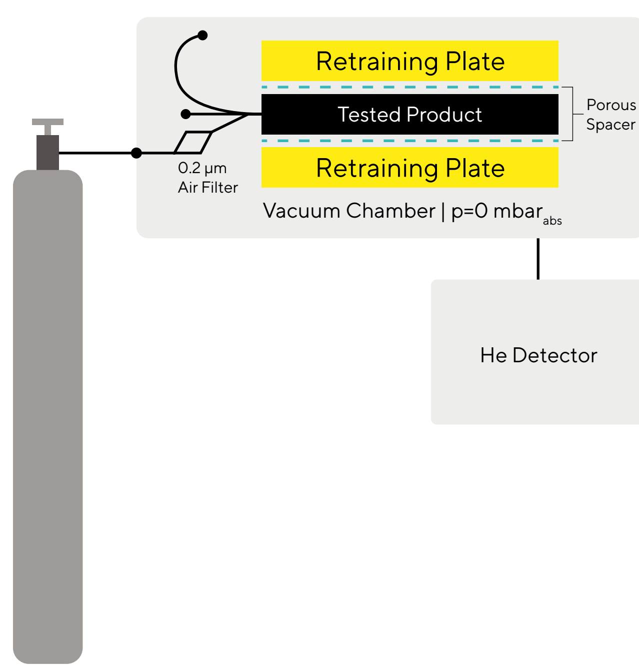

Figure 1: Schematic diagram of the helium test

Test procedure

The SUS is connected to the helium-delivering system.

The test sequence is executed in the following steps:
- Test compartment evacuation to a very low vacuum
- Helium background evacuation
- Helium filling into the SUS and leak rate measurement
- Helium removal from the SUS
- Flooding to ambient pressure

During the test phase, the leak rate for a defective SUS starts to raise earlier, while the permeation of the non-defective SUS follows delayed with a clear separation (see figure 2).

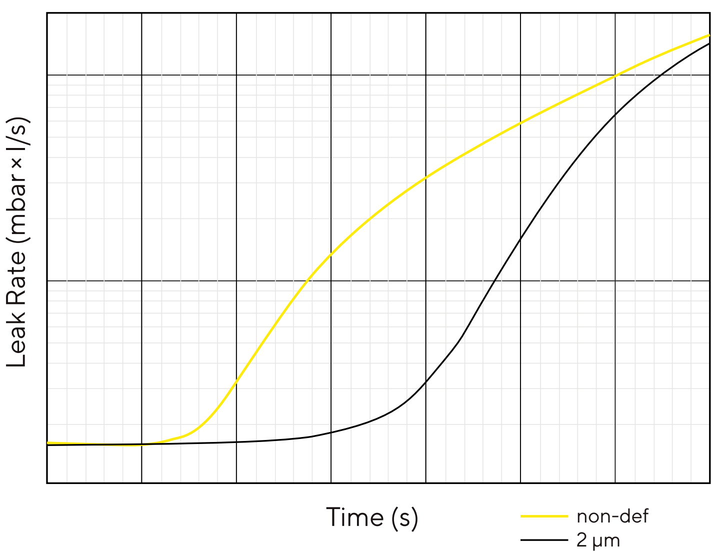

Figure 2: Illustration of the leak rate test during test phase

SUS Configurations Validated With Supplier Integrity Testing

Applications

Flexsafe® 3D for Storage | Shipping and Flexsafe® for Mixing are used in a variety of process steps and applications:
- Buffer and media storage, shipping and mixing
- Harvest storage, shipping and mixing
- Process intermediate storage, shipping and mixing
- Drug substance and drug product, storage and mixing

The Supplier Integrity Test of SUS is especially recommended in SUS applications where assurance of integrity is critical. Evaluation of the criticality and potential need for this Supplier Integrity Test is based on the end-user's risk assessment.

The requirement for supplier integrity testing of SUS is more prevalent in:
- Aseptic processes
- Drug product formulation in final filling and finish steps
- Critical process steps of drug substance purification in downstream processing
- Applications dealing with hazardous products

Description of Flexsafe® 3D for Storage | Shipping validated design for SIT

Flexsafe® 3D for Storage | Shipping from 100 L to 500 L, intended to be integrity tested, need to meet specific design requirements (see figure 3 and table 1).

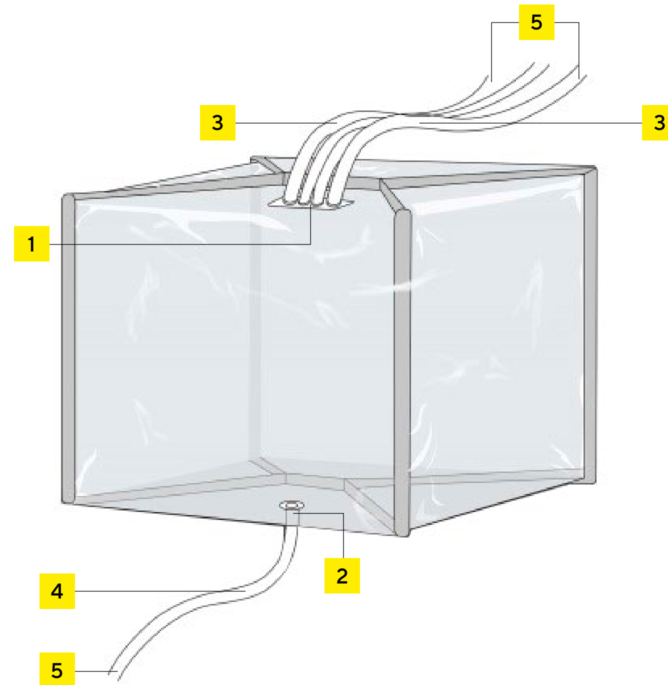

Figure 3: Representation of a Flexsafe® 3D for storage | shipping

Item	Position	Description
SUS Volumes		100 L, 200 L, 500 L
SUS format		3D Palletank®
SUS film		Flexsafe® film
Number | Type of Ports	1	Top flange with 4 ports ▪ 1 to 4 hose barbs with following dimensions (¼", ⅜", ½", plug)
	2	Bottom flange with 1 port ▪ 1 hose barb with following dimensions (¼", ⅜", ½")
Lengths | Type of Tubing	3	Top lines up to 4× ▪ Thermo Plastic Elastomer (TPE) ▪ ≤ 2,500mm* ▪ ¼" ≤ ID ≤ ½"
	4	Bottom line 1× ▪ TPE ▪ ≤ 2,500 mm* ▪ ¼" ≤ ID ≤ ½"
		Minimum tubing lengths, clear of component: 1,000 mm *At least one tubing line will be dedicated to helium injection in the SUS: ▪ Its length should be between 1,000 mm and 1,500 mm ▪ Its end must be open ▪ It can be either on top or bottom
Distal Connectors & Components	5	Distal components that close a line can be helium-tested (e.g. plug, quick-coupler with plug, tri-clamp with plug). Components that do not close the line (e.g. aseptic connectors with membrane) cannot be tested with helium. In such cases, the line will be clamped as close as possible to this connector to allow the test to be performed on the SUS, excluding this connector.

Table 1: Flexsafe® 3D for Storage | Shipping validated design

Description of Flexsafe® for Mixing validated design

Flexsafe® for Mixing from 50 L to 650 L, intended to be integrity tested, need to meet specific design requirements (See figure 4 and table 2).

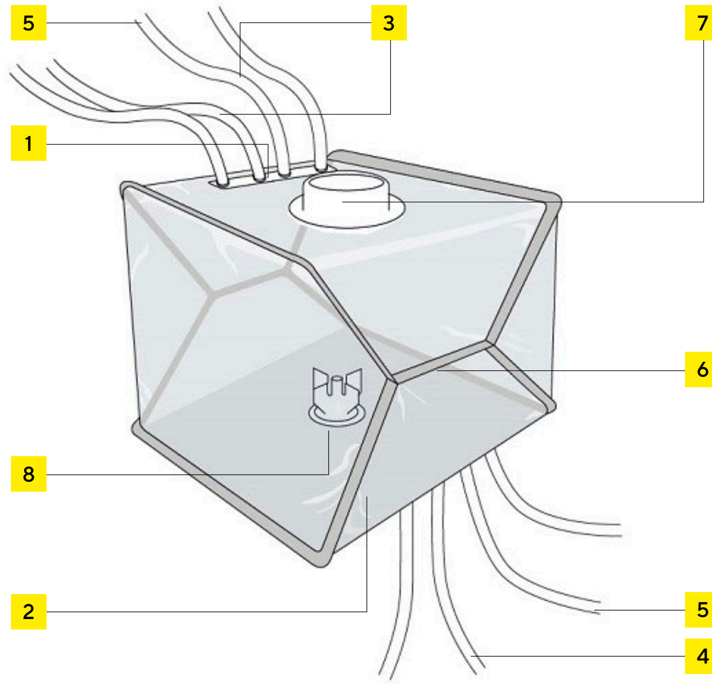

Figure 4: Representation of a Flexsafe® 3D for mixing.

Item	Position	Description
SUS Volumes		50 L, 100 L, 200 L, 400 L, 650 L
SUS format		Cubical
SUS film		Flexsafe® film
Number | Type of Ports	1	Top flange with 4 ports for all SUS volumes (except for 50 L where no top flange is available for SIT) ▪ 1 to 4 hose barbs with following dimensions (¼", ⅜", ½", plug)
	2	Bottom flange with 4 ports ▪ 1 to 4 hose barbs with following dimensions (¼", ⅜", ½", plug)
Lengths | Type of Tubing	3	Top lines up to 4x (except for 50L where no top flange is available for SIT) ▪ TPE ▪ 100 mm or 1,500 - 2,500 mm* ▪ ¼" ≤ ID ≤ ½"
	4	Bottom line 1x ▪ TPE ▪ ≤ 2,500 mm* ▪ ¼" ≤ ID ≤ ½"
		Minimum tubing lengths, clear of component: 1,000 mm *At least one tube will be dedicated to helium injection in the SUS: ▪ Its length should be between 1,000 mm and 1,500 mm ▪ Its end must be open ▪ It can be either on top or bottom of any kind of SUS
Distal Connectors & Components	5	Distal components that close a line can be helium-tested (e.g. plug, quick-coupler with plug, tri-clamp with plug). Components that do not close the line (e.g. aseptic connectors with membrane) cannot be tested with helium. In such cases, the line will be clamped as close as possible to this connector to allow the test to be performed on the SUS, excluding this connector.
Sensors	6	No sensor
Canotier	7	Closed
Mixing system	8	Pro Mixer, LevMixer® & Magnetic Mixer

Table 2: Flexsafe® for Mixing validated design

Results

Validation Approach

Worst-case leak location design of experiment

A DOE has been carried out to determine the worst-case location of puncture in film of the SUS, using 2 µm calibrated defects sealed at different locations on the SUS bag (see figure 5).

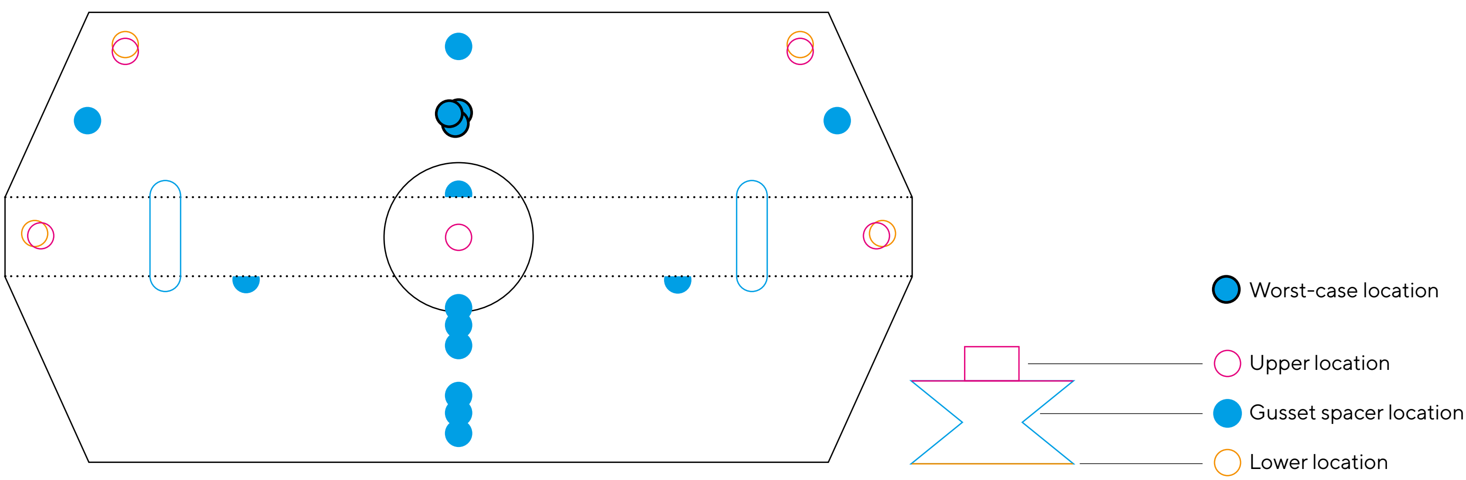

Figure 5: Patch mapping on a 650 L 3D Flexsafe® SUS as part of the worst-case leak location design of experiment

The volume of SUS chosen for the DOE corresponds to 650 L, which is the largest size of SUS in the qualified range of SUS for helium test and corresponds to the worst case for the detection of a hole in the film.

It has been discovered that a 2 µm puncture in the gusset area of the 650 L SUS gives the lowest Helium leak rate. Accordingly, this worst-case location for a puncture in film has been considered for the next steps of the validation plan.

Research of helium filling parameters range

In that phase, the range of available settings leading to different filling conditions of the considered range of SUS has been investigated. Worst cases of helium filling conditions (filling speed and pressure) in combination with the SUS have been determined.

The determination of reject threshold

After the identification of worst case for leak detection, repeats of measurement on defective and non-defective 650 L SUS have been carried out to:

1) Statistically check the robustness of the detection process, which has proven an ability to segregate defective SUS from conforming SUS with a confidence interval superior to 6σ
2) Reject threshold determination

Following the recommendations of ASTM E3244, we used negative controls made of tight SUS and positive controls made of tight SUS with a defect of a known leak size attached to it. Both capillaries, representing a channel in tubing line or laser drilled patches representing a puncture in the SUS film have been measured with the positive controls.

These defects have been calibrated before helium testing to ensure they correspond to an equivalent 2 µm leak size. The tested SUS have been chosen as per the previous worst-case DOE study.

The worst-case sets of parameters have been applied during the measurement, enabling the validation of our range of recipe parameters.

Repeated measurements have been performed to calculate the average value and standard deviation of each control.

The outcome of the study is:
1) The determination of an optimal test time, enabling segregation of negative and positive controls with a confidence interval of 6σ.
2) The determination of the reject threshold between worst case of positive controls and negative controls (see figure 6).

Reject Threshold Determination

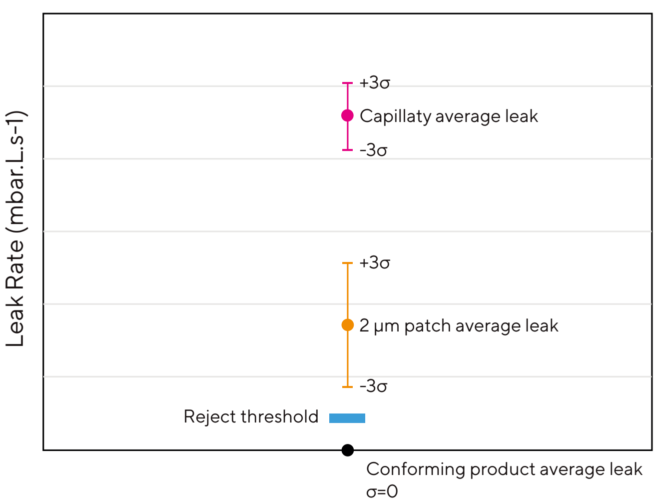

Figure 6: Leak rate chart as part of reject threshold determination

Item	Number of Tested Samples				
	Determination of Test Parameters		Validation of Threshold Values		
SUS volume	Leak worst-case location	Filling worst-case condition	Negative controls	Positive controls capillary	Positives controls patch
650 L	27	13	-	19	19
200 L	-	5	16	-	-
100 L	-	12	-	-	-
Sub-total	57		54		
Total	111				

Table 3: Number of tested samples for the validation of the method

Conclusion

Sartorius Supplier Integrity Test, based on helium tracer gas leak detection, for Flexsafe® 3D for Storage | Shipping and Flexsafe® for Mixing assemblies was successfully validated and proved to be a robust method for reliable detection of leaks.

The Design of Experiments and the test validation study passed all acceptance criteria and allowed to establish reliable and robust test parameters, methods and specifications and the application of a 6-sigma confidence interval for critical parameters.

Non-defective SUS showed results below the maximum helium leak rate specification and SUS with a deliberate 2 µm defect showed results above the maximum helium rate specification and failed the test.

The detection limit of 2 µm is correlated to microbial ingress and liquid leakages. Sartorius extensive scientific studies have shown that 2 µm is the Maximum Allowable Leakage Limit (MALL)* under the worst case process conditions of single-use systems (Single-use System Integrity: Using a Microbial Ingress Test Method to Determine the Maximum Allowable Leakage Limit[1,2,3])

*MALL is the greatest leakage rate (or leak size) tolerable for a given product package to maintain its barrier properties under its use-case conditions (e.g. prevent any risk to product safety, product quality or operator and environmental safety, ASTM E3244.

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