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Application Note

January 26, 2024

Keywords or phrases:
Integrity testing, Helium testing, Celsius® bags, Freeze and thaw

Sartorius Supplier Integrity Testing (SIT) for Celsius® Bag Assemblies for Frozen Storage and Shipping

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Abstract

Single-use frozen storage and shipping solutions are increasingly employed in bioprocesses owing to their robustness, flexibility, and ease of use. As they are in direct contact with drug substance | drug product, the integrity of these containers is crucial for drug quality and operator exposure.

This application note summarizes the validation of our integrity testing method (which uses a helium tracer gas test) employed during the production of our Celsius® FFT single-use assemblies. With its 2 µm detection limit, the test provides integrity assurance correlated to microbial ingress and liquid leaks under the most severe use-case conditions of these single-use assemblies.

Introduction

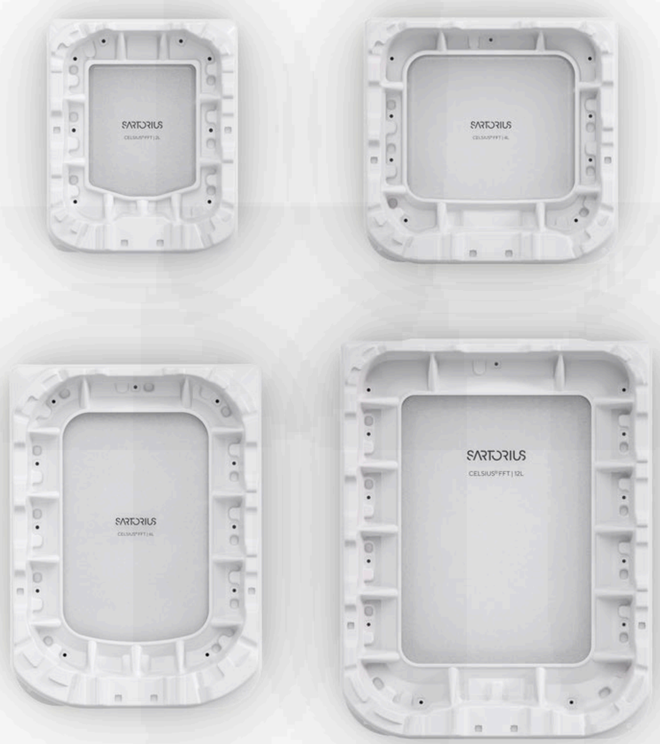
Supplier integrity testing (SIT) of single-use (SU) systems is especially recommended in applications where integrity assurance is critical. Integrity testing of SU systems is most often required in aseptic processes, drug product formulation in final filling and finish steps, applications dealing with hazardous products, and drug substance or drug product storage and shipping,

Celsius® bag assemblies are SU systems used for the storage and shipping of frozen bulk drug substance or product. Their integrity is critical for the safe delivery and storage of drugs. We validated an integrity test method to detect potential leaks in Celsius® bag assemblies using the helium tracer gas method. The study is aimed at validating a leak size detection of 2 µm across the following products:

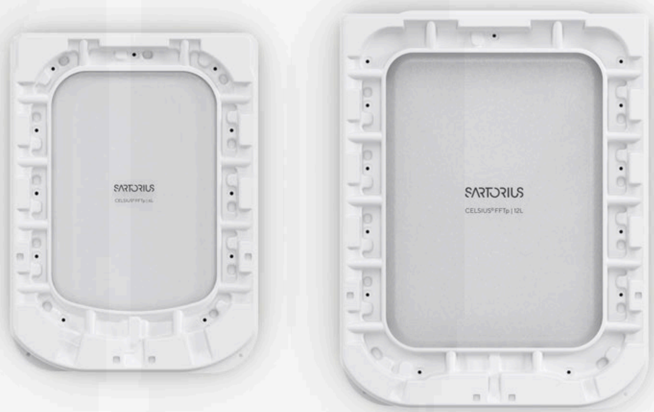
- Celsius® FFT bag assemblies with volumes between 2 and 12 L
- Celsius® FFTp bag assemblies with volumes between 6 and 12 L
- Celsius® Pak bag assemblies with volumes between 1 and 16.6 L

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 most severe use-case conditions of SU systems[1,2,3,4]. The MALL represents 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), according to ASTM E3244.

This application note summarizes the methods used to establish the robustness of the validation study and the reliability of the helium tracer gas test for validating the integrity of Celsius® products.



Celsius® FFT bags with volumes of 2 L, 4 L, 6 L and 12 L



Celsius® FFTp bags with volumes of 6 L and 12 L

Materials and Methods

Helium Integrity Testing for SU Systems

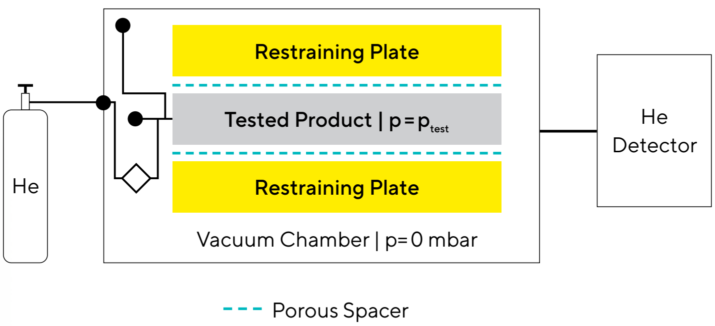
Test Principle

The integrity test method is derived from ASTM E3336-2022. The helium test machine has a test compartment that holds the SU system, which consists of a bag, tubing, fittings, and attached components. The test compartment was equipped with restraining plates to limit the inflation volume and mechanically support the SU system under test pressure. The area for the SUS bag chamber was 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 a leak in the SU system. Due to the thin film thicknesses of only a few hundred microns, we had to differentiate between the natural leak rate of an integral part of the SU system (caused by gas permeation through the film) and the leak rate caused by a defect.

The SU system was placed inside a well-sealed, rigid vacuum compartment (vacuum chamber) and connected through a valve to a helium source. We used restraining plates to reduce stress on the film while increasing the allowable test pressure. Porous spacers were inserted between the film surface and the restraining plates to avoid masking any leaks and allow testing of the entire film surface (Figure 1).

Figure 1: *Schematic Diagram of the Helium (He) Test*



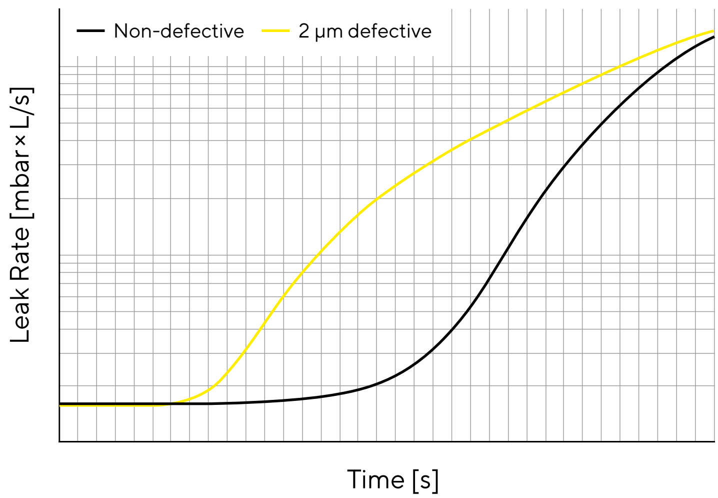
Test Procedure

The SU system was connected to the helium-delivering system, and the test sequence was executed in the following steps:

- Test compartment evacuation to reach vacuum
- Helium background evacuation
- Helium filling into the SU system and leak rate measurement
- Helium removal from the SU system
- Flooding to ambient pressure

During the test phase, the leak rate for a defective SU system increased earlier, while the permeation of the non-defective SU system was delayed (Figure 2). The area between the two lines represents the difference between a non-defective SU system and a SU system with a 2 µm defect.

Figure 2: *Illustration of the Leak Rate During the Test Phase (Log Scale)*



SU System Configurations Validated With Supplier Integrity Testing

Applications

Description of Celsius® Bag Assemblies Subject to Integrity Testing

The following tables describe the validated design space for the Celsius® products subject to integrity testing (Tables 1 and 2, Figures 3 and 4). The design space for SIT testing is broader than the actual design space for Celsius® products. Limitations in lengths of tubing lines, etc., might be required due to the protective shells or other constraints.

a) Celsius® FFT | FFTp

Figure 3: *Representation of a Celsius® FFT | FFTp With Safecore™*

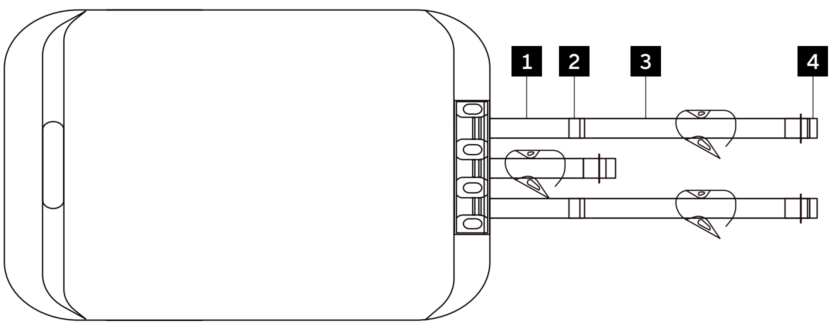


Table 1: *SIT Design Range Validated for Celsius® FFT | FFTp With Safecore™ Technology*

Item	Position	Description
Volumes		2 – 12 L
SU System Format		Celsius® FFT and FFTp with Safecore™ Technology
SU System Film		S71 film (360 µm)
Bag Tubings	1	EVA tubing lines welded on the bag
Connector	2	Connection between EVA tubing lines from the bag chamber and Silicone | TPE tubing lines
Type of Tubing (Silicon or TPE)	3	▪ For 2 L (FFT only): 2 tubing lines, ≤ 1,075 mm, ¼" ID to ⅜" ▪ For 4 L (FFT only): 3 tubing lines, ≤ 2,150 mm, ¼" ID to ⅜" ▪ For 6 L: 2 tubing lines, ≤ 2,150 mm, ¼" ID to ⅜" ▪ From 12 L: 3 tubing lines, ≤ 2,150 mm, ¼" ID to ⅜"
Distal Connectors & Components	4	▪ Tight distal components (e.g., plugs) are compatible with the helium tracer gas test. ▪ Components that do not close the line (e.g., aseptic connectors with permeable membrane) cannot be tested with helium.

b) Celsius® Pak

Figure 4: *Representation of a Celsius® Pak*

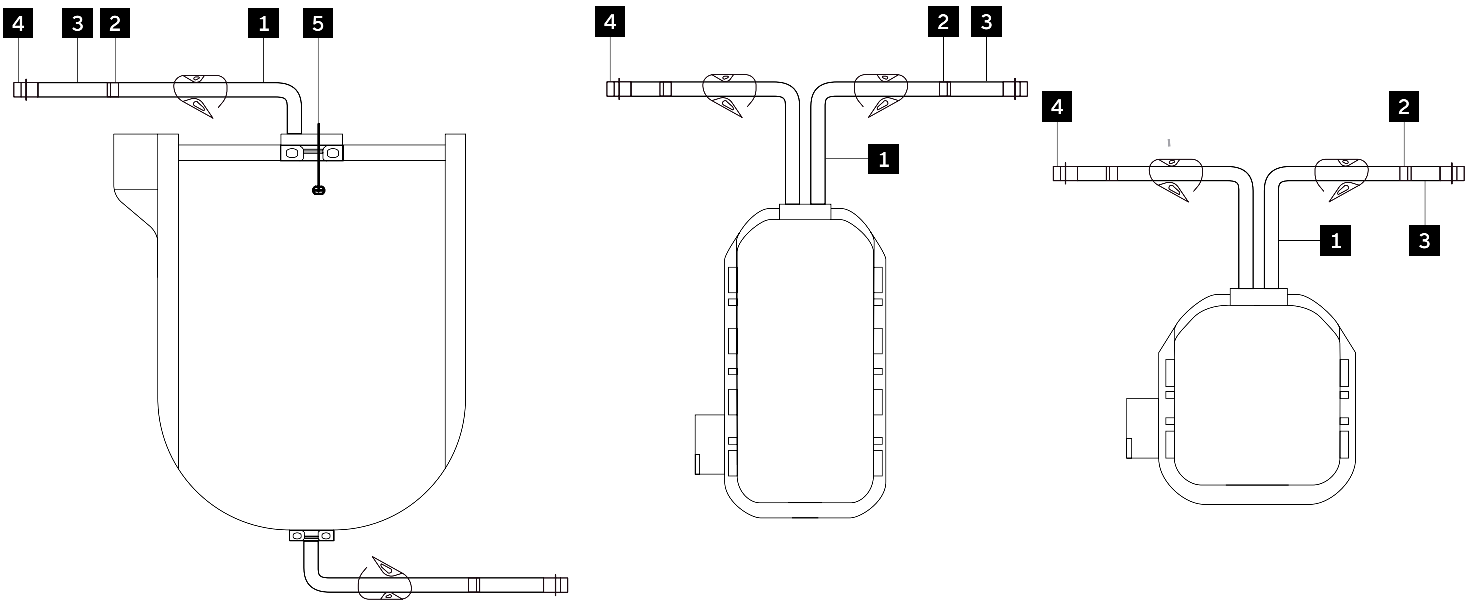


Table 2: *SIT Design Range Validated for Celsius® Pak*

Item	Position	Description
Volumes		1 – 16.6 L
SU System Format		Celsius® Pak bags
SU System Film		S71 film (360 µm)
Bag Tubings	1	EVA tubing lines welded on the bag
Connector	2	Connection between EVA tubing lines from the bag chamber and Silicone | TPE tubing lines
Type of Tubing (Silicon or TPE)	3	▪ From 1 – 2 L: 2 tubing lines, ≤ 1,075 mm, ¼" ID to ⅜" ▪ From 8.3 – 16.6 L: 2 tubing lines, ≤ 2,150 mm, ¼" ID to ⅜"
Distal Connectors & Components	4	▪ Tight distal components (e.g., plugs) are compatible with the helium tracer gas test. ▪ Components that do not close the line (e.g., aseptic connectors with permeable membrane) cannot be tested with helium.
Other Components	5	▪ Celsius® bag Thermowell

Validation Approach

The validation was carried out in several steps:

1. Determination of the type of defect to be used for the validation and verification that the porous spacer do not impact the leak detection
2. Definition of groups of products (clusters) for all the 2D bag assemblies, each film material, and then by determination of filling parameters
3. Implementation of a design of experiments (DoE) strategy to evaluate the impact of the product design on the Helium leak rate for the worst-case leak
4. Determination of the reject threshold for each product cluster

The same approach was applied to qualify the supplier integrity testing method for Flexsafe® 2D, Flexboy® 2D products (studies not shown), and the Celsius® products described herein.

Initial Qualification of Specific Designs: Worst-Case Defect Determination

We carried out individual qualifications of designs covering the range of volumes in the scope of the 2D supplier integrity test method. The test setup is represented in Figure 1. During the qualifications, two types of defects for positive controls were tested:

- 2 µm laser drilled patches of film, representing a puncture in the bag chamber.
- 20 µm × 3 cm capillary tubes, representing a channel in connections.

Both defects were calibrated before and after being used in the qualification with the Helium tester. They both exhibited a similar leak rate during calibration. The defects were attached at the end of one of the tubing lines of each product sample configuration.

To mimic the actual test conditions in the helium test machine, we placed the 2 µm laser drilled patch inside a patch holder with the same porous spacer as the one used in the test machine. We tested 32 positive controls and 32 negative controls for each product design, generating leak rate curves, which were statistically analyzed to determine the optimal test time and to separate the positive from negative controls with a 6-sigma confidence interval.

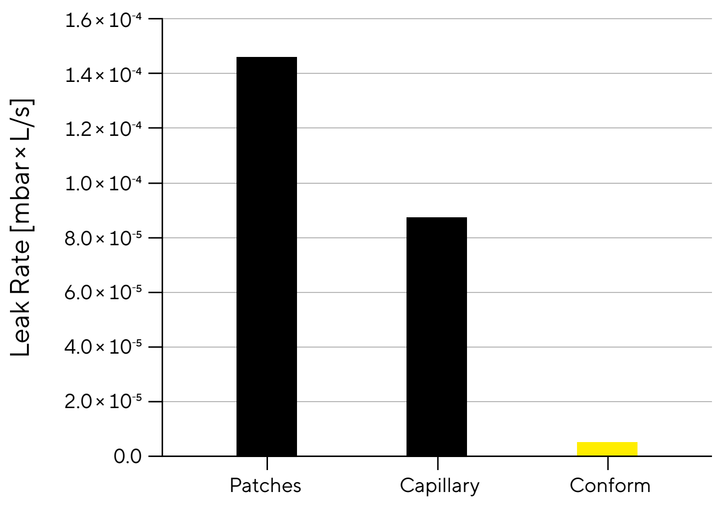
For each design, the following samples were tested:

- 16 samples with capillaries (positive controls)
- 16 samples with laser-drilled patches (positive controls)
- 32 tight samples (negative controls)

These qualifications show that, with a 6-sigma confidence interval, the helium tester can detect a 2 µm hole in a film pressed against the porous spacer. Thus, the porous spacer, although placed in direct contact with the bag, does not block a 2 µm hole (tested in the patch holder).

Secondly, although the 20 µm × 3 cm capillary and the 2 µm laser drilled patch both displayed the same leak rate during the calibrations performed under atmospheric conditions (0.07 cm³/min under 1 bar g (15 psig)), they did not induce the same leak rate under vacuum. Using the same test conditions, the positive controls with capillaries displayed around half the helium leak rates induced by the laser-drilled patches of film (Figure 5).

Figure 5: *Leak Rate of Capillaries and Laser Drilled Patches Compared to Negative Controls.*



Accordingly, to further qualify the Helium test method on 2D bags, we performed tests using the worst-case defect of 20 µm × 3 cm capillary, calibrated at 0.07 sdt.cm³/min under 15 PSIG, which corresponds to a 2 µm flow equivalent diameter.

Determination of Filling Parameters

The range of relevant products was split by volume into clusters (Table 3). Inside a given cluster, each product configuration was tested under the same conditions (Helium test pressure and filling speed).

Table 3: *Celsius® Product Clusters*

Product Family	Cluster	Bag Volumes	Bag Film (Thickness)
Celsius®	Cluster 3	1 – 8.3 L	S71 (360 µm)
	Cluster 4	12 – 16.6 L	S71 (360 µm)

Design of Experiments (DoE) to Determine the Impact of Product Design on Helium Leak Rate

For each film family, we performed a design of experiments (DoE) to assess different combinations of bag chamber size and tube configurations. The results showed that the bag size had minimal effect on the helium leak rate. In contrast, the tube dimensions (ID and length) have a more significant impact: the smaller diameter and longer the tubes, the lower the leak rate.

Thus, a set of DoEs helped determine the worst-case product configuration for positive and negative controls in this validation phase. The range of settings leading to different filling conditions of the SU systems was investigated. Worst-case conditions of helium filling (filling speed and pressure) were determined in combination with the SU system design.

The Determination of the Reject Threshold

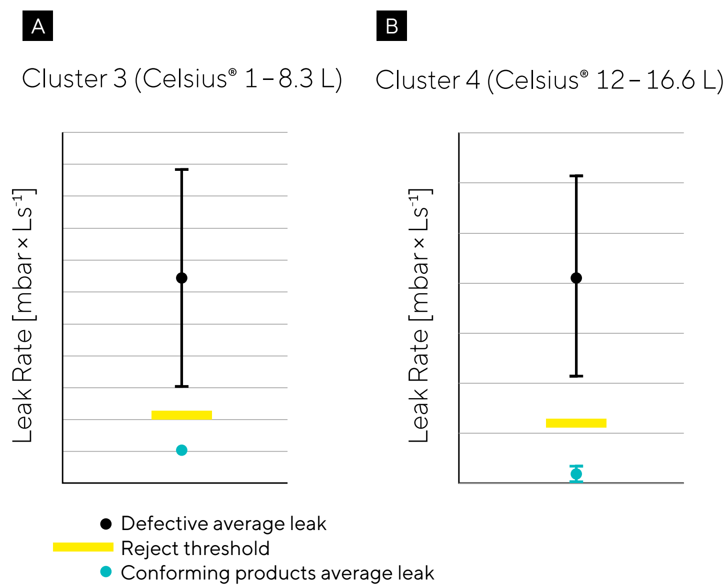
We then repeated the same tests for each cluster, using 16 positive and 16 defective controls to statistically check the robustness of the detection process and determine the reject threshold. Following the recommendations of ASTM E3244, we used negative controls, made of tight SU systems, and positive controls, made of tight SU systems with a defect of a known leak size attached. The previously defined worst-case defect (20 µm × 3 cm capillary) was chosen for the positive controls. These defects were calibrated before helium testing to ensure they corresponded to an equivalent 2 µm leak size. The numbers of tested samples for Celsius® products are shown in Table 4.

Table 4: *Numbers of Tested Samples for Celsius® Products*

Product Family	Cluster	Positive Controls	Negative Controls
Celsius®	Cluster 3	16	16
	Cluster 4	16	16

The same test procedure was performed on each sample using the same test conditions (Helium pressure and filling speed) for each product cluster. The test results were used to calculate the average value and standard deviation of each control. This study allowed us to determine the optimal test time, segregate the negative and positive controls with a confidence interval of six times the standard deviation (6 sigma), and determine the reject threshold between the worst-case positive controls and negative controls (Figure 6A and 6B).

Figure 6: *Leak Rate Chart as Part of Reject Threshold Determination of Celsius®*



Conclusion

In this application note, we demonstrate the successful validation of SIT testing based on helium tracer gas leak detection. We determined that this approach was a robust method for the detection of leaks in Celsius® bag assemblies. This test is performed during the standard manufacturing of Celsius® with Safecore™ Technology and other Celsius® products upon request.

The DoE and test validation study passed all acceptance criteria and allowed us to establish reliable and robust test parameters, methods, and specifications and apply a 6-sigma confidence interval for critical parameters. Non-defective SU systems showed results below the maximum helium leak rate specification. SU systems with a deliberate 2 µm defect showed results above the maximum helium rate specification, thereby failing the test.

Thus, this testing regime provides integrity assurance for SU frozen storage and shipping solutions.

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Status: 01 | 2024