Biotech

Validation Guide

USTR 3261a

Pall HIT™ System for Helium Integrity Testing in Allegro™ 2D Single-Use System Manufacturing

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Contents

1 Qualification Overview

1.1 Introduction

To meet the demand for an increased assurance of integrity of single-use products for critical applications, Pall Biotech now offers a helium integrity test (using proprietary Pall HIT technology) for Allegro single-use systems (SUS) for specific end-user requests. This guide contains data applicable to the Allegro 2D biocontainer SUS and filling sets which have been tested at the Pall Medemblik (Netherlands), SUS manufacturing site, by the HIT method to increase assurance of integrity. The purpose of this report is to document testing that has been performed to demonstrate the ability of the HIT system to detect defects as small as 2 micrometers (µm).

The scope of this document is limited to the Pall HIT system which is used to test the Allegro 2D SUS. These SUS include biocontainers with associated transfer lines and filling sets with surge biocontainers. When dealing with complex SUS, e.g. including manifolds, filter capsules, etc., additional tests using a high sensitive pressure decay test may be required. Pall can also offer these tests for specific end-user requests.

A 3-step approach was followed to verify the capability of the HIT system to detect 2 µm defects:

1. **Design Verification.** Performed by testing 60 biocontainer assemblies of each size to confirm a single pass / fail threshold could be applied for all sizes.
2. **Operational Qualification (OQ).** Performed after installation in the factory to refine the pass / fail threshold value and confirm the equipment accuracy, recall and precision.
3. **Performance Qualification (PQ).** To confirm the working capacity of the equipment on a larger quantity of biocontainer assemblies.

Once the system was qualified for the detection of defective biocontainer assemblies, two additional qualifications were carried out to demonstrate the capabilities of the test system:

1. **Transfer Line Length Qualification**. To extend the allowable length of transfer line that can be tested.
2. **Filling Set Qualification**. To allow complex filling sets with transfer line lengths outside of the qualification range to be tested at a 10 µm defect size.

1.2 Summary of Conclusions

1.2.1 HIT System Design Verification

Sixty (60) Allegro 2D biocontainer assemblies of each size were tested, 30 with a 2 µm equivalent nominal diameter orifice size defect and 30 without a defect, representing 540 tested biocontainer assemblies in total.

The HIT system can consistently distinguish integral 2D biocontainer assemblies from those with a 2 µm equivalent nominal diameter orifice size defect, thus confirming the capability of the HIT method for this purpose. A single pass / fail threshold can be applied to all biocontainer sizes. Therefore, the operational qualification of the instrument (next step) to confirm this threshold has been carried out following a bracketed approach, with only the largest and smallest biocontainers within the test range.

1.2.2 HIT System Operational Qualification

A statistically significant number of control Allegro 2D biocontainer assemblies (n=49), and Allegro 2D biocontainer assemblies with a 2 µm equivalent nominal diameter orifice size defect (n=46), were tested using the HIT system at the Pall Medemblik manufacturing site. A combination of 50 mL and 20 L biocontainers (the smallest and largest sizes in the test range) were tested as representative of all intermediate sizes.

Using this data, a pass / fail leak rate specification was selected to minimize false negatives (product deemed acceptable but actually defective) and reduce the risk of false positives (product deemed to be defective but actually integral). Both control and defect data are a minimum of 3 standard deviations from the pass / fail specification.

All biocontainer assemblies tested were correctly classified as defective or non-defective. Based on the scoring methods for a binomial response variable, the OQ results demonstrated 100% accuracy, 100% recall and 100% precision for the assemblies tested. Defects at, or above, 2 µm equivalent nominal diameter orifice size can be detected within Allegro 2D biocontainer systems between 50 mL and 20 L.

1.2.3 HIT System Performance Qualification

An additional 150 Allegro 2D biocontainer assemblies were produced and tested at the Pall Medemblik manufacturing site for the performance qualification. These 1 L and 10 L biocontainer assemblies followed the full manufacturing procedure from order receipt to final packaged product, including the helium integrity test.

The Allegro 2D biocontainer assemblies manufactured during PQ all passed the HIT specification, with no biocontainer assemblies classified as defective.

1.2.4 HIT System Transfer Line Qualification

During design verification, and OQ, of the extended transfer line study scope a total of 144 Allegro 2D biocontainer assemblies were tested using the HIT system at the Pall Medemblik manufacturing site. Half of these assemblies included minimum length transfer lines without a defect. The other half included maximum length transfer lines and were equipped with a 2 µm equivalent nominal diameter orifice size defect. A further 36 Allegro 2D biocontainer assemblies with maximum transfer line lengths were produced according to full manufacturing procedures and tested during PQ. All sizes of biocontainers in scope from 50 mL to 20 L were included in each stage of testing.

All biocontainer assemblies were correctly classified as defective or non-defective. Based on the scoring methods for a binomial response variable, the transfer line length design verification and OQ results both demonstrated 100% accuracy, 100% recall and 100% precision for the assemblies tested. Defects at, or above, 2 µm equivalent nominal diameter orifice size can be detected within an Allegro 2D biocontainer system up to the maximum permitted transfer line lengths for all sizes between 50 mL and 20 L.

No biocontainer assemblies exceeded the maximum manufacturing pressure threshold during testing. This confirms that the minimum transfer line lengths permitted can be used without risk to the biocontainer assembly integrity.

The Allegro 2D biocontainer assemblies manufactured during the transfer line length PQ all passed the HIT specification, with no biocontainer assemblies classified as defective.

1.2.5 HIT System Filling Set Qualification

During design verification and OQ of filling sets, a total of 38 filling sets with Allegro 2D surge biocontainers were tested using the HIT system at the Pall Medemblik manufacturing site. Ten (10) filling sets were controls without a defect and 28 were equipped with a 10 µm nominal diameter orifice size defect. A further 20 control filling set assemblies with Allegro 2D surge biocontainers were produced according to full manufacturing procedures and tested during PQ. All sizes of filling sets in scope from 500 mL to 10 L biocontainers were included in OQ and PQ testing.

All filling sets with a defect were correctly classified according to the pass / fail specification determined during HIT system OQ. Based on the scoring methods for a binomial response variable, the filling set design verification demonstrated 100% accuracy, 100% recall and 100% precision for the assemblies

tested. The filling set OQ demonstrated 97% accuracy, 100% recall and 95% precision for the assemblies tested. The lower scoring results indicate a reduced manufacturing yield. The 100% recall is confirmation that all defect test assemblies were detected. Defects at or above 10 µm equivalent nominal diameter orifice size can be detected within filling sets with Allegro 2D surge biocontainers up to the maximum permitted transfer line lengths.

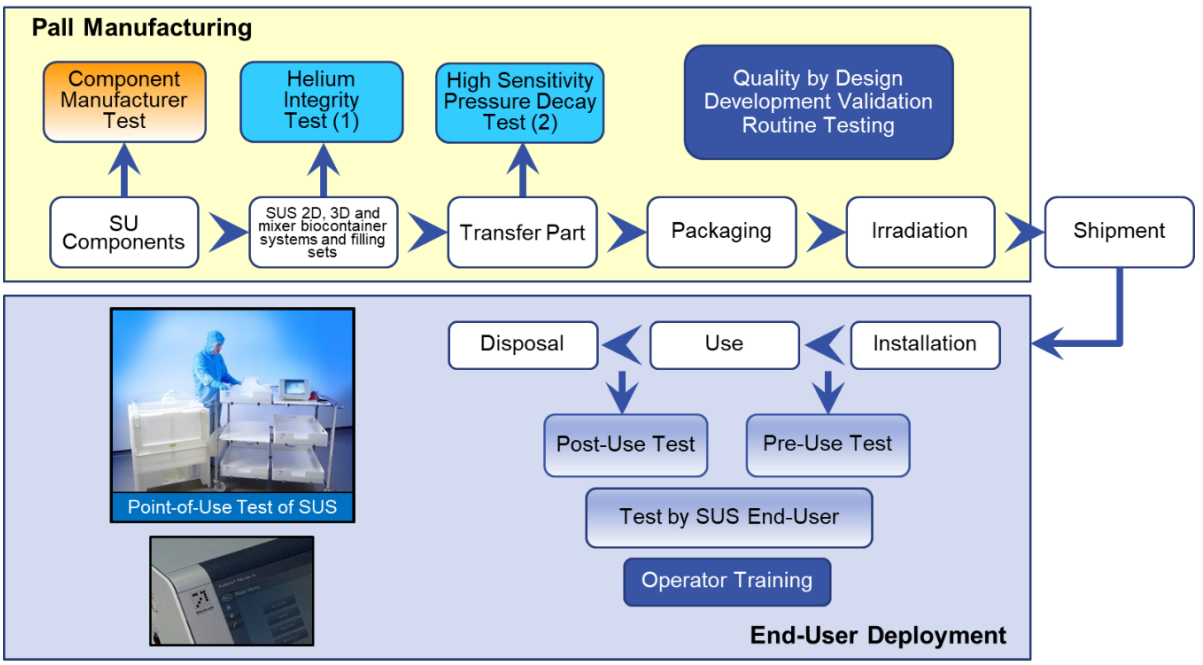
The filling sets with 2D Allegro surge biocontainers manufactured during the filling set PQ all passed the HIT specification, with no filling sets classified as defective.

1.3 Background

Single-use technologies have seen a very rapid market acceptance in both clinical and manufacturing operations, even in the most critical steps dealing with highly purified and/or sterile drug substances. This broad adoption has led to an increased need for a higher level of integrity assurance for the SUS implemented in these applications. Drug manufacturer risk assessments and strategies for the control and assurance of integrity of SUS have led to requests to suppliers for the availability of an extremely sensitive integrity test of each single-use biocontainer assembly at the SUS supplier's manufacturing site [1].

Pall Biotech constantly strives to meet the needs of the drug manufacturer. The Pall high sensitivity helium integrity test (using Pall HIT technology) is implemented at the Pall Medemblik SUS manufacturing site. The HIT system was designed for biocontainer assemblies, including the joints of the transfer lines to the biocontainer, and is capable of detecting defects as small as 2 µm in diameter. This additional test can be performed to further strengthen the assurance of integrity of SUS designs which fall within the validated envelope for biocontainer size and transfer line configuration. The test occurs at the Pall site during manufacturing of the Allegro SUS. Figure 1 shows the life cycle of a Pall Allegro SUS with the Pall manufacturing steps in the upper region and the SUS deployment steps at the end-user site in the lower region of the figure. The HIT system is designed to provide the highest level of sensitivity for process steps that incorporate SUS, especially important for the most critical step of bulk drug substance or drug product storage.

Figure 1.
Life cycle of Allegro 2D biocontainer assemblies from manufacture to disposal



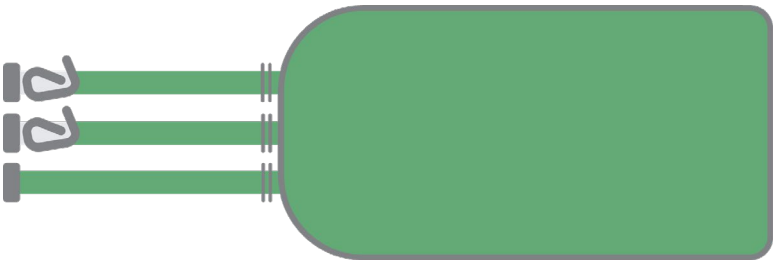
Note: (1) and (2) are tests performed upon customer request

1.4 HIT Method Principle

To achieve sufficient sensitivity for detecting defects as small as 2 µm, a tracer gas is employed. The tracer gas detection method, in vacuum mode, is a method of choice recommended in the USP sub chapter <1207.2> Package integrity leak test technologies [2, 3, 4]. Helium is used as the tracer gas, due to its favorable properties [5, 6], in an inside-out test method, where helium leaking from the defects on the biocontainer is collected in the test chamber [7]. A highly sensitive helium mass spectrometer (MS) connected to the test chamber quantifies helium gas concentration. The time point for each assembly type is optimized for maximal test sensitivity. Additional critical test parameters, including test pressures, are optimized for each Allegro 2D biocontainer size. A leak rate calculated from the helium concentration in the test chamber is converted into a pass/fail result based on the predetermined acceptance criteria.

Seams and connection points are the most likely biocontainer assembly defect locations. These regions must be fully exposed during the test, that is, not masked due to contact with the surface of the test chamber or biocontainer restraining support. The Pall HIT system was designed to ensure maximum exposure of all seams and welds in the biocontainer assembly, as well as the port connections (Figure 2). Additionally, it was designed to maximize the film surface exposed during the test, contributing to a high confidence in the integrity of the biocontainer assembly.

Figure 2.
Schematic of a single-use assembly with the HIT tested area (in green)



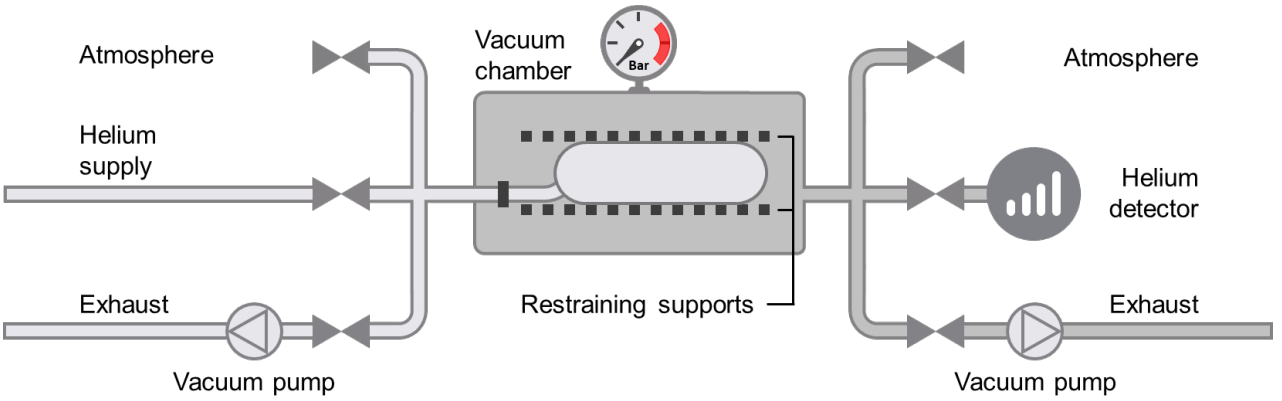
1.5 HIT Equipment Description

The system is composed of the following components:

- HIT controller to run the integrity test automatically using pre-set parameters determined based on specific biocontainer assembly
- Vacuum chamber (test chamber) for Allegro 2D biocontainer assemblies from 50 mL to 20 L
- Biocontainer restraining supports, designed for minimal masking of the surface and stress reduction on the biocontainer
- Helium detector (inside the controller)
- Vacuum pumps

All testing presented in this validation guide was carried out using this equipment.

Figure 3.
Schematic of the HIT system



1.6 Defect Testing Approach

There are two main ways to create a 2 µm defect in a flexible 2D biocontainer assembly. One way is to laser drill a 2 µm defect in the biocontainer film and another is to attach a rigid assembly (e.g. a stainless steel disc) with a 2 µm laser drilled orifice defect via transfer line on the biocontainer. Evaluation of laser drilled 2 µm holes in film showed shape variation and significant size variation (from 1.5-2.5 µm), ultimately resulting in large variation in the observed leak rate. To evaluate the true HIT process capability, it is important to keep the artificial defect size constant and accurate and, therefore, laser drilled defects in film were not used at any point in this qualification. Instead, qualification tests were performed by attaching a reference calibrated, 2 µm equivalent nominal diameter orifice size, stainless steel defect assembly to the end of a transfer line (the defect line) on the test biocontainer assembly. Due to their complex design and long transfer line, the sensitivity had to be adapted, and filling sets with 2D Allegro surge biocontainers were qualified using a 10 µm equivalent nominal diameter orifice size stainless steel defect. Based on Pall experiments and application specific risk assessment, it is Pall's technical opinion that this sensitivity adequately covers transfer applications (the category of applications where filling sets are used).

It is important to distinguish that the reference calibrated stainless-steel orifice defects used during qualification tests are all flow calibrated. This flow (measured in standard cubic centimeters per second, sccs) is used to calculate a nominal diameter orifice size assuming a leak path of negligible length. This calculation uses the same equations as per USP <1207.1> Section 3.9 and aligns with the USP method of defining defects based on their dry air flow. To qualify the nominal orifice size of a reference leak, the dry air leak rate through the leak is measured under 1 bar differential pressure. This value is compared to the leak flow rate measured through a reference orifice certified by the National Institute of Standards and Technology (NIST).

Placing the defect at the end of the longest transfer line represents the worst-case scenario for defect location on the biocontainer assembly due to the distance from the helium supply. Transfer line up to the lengths tested in the scope of this qualification guide can be included in the HIT test area and the qualification approach will demonstrate that defects can be detected along the full length of that transfer line.

2 HIT System Design Verification

2.1 Introduction

The purpose of these tests is to verify that the HIT system can differentiate between non-defective Allegro 2D biocontainer assemblies and Allegro 2D biocontainer assemblies containing a 2 µm defect.

2.2 Summary of Methods

Sixty (60) Allegro 2D biocontainer assemblies of each size were tested using the HIT system described in Section 1.5, thirty (30) with a 2 µm equivalent nominal diameter orifice size defect connected and thirty (30) without a defect. A total of 540 tests were conducted with the 9 sizes of Allegro 2D biocontainers: 50, 100, 250, and 500 mL, and 1, 2, 5, 10 and 20 L.

The test assemblies (control or defect) are placed inside the test chamber. The helium line on the test assembly is connected to the helium supply and then subjected to the standard test method using parameters defined during method development. The measured leak rate of helium is then recorded for a defined time period. The leak rate at this defined time, is reported for all tested assemblies and results are analyzed.

2.2.1 Biocontainer Assemblies

The HIT system is designed to detect defects on the biocontainer film surface, at seals, and at joints with transfer lines. The test biocontainer assemblies used during design verification testing include all these components, as shown in Figures 4 and 5. Longer lengths of transfer lines, in addition to the joints of these transfer lines, are qualified in Section 5.

Figure 4.
Control (top) and defect (bottom) biocontainer assembly configurations for 50 mL, 100 mL, 250 mL, 500 mL, and 1 L sizes during HIT system design verification

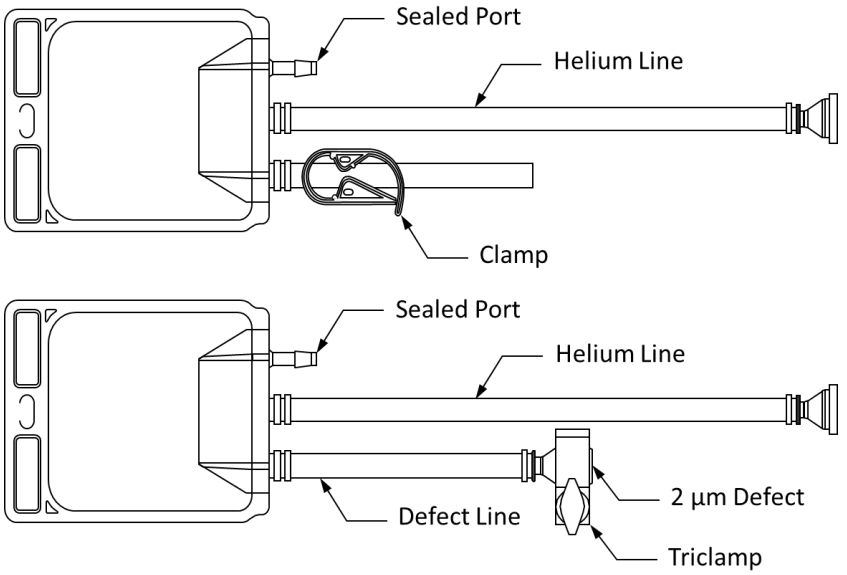
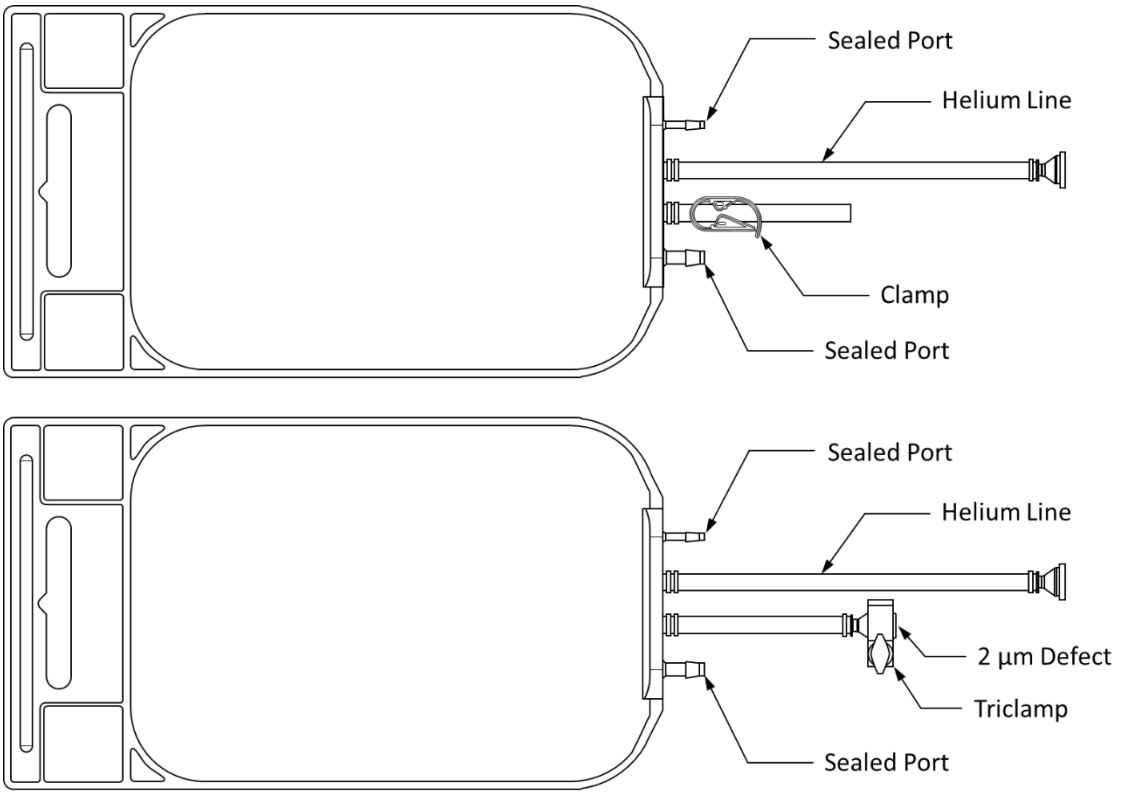


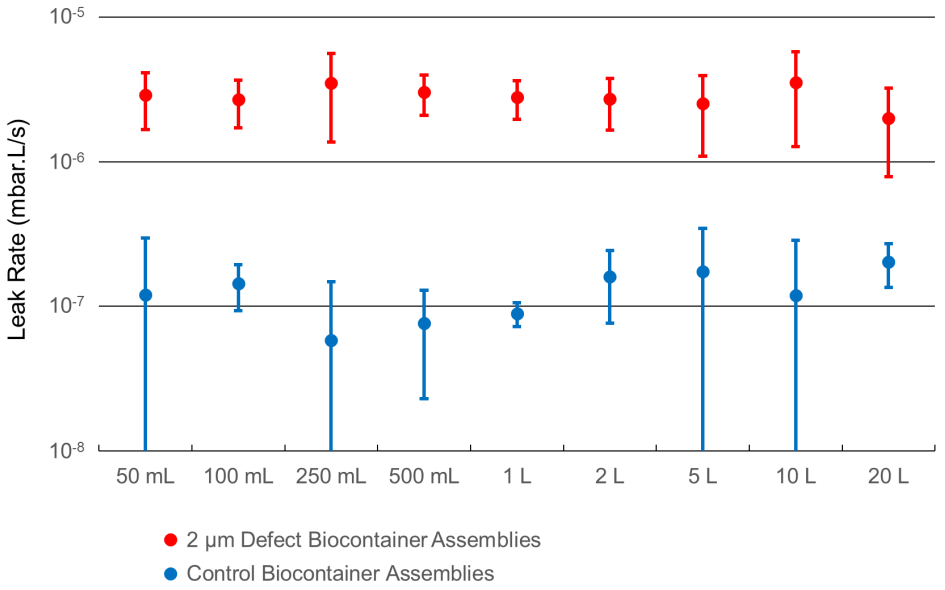
Figure 5.
Control (top) and defect (bottom) biocontainer assembly configurations for 2 L, 5 L, 10 L, and 20 L sizes during HIT system design verification.



2.3 Results

Results from tests conducted for 2 µm defect detection are summarized in Figure 6. All biocontainer sizes show a clear separation of leak rates from control biocontainer assemblies (blue data points) and the 2 µm defect biocontainer assemblies (red data points) beyond the error bars representing three standard deviations. There is also clear separation between the leak rate of any control biocontainer from any 2 µm defect biocontainer assembly, demonstrating that the test procedures for each biocontainer volume allow for a single pass / fail threshold that applies to all Allegro 2D biocontainer sizes.

Figure 6.
Leak rate for control and 2 µm defect biocontainer assemblies using the high sensitivity HIT system



Filled circles show the mean data. Error bars indicate ± 3 standard deviations from the mean. Note that error bars are asymmetric due to the logarithmic scale.

2.4 Conclusions

The HIT system can consistently distinguish integral 2D biocontainer assemblies from those with a 2 µm equivalent nominal diameter orifice size defect, thus confirming the capability of the HIT method for this purpose. A single pass / fail threshold can be applied to all biocontainer sizes. Therefore, the operational qualification of the instrument (next step) to confirm this threshold has been carried out following a bracketed approach, with only the largest and smallest biocontainers within the test range.

3 HIT System Operational Qualification

3.1 Introduction

The purpose of these tests is to qualify the HIT system after installation at the Pall manufacturing site and verify the pass / fail specification for classifying Allegro 2D biocontainer assemblies as non-defective or defective.

3.2 Summary of Methods

The HIT system was installed and qualified at the Pall manufacturing site for Allegro SUS, to give drug manufacturers the option of having their single-use assemblies undergo this highly sensitive integrity test. The post-installation process included a full system qualification, full check of correct installation and calibration of the HIT system, introduction of standard operating procedures (SOPs), training of operators and operational qualification using 50 mL and 20 L biocontainers (the smallest and largest sizes in the test range).

A total of 95 Allegro 2D biocontainer assemblies were tested using the HIT system described in Section 1.5, 46 with a 2 µm equivalent nominal diameter orifice size defect and 49 without a defect. The specific volumes and sample sizes of control and defect biocontainer assemblies used are summarized in Table 1.

The test assemblies (control or defect) are placed inside the test chamber. The helium line on the test assembly is connected to the helium supply and then subjected to the standard test method using parameters optimized during installation qualification and fixed for manufacturing. The measured leak rate of helium is then recorded for a defined time period. The leak rate is reported for all tested assemblies and results are analyzed.

Control of environmental conditions within the manufacturing clean-room significantly decreased the noise in low leak rate data from the control biocontainer assemblies compared to the design verification. Therefore, control data generated during the OQ are lower than the comparable data in the design verification.

3.2.1 Biocontainer Assemblies

Operational qualification used the same biocontainer assembly designs as the design verification testing. Refer to Figures 4 and 5.

3.3 Results

A the OQ test data from the 50 mL and 20 L Allegro biocontainer assemblies (listed in Table 1) is shown in Figure 7. The data confirms the clear separation of control and defect samples based on leak rate and demonstrates the characteristics of a binomial response variable:

- The experiment consists of a number of repeated trials
- Each trial can result in only two possible outcomes:
    - No defect (success)
    - Identify defect (failure)
- The probability of success is the same on all trials due to fixed experimental conditions
- The trials are independent since the equipment does not degrade during use and is regularly calibrated

Selection of a pass / fail leak rate specification, as shown in Figure 7, was carried out using the control and defect data as well as other considerations and parameters, including statistical analysis of the margin of error in the test data. The selected specification line is closer to the control data based on the greater importance given to the detection of defective biocontainer assemblies compared to the manufacturing yield.

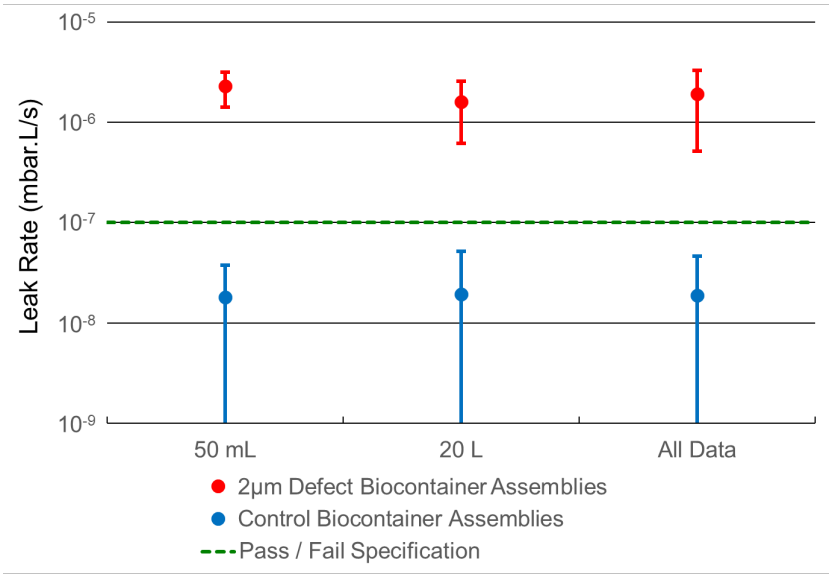
Table 1 shows the binary pass or fail results for the HIT test, demonstrating no false negatives (product deemed acceptable but actually defective) and no false positives (product deemed to be defective but

actually integral). The binomial response scoring method results are summarized in Table 2, showing 100% scoring for all methods due to the correct classification of every Allegro 2D biocontainer assembly tested.

Table 1.
Results of HIT system operational qualification testing at the manufacturing site

Allegro 2D Biocontainer Assembly	Number of Assemblies Tested	Number of HIT Failures (Positive Leak Detection)	Number of HIT Passes (Negative Leak Detection)
50 mL, control	21	0	21
50 mL, defect	21	21	0
20 L, control	28	0	28
20 L, defect	25	25	0

Figure 7.
Results from the operational qualification of the high sensitivity HIT system at the manufacturing site



Filled circles show the mean data. Error bars indicate ± 3 standard deviations from the mean. Note that error bars are asymmetric due to the logarithmic scale.

Table 2.
Bionomial response variable scoring method results for the HIT system OQ

Scoring Method	Description	Result
Accuracy	Percentage of biocontainer assemblies correctly classified	100%
Recall	The true positive rate or sensitivity of the test. Percentage of biocontainer assemblies correctly classified out of the total number of biocontainer assemblies that are actually defective.	100%
Precision	The true negative rate or specificity of the test. Shows the percentage of biocontainer assemblies correctly classified out of the total number of biocontainer assemblies that are classified as defective.	100%

3.4 Conclusions

Using the OQ, a pass / fail leak rate specification was selected to minimize false negatives (product deemed acceptable but actually defective) and reduce the risk of false positives (product deemed to be defective but actually integral). Both control and defect data are a minimum of 3 standard deviations from the pass / fail specification.

All biocontainer assemblies tested were correctly classified as defective or non-defective. Based on the scoring methods for a binomial response variable, the OQ results demonstrated 100% accuracy, 100% recall and 100% precision for the assemblies tested. Defects at or above 2 µm equivalent nominal diameter orifice size can be detected within Allegro 2D biocontainer systems between 50 mL and 20 L.

4 HIT System Performance Qualification

4.1 Introduction

The purpose of these tests is to confirm the successful operation of the HIT system at the Pall Medemblik manufacturing site under the full control of the manufacturing team according to standard work procedures. The performance qualification PQ process followed the full manufacturing procedure from order receipt to final packaged product.

4.2 Summary of Methods

An additional 150 Allegro 2D biocontainer assemblies were produced and tested at the Pall Medemblik manufacturing site for the performance qualification. These 1 L and 10 L biocontainer assemblies followed the full manufacturing procedure from order receipt to final packaged product, including the helium integrity test.

The manufactured product assemblies are placed inside the test chamber. The production biocontainer test assembly is connected to the helium supply and then subjected to the standard test method as defined in the manufacturing standard operating procedures. The measured leak rate of helium is then recorded for a defined specific time and tested against the pass / fail specification. The HIT system PQ was run using the manufacturing program, which terminates the test before the specified time if a leak is detected.

4.3 Results

Table 3 summarizes the percentage of biocontainer assemblies which passed the helium leak test during the PQ manufacturing process.

Table 3.
Summary of HIT system performance qualification results using manufactured product

Allegro 2D Biocontainer Assembly Size	Number of SUS Tested	% HIT Pass
10 L	20	100%
1 L	130	100%

4.4 Conclusions

The Allegro 2D biocontainer assemblies manufactured during PQ all passed the HIT specification, with no biocontainer assemblies classified as defective.

5 HIT System Transfer Line Qualification

5.1 Introduction

The purpose of these tests is to qualify the HIT process for an extended range of transfer line lengths. Due to the complex nature of fitting the long transfer line into the HIT system chamber, the transfer line qualification includes the following:

- A design verification to confirm that:
  - The transfer line can fit into the HIT chamber and proposed handling procedures are effective
  - The HIT system is capable of detecting defects at the end of the maximum transfer line lengths (worst-case scenario for detecting a defect).
  - Safe differential pressures are not exceeded for minimum transfer line lengths (lowest possible volume and worst-case scenario for high differential pressures).
- An operational qualification to confirm the instrument functions according to specifications for minimum and maximum transfer line lengths
- A performance qualification to confirm effective operation of the HIT process for maximum transfer line lengths under full manufacturing conditions

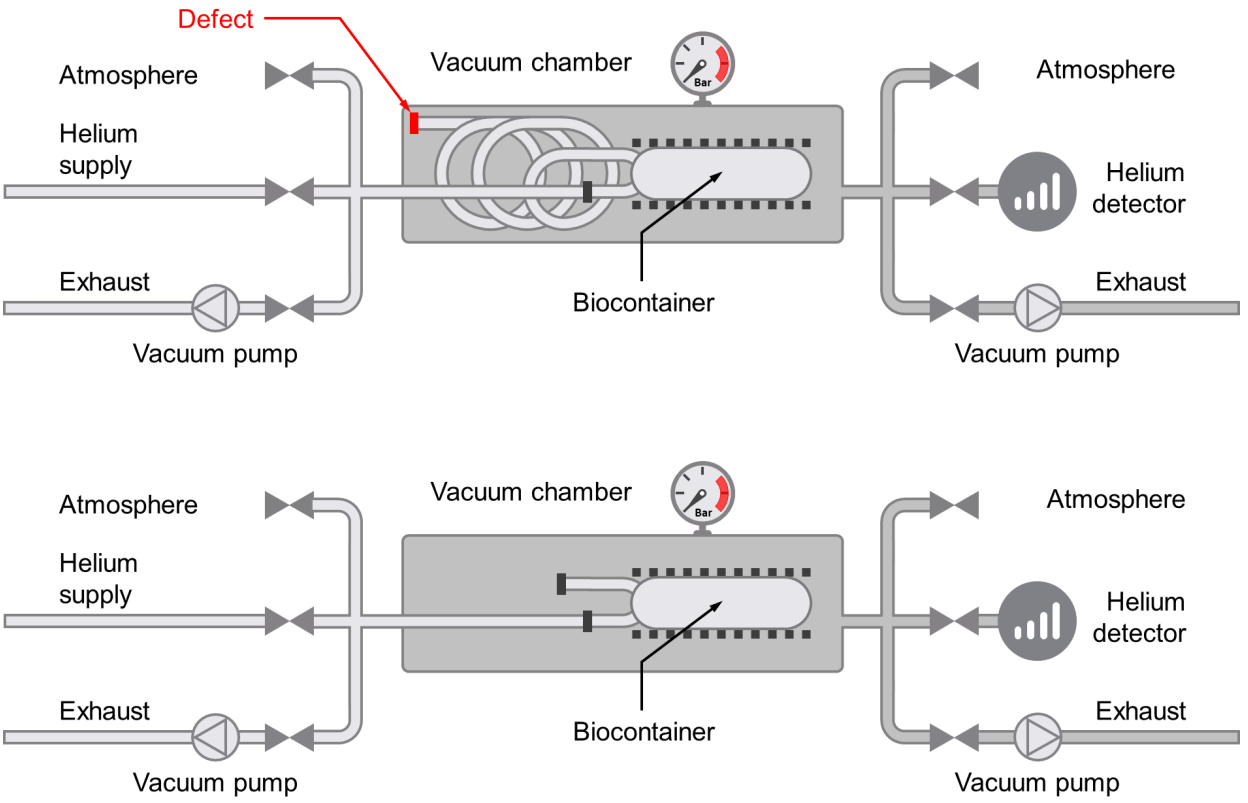
5.2 Summary of Methods

The tests were carried out using the HIT system as described in Section 1.5. The test assemblies (control or defect) are placed inside the test chamber. The helium line on the test assembly is connected to the helium supply and then subjected to the standard test method fixed during HIT system OQ, with modified test parameters to allow processing of both long and short transfer line length assemblies. The measured leak rate of helium is then recorded for a defined time period. The transfer line length OQ and PQ were both run using the manufacturing program which terminates the test before the specified time if a leak is detected.

Figure 8 shows a schematic of the biocontainer assembly within the HIT chamber for short transfer line lengths and maximum transfer line lengths, including demonstration of the positioning of the defect in the chamber at the furthest point from the helium detector. This illustrates that the HIT system transfer line qualification incorporates the following worst-case scenarios:

- Defect size corresponding to the smallest effective nominal diameter orifice size defect to be detected
- The maximum distance between the helium source and the defect, giving the worst-case path length for mass transfer:
  - Defect placed at the end of the longest transfer line
  - SUS design using the longest permitted transfer line length
- Positioning of the defect within the chamber to give the worst-case maximum distance between the defect and the helium detector.

Figure 8.
Schematic of the incorporation of biocontainer assemblies into the HIT chamber with a maximum transfer line length defect assembly with worst-case defect location (top) and a short transfer line length assembly during a typical manufacturing tests with the potential for a defect location anywhere on the assembly (bottom)



5.2.1 Transfer Line Design Verification

A total of 72 Allegro 2D biocontainer assemblies were tested using the HIT system as described in Section 1.5. Thirty-six (36) with a 2 µm equivalent nominal diameter orifice size defect and maximum allowable transfer line lengths. Thirty-six (36) without a defect with minimum transfer line lengths. The specific volumes and samples sizes of control and defect biocontainer assemblies used are summarized in Table 4.

5.2.2 Transfer Line Operation Qualification

A total of 72 Allegro 2D biocontainer assemblies were tested using the HIT system as described in Section 1.5. Thirty-six (36) with a 2 µm equivalent nominal diameter orifice size defect and maximum allowable transfer line lengths, and 36 without a defect with minimum transfer line lengths. The specific volumes and samples sizes of control and defect biocontainer assemblies used are summarized in Table 4.

5.2.3 Transfer Line Performance Qualification

An additional 36 production Allegro 2D biocontainer assemblies with maximum allowable transfer line lengths were produced and tested at the manufacturing site for the performance qualification. These biocontainer assemblies followed the full manufacturing procedure from order receipt to final packaged product, including the HIT method. The specific volumes and samples sizes of control and defect biocontainer assemblies used are summarized in Table 8.

5.2.4 Biocontainer Assemblies

The HIT system is designed to detect defects on the biocontainer film surface, at seals, and at joints with transfer lines. The test filling sets used during transfer line qualification include all these components, as shown in Figure 9 and Figure 10.

Figure 9.
Control with minimum transfer line lengths (top) and defect with maximum transfer line lengths (bottom) biocontainer assembly configurations for 50 mL, 100 mL, 250 mL, 500 mL, and 1 L sizes during HIT system transfer line design verification

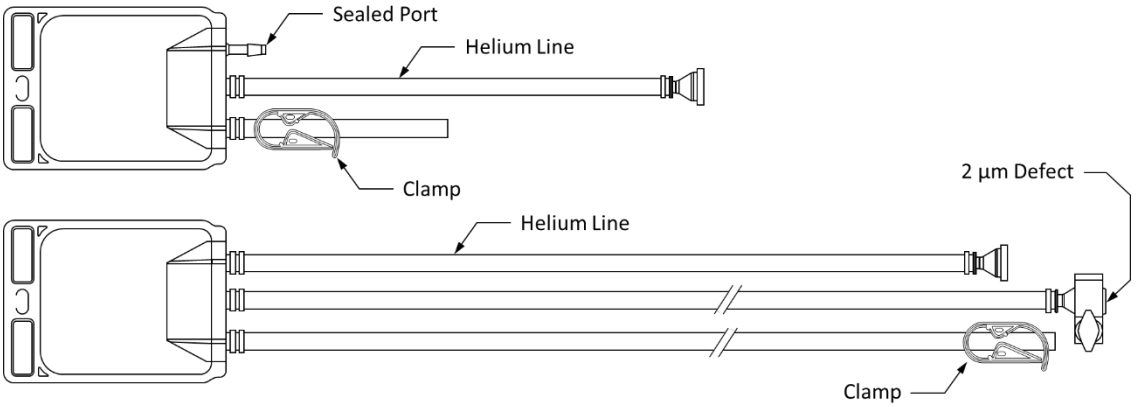
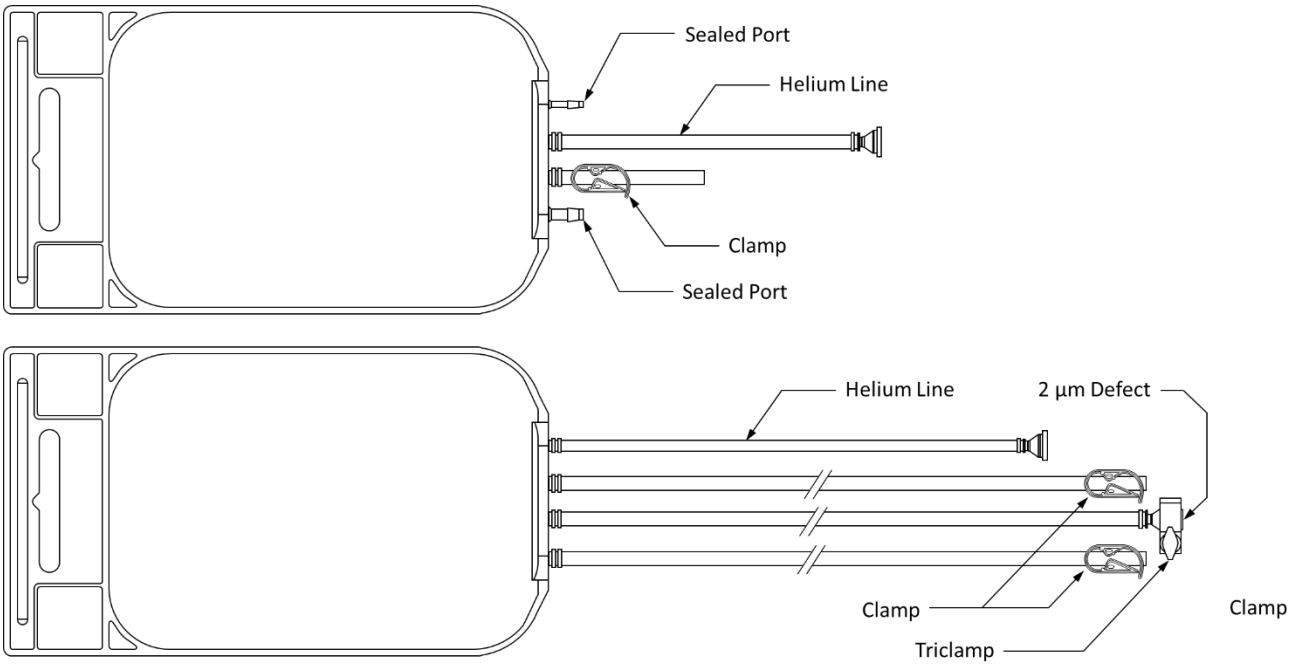


Figure 10.
Control with minimum transfer line lengths (top) and defect with maximum transfer line lengths (bottom) biocontainer assembly configurations for 2 L, 5 L, 10 L, and 20 L sizes during HIT system transfer line design verification



5.3 Results

Table 4 and Table 6 show the binary pass or fail results for the HIT method during design verification and operational qualification of the extended transfer line length scope. The data demonstrate no false negatives (product deemed acceptable but actually defective) and no false positives (product deemed to be defective but actually integral). The binomial response scoring method results are summarized in Table 5 and Table 7, showing 100% scoring for all methods due to the correct classification of every Allegro 2D biocontainer assembly tested.

The performance qualification test results are shown in Table 8, confirming that every Allegro 2D biocontainer assembly passed during the HIT process, as expected.

No biocontainer assembly tested exceeded internally defined maximum pressures for operation, even with minimum transfer line lengths and minimum possible volumes for the biocontainer assemblies tested.

Table 4.
Results of HIT transfer line design verification testing

Allegro 2D Biocontainer Assembly	Transfer Line Lengths	Number of Assemblies Tested	Number of HIT Failures (Positive Leak Detection)	Number of HIT Passes (Negative Leak Detection)
50 mL, control	Minimum	4	0	4
50 mL, defect	Maximum	4	4	0
100 mL. control	Minimum	4	0	4
100 mL, defect	Maximum	4	4	0
250 mL, control	Minimum	4	0	4
250 mL, defect	Maximum	4	4	0
500 mL, control	Minimum	4	0	4
500 mL, defect	Maximum	4	4	0
1 L, control	Minimum	4	0	4
1 L, defect	Maximum	4	4	0
2 L, control	Minimum	4	0	4
2 L, defect	Maximum	4	4	0
5 L, control	Minimum	4	0	4
5 L, defect	Maximum	4	4	0
10 L, control	Minimum	4	0	4
10 L, defect	Maximum	4	4	0
20 L, control	Minimum	4	0	4
20 L, defect	Maximum	4	4	0

Table 5.
Binomial response variable scoring method results for the HIT transfer line length design verification

Scoring Method	Description	Result
Accuracy	Percentage of biocontainer assemblies correctly classified	100%
Recall	The true positive rate or sensitivity of the test. Percentage of biocontainer assemblies correctly classified out of the total number of biocontainer assemblies that are actually defective.	100%
Precision	The true negative rate or specificity of the test. Shows the percentage of biocontainer assemblies correctly classified out of the total number of biocontainer assemblies that are classified as defective.	100%

Table 6.
Results of HIT transfer line length operational qualification testing

Allegro 2D Biocontainer Assembly	Transfer line Lengths	Number of Assemblies Tested	Number of HIT Failures (Positive Leak Detection)	Number of HIT Passes (Negative Leak Detection)
50 mL, control	Minimum	4	0	4
50 mL, defect	Maximum	4	4	0
100 mL. control	Minimum	4	0	4
100 mL, defect	Maximum	4	4	0
250 mL, control	Minimum	4	0	4
250 mL, defect	Maximum	4	4	0
500 mL, control	Minimum	4	0	4
500 mL, defect	Maximum	4	4	0
1 L, control	Minimum	4	0	4
1 L, defect	Maximum	4	4	0
2 L, control	Minimum	4	0	4
2 L, defect	Maximum	4	4	0
5 L, control	Minimum	4	0	4
5 L, defect	Maximum	4	4	0
10 L, control	Minimum	4	0	4
10 L, defect	Maximum	4	4	0
20 L, control	Minimum	4	0	4
20 L, defect	Maximum	4	4	0

Table 7.
Binomial response variable scoring method results for the HIT transfer line length design verification

Scoring Method	Description	Result
Accuracy	Percentage of biocontainer assemblies correctly classified	100%
Recall	The true positive rate or sensitivity of the test. Percentage of biocontainer assemblies correctly classified out of the total number of biocontainer assemblies that are actually defective.	100%
Precision	The true negative rate or specificity of the test. Shows the percentage of biocontainer assemblies correctly classified out of the total number of biocontainer assemblies that are classified as defective.	100%

Table 8.
Summary of HIT transfer line length performance qualification results using manufactured product

Allegro 2D Biocontainer Assembly Size	Transfer line Lengths	Number of SUS Tested	% HIT Pass
50 mL	Maximum	4	100%
100 mL	Maximum	4	100%
250 mL	Maximum	4	100%
500 mL	Maximum	4	100%
1 L	Maximum	4	100%
2 L	Maximum	4	100%
5 L	Maximum	4	100%
10 L	Maximum	4	100%
20 L	Maximum	4	100%
All Biocontainer Assemblies	**Maximum**	**36**	**100%**

5.4 Conclusions

All biocontainer assemblies tested were correctly classified as defective or non-defective. Based on the scoring methods for a binomial response variable, the transfer line length design verification and OQ results both demonstrated 100% accuracy, 100% recall and 100% precision for the assemblies tested. Defects at or above 2 µm equivalent nominal diameter orifice size can be detected within an Allegro 2D biocontainer system up to the maximum permitted transfer line lengths for all sizes between 50 mL and 20 L.

No biocontainer assemblies tested exceeded the maximum manufacturing pressure threshold during testing. This confirms that the minimum transfer line lengths permitted can be used without risk to the biocontainer assembly integrity.

The Allegro 2D biocontainer assemblies manufactured during the transfer line length PQ all passed the HIT specification, with no biocontainer assemblies classified as defective.

6 HIT Filling Set Qualification

6.1 Introduction

Complex transfer line configurations and longer total transfer line length biocontainer assemblies can be present in filling sets with 2D Allegro surge biocontainers. These single-use assemblies cannot be fully tested to a 2 µm defect size with sufficient capability and therefore the following tests were carried out using a flow calibrated 10 µm equivalent nominal diameter orifice size defect.

- A design verification to confirm that:
  - The transfer line from complex filling sets can fit into the HIT chamber and proposed handling procedures are effective
  - The new recipes for filling set testing using the HIT system are capable of detecting 10 µm defects
- An operational qualification to confirm the instrument functions according to specifications and is capable of detecting defective filling sets.
- A performance qualification to confirm effective operation of the HIT process for filling sets under full manufacturing conditions

6.2 Summary of Methods

The tests were carried out using the HIT system as described in Section 1.5. The test assemblies (control or defect) are placed inside the test chamber. The helium line on the test assembly is connected to the helium supply and then subjected to the standard test method using parameters developed for filling set testing and fixed during design verification. The measured leak rate of helium is then recorded for a defined specific time. The filling set OQ and PQ were both run using the manufacturing program which terminates the test before the specified time if a leak is detected.

6.2.1 Filling Set Design Verification

Eight (8) filling sets with Allegro 2D surge biocontainers and equipped with a 10 µm equivalent nominal diameter orifice size defect were tested using the HIT system described in Section 1.5. Control assemblies from the transfer line design verification have equivalent helium exposure areas and data from eight (8) assemblies were used for design verification analysis. The specific volumes and samples sizes of control and defect filling sets used are summarized in Table 9.

6.2.2 Filling Set Operation Qualification

A total of 30 filling sets with Allegro 2D surge biocontainers were tested using the HIT system described in Section 1.5, 20 with a 10 µm equivalent nominal diameter orifice size defect and 10 without a defect. The specific volumes and samples sizes of control and defect filling sets used are summarized in Table 11.

6.2.3 Filling Set Performance Qualification

An additional 20 production filling sets with Allegro 2D surge biocontainers were produced and tested at the manufacturing site for the performance qualification. These filling sets followed the full manufacturing procedure from order receipt to final packaged product, including the helium integrity test. The specific volumes and samples sizes of control and defect biocontainers used are summarized in Table 13.

6.2.4 Filling Set Assemblies

The HIT system is designed to detect defects on the biocontainer film surface, at seals, and at joints with transfer lines. The test filling sets used during qualification testing include all these components, as shown in Figure 11 and Figure 12. Defect assemblies with maximum transfer line lengths were

tested with the clamps next to the biocontainer open. Control assemblies tested during OQ with minimum transfer line lengths were tested with the clamps next to the biocontainer closed. Assemblies during PQ were tested with the clamps next to the biocontainer open and the defect line clamped at the end.

Figure 11.
Filling set assembly configurations for 500 mL and 1 L filling sets

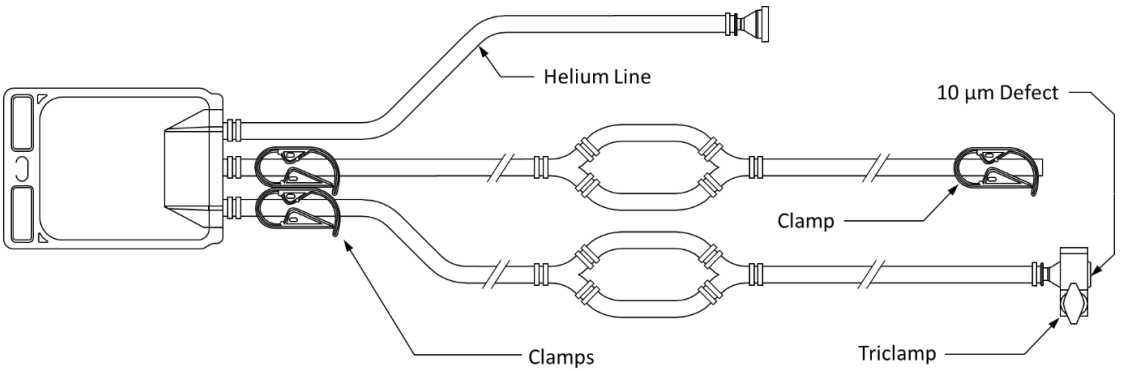
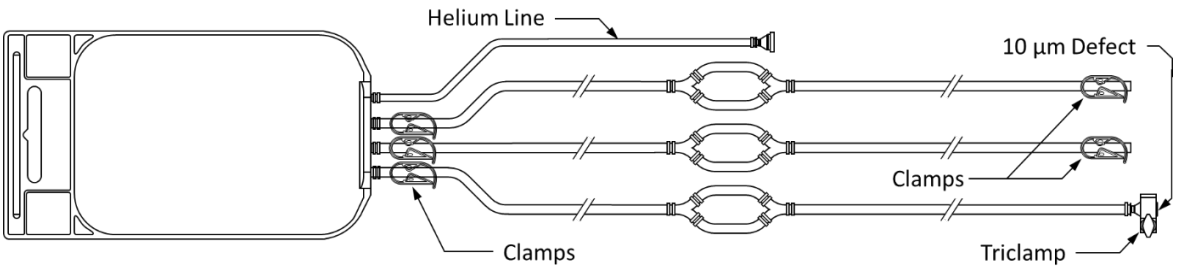


Figure 12.
Filling set assembly configurations for 2 L, 5 L, and 10 L filling sets



6.3 Results

Table 9 and Table 11 show the binary pass or fail results for the HIT method during design verification and operational qualification of the extended transfer line length scope. The data demonstrate no false negatives (product deemed acceptable but actually defective). The binomial response scoring method results are summarized in Table 10 and Table 12, showing 100% scoring for recall (100% detection of defective filling sets) and ≥ 95% accuracy and precision.

The reduced accuracy and precision are due to a single false positive (product deemed to be defective but actually integral). Analysis of the evolution of the leak rate over time confirmed that there is a clear and genuine leak and that the HIT system itself is not considered the cause of the false positive and is operating correctly. This false positive filling set was either due to operator failure to seal the test assembly correctly or the presence of a genuine defect on the filling set. Although precision and accuracy of the test process and overall yield of the process are reduced, the qualified pass / fail decision line correctly identifies all of the defective filling sets (100% recall) and protects the quality of the final shipped product. Since the HIT system itself has not caused the false positive result and there is no impact on the quality of the filling sets supplied, the qualification is considered successful.

The performance qualification test results are shown in Table 13, confirming that every filling set with an Allegro 2D biocontainer passed during the HIT process, as expected.

Table 9.
Results of HIT system design verification testing for filling sets

Allegro 2D Biocontainer Size	Filling Set Transfer Line Lengths	10 µm Defect?	Number of Assemblies Tested	Number of HIT Failures (Positive Leak Detection)	Number of HIT Passes (Negative Leak Detection)
500 mL	Minimum*	No	4	0	4
500 mL	Maximum	Yes	4	4	0
10 L	Minimum*	No	4	0	4
10 L	Maximum	Yes	4	4	0

** Data taken from the transfer line OQ using assembly designs with comparable HIT exposure areas*

Table 10.
Binomial response variable scoring method results for the HIT system design verification for filling sets

Scoring Method	Description	Result
Accuracy	Percentage of filling sets correctly classified	100%
Recall	The true positive rate or sensitivity of the test. Percentage of filling sets correctly classified out of the total number of filling sets that are actually defective.	100%
Precision	The true negative rate or specificity of the test. Shows the percentage of filling sets correctly classified out of the total number of filling sets that are classified as defective.	100%

Table 11.
Results of HIT system operational qualification testing for filling sets

Allegro 2D Biocontainer Size	Filling Set Transfer Line Lengths	10 µm Defect?	Number of Assemblies Tested	Number of HIT Failures (Positive Leak Detection)	Number of HIT Passes (Negative Leak Detection)
500 mL	Maximum	No	2	0	2
500 mL	Maximum	Yes	4	4	0
1 L	Maximum	No	2	0	2
1 L	Maximum	Yes	4	4	0
2 L	Maximum	No	2	0	2
2 L	Maximum	Yes	4	4	0
5 L	Maximum	No	2	1†	1
5 L	Maximum	Yes	4	4	0
10 L	Maximum	No	2	0	2
10 L	Maximum	Yes	4	4	0

† *A leak was detected in one 5 L control filling set, see Section 6.3 for further details.*

Table 12.
Binomial response variable scoring method results for the HIT operational qualification for filling sets

Scoring Method	Description	Result
Accuracy	Percentage of filling sets correctly classified	97%
Recall	The true positive rate or sensitivity of the test. Percentage of filling sets correctly classified out of the total number of filling sets that are actually defective.	100%
Precision	The true negative rate or specificity of the test. Shows the percentage of filling sets correctly classified out of the total number of filling sets that are classified as defective.	95%

Table 13.
Summary of HIT performance qualification results for filling sets using manufactured product

Allegro 2D Biocontainer Size	Filling Set Transfer Line Lengths	Number of SUS Tested	% HIT Pass
500 mL	Maximum	4	100%
1 L	Maximum	4	100%
2 L	Maximum	4	100%
5 L	Maximum	4	100%
10 L	Maximum	4	100%
All Biocontainer Sizes	**Maximum**	**20**	**100%**

6.4 Conclusions

All filling sets tested with a defect were correctly classified according to the pass / fail specification determined during HIT system OQ. Based on the scoring methods for a binomial response variable, the filling set design verification demonstrated 100% accuracy, 100% recall and 100% precision for the assemblies tested. The filling set OQ demonstrated 97% accuracy, 100% recall and 95% precision for the assemblies tested. The lower scoring results indicate a reduced manufacturing yield. The 100% recall is confirmation that all defect test assemblies were detected. Defects at or above 10 µm equivalent nominal diameter orifice size can be detected within filling sets with Allegro 2D surge biocontainers up to the maximum permitted transfer line lengths.

The filling sets with Allegro 2D surge biocontainers manufactured during the filling set PQ all passed the HIT specification, with no biocontainers classified as defective.

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