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White Paper

Novel Methods of Testing Container Closure Integrity in Large Volume Containers

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In this article, we discuss the Helium leak and dye ingress CCIT methods in relation to larger volume containers while reviewing potential strategies to address any issues. We will include technical considerations and case studies, as well as explaining how partnering with Lonza may help to overcome some of the challenges associated with CCIT of large volume containers.

Introduction

The goal of container-closure integrity testing (CCIT) is to assure the sterility and quality of a biotherapeutic drug product (DP) inside a container during its shelf life and before use in humans. Ensuring closure container integrity (CCI) is a regulatory requirement that helps assure a DP is safe, of an appropriate product quality and is in the correct dosage range at the point of patient administration. The most commonly used guidelines for CCIT are USP 71 for assuring product sterility [1] and USP 1207 [2] which includes guidance on selection and validation of leak testing methods, as well as tests for seal quality.

USP 71 guidelines have been used for CCIT to ensure sterility. This type of testing works by demonstrating the absence of microbial contamination through artificially created leaks. However, there are several limitations associated with this method of testing. One issue is that this type of CCIT will only detect viable microorganisms present, as well as those which can grow in the DP or test medium used. Additionally, sterility CCIT is subject to false positive results due to accidental microbial contamination introduced by operators at the time of testing.

The limitations associated with USP 71 sterility testing have prompted the development of physical tests, recommend in USP 1207 guidelines. These include the Helium leak test and the dye ingress test which are suitable for CCIT on small containers that have well-established sensitivity and acceptance criteria.

The growing demand for biotherapeutics and cell therapies has led to an increase in the use of large volume containers, such as flexible bags or plastic bottles shown in Figure 1, next page. However, CCIT methods are less well defined for these and pose specific challenges, such as bigger volumes, larger head space and the container's flexible nature. These issues have resulted in the need to determine, validate and even modify CCIT methods to suit large volume containers.

Figure 1
Examples of flexible Bags (left) and media fill bottles (right) used with biotherapeutic DPs.

Helium leak CCIT

The Helium leak CCIT method is used to find small leaks or larger leaks in bigger volumes. Helium is used as a tracer gas and its concentration is measured with a detector (Figure 2). This method is typically used to test for leaks with primary packaging and process development equipment such as single-use bioreactors. It has become popular as a leak detection method because it is an accurate test that is recommended in USP 1207 guidelines and has shown good correlation to USP 71 sterility testing [3]. It is also useful as it can be used as an accurate method for detecting artificially created leaks, as well as testing for unexpected stress-induced leaks at sealing interfaces such as sealing clamps and seams in single-use infusion bags.

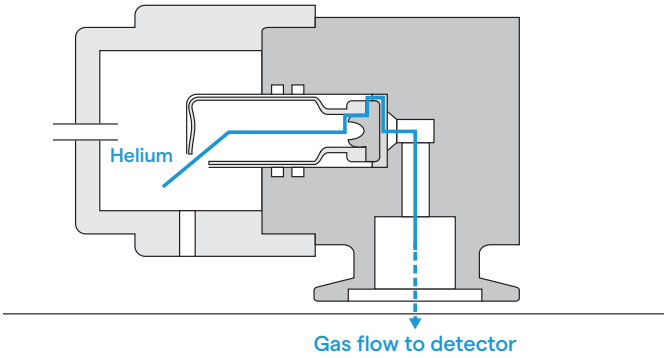


Figure 2
Example of typical set for helium leak CCIT on small containers (e.g. glass vials).

Application: Development of a Helium Leak CCIT Method for Media Fill Bags

Introduction

In this set of case studies, we developed and optimized a Helium Leak CCIT method to assess CCI in the filling tubes of media fill bags.

In our first study, we wanted to demonstrate CCI of the intermediate manual closure method, which consists of the miniature protector connector (MPC) assembly and pinch clamp (Figure 3) before a radio frequency (RF) seal was applied to the media fill bag.

This method involved removing and discarding the MCP, attaching the fill line to the helium gas, filling the product bag with helium, closing the pinch clamp and then capping the fill line with a new MCP (see Figure 4 for workflow).

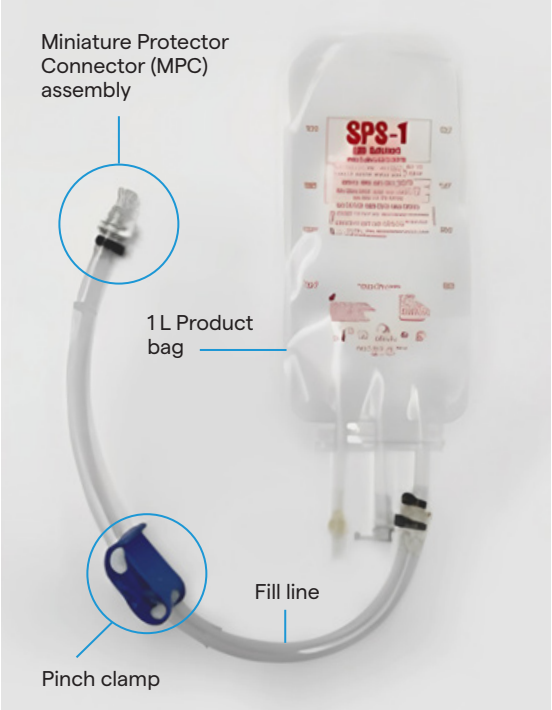


Figure 3
Manual closure points on the filling line attached to a media fill bag.

Purpose: Demonstrate suficient CCI of the intermediate manual closure method (MPC + pinch clamp) before RF sealing.

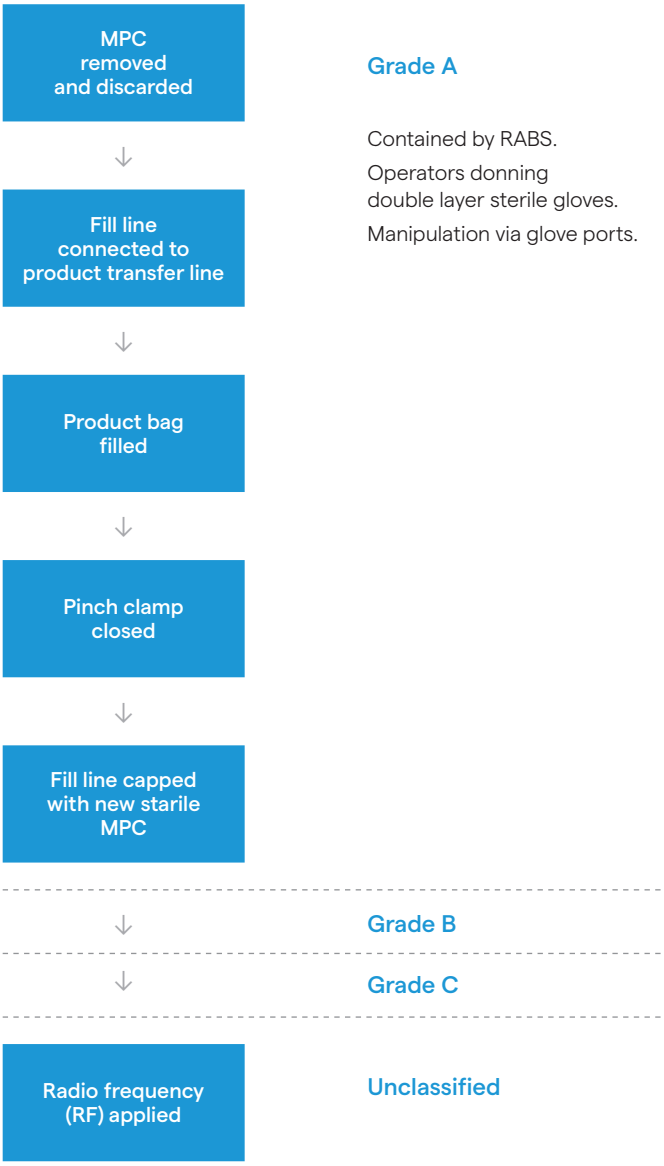


Figure 4
Workflow for CCIT of Media Fill Bags.

Case Study 1: Helium gas diffusion characterization

In this study, we wanted to determine the helium diffusion rate over time through standard polymer tubing where there were no artificially created leaks in the tubing. To develop an optimal result readout time, we allowed helium inside the test chamber 10 seconds after turning on the vacuum to ensure air elimination and gas stabilization and then set the readout time to every 10 seconds. This test provided baseline standard helium gas diffusion rates (see Figure 5) which are below the acceptance criteria (1.0×10-7 mbar L/s) for both the MCP and pinch clamp within 20 seconds.

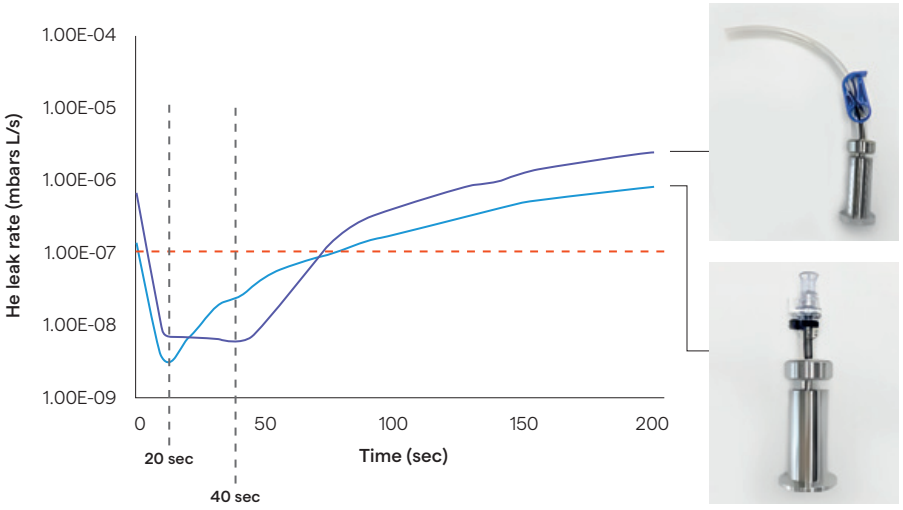


Figure 5
Helium diffusion rate through standard polymer tubing.

Case Study 2: Clamping process characterization

In this study, we wanted to determine the effect that the number of clicks used to secure the pinch clamp would have on helium diffusion through standard polymer tubing without any leaks. We tested 6–9 clicks and found that 8 clicks provided the lowest rate of helium diffusion (Figure 6), while with 6 clicks the helium diffusion rates at 2×10-6 mbar L/s was several orders of magnitude higher and is above the acceptance criteria (1.0×10-7 mbar L/s).

9 clicks	8 clicks	7 clicks	6 clicks
Helium rate (mbar L/s)			
8.80E-09	7.60E-09	8.50E-09	2.60E-06

Figure 6
Helium diffusion rate with a range of clicks of the pinch clamp.

Case Study 3: Sensitivity assessment

To determine the sensitivity of our helium CCIT method, in this study, we used 40µm copper wire to make a hole in between the pinch clamp (set at 8 clicks) and tubing and 20µm fused capillary to make another hole in between the male and female MPC assembly to create artificial leaks in the tubing. Our readout time was set at 10 seconds, and our results showed good sensitivity within 20 seconds of measuring helium diffusion for both 20µm (~1x 1.0×10-3 mbar L/s) and 40µm (~1x 1.0×10-5 mbar L/s) leak sizes (figure 7) which are above the leak acceptance criteria (1.0×10-7 mbar L/s).

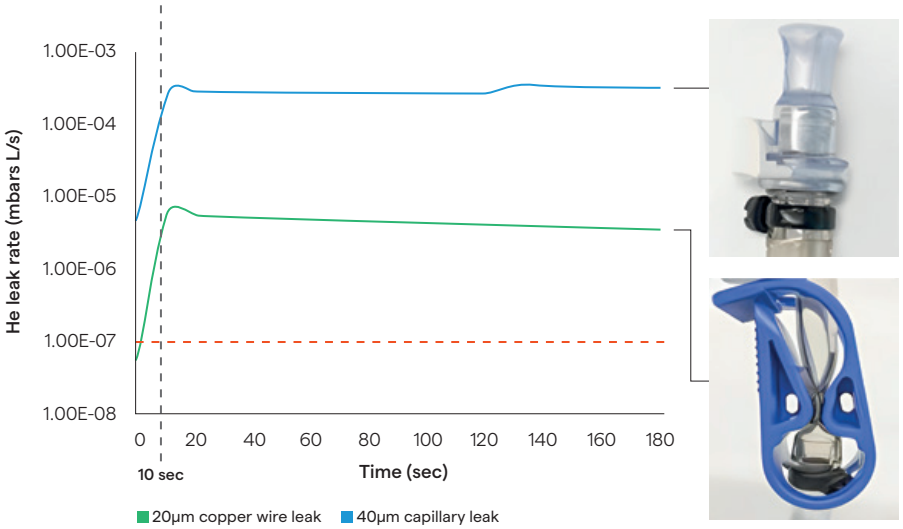


Figure 7
Helium diffusion rates through polymer tubing with artificially manufactured leaks.

Results

These results indicate that our helium leak CCIT, when optimized for readout time (Figure 5) and pinch clamp closure (Figure 6) can provide a sensitive test for measuring 20µm and 40µm leak sizes (Figure 7). Since the test is highly sensitive and well suited to use with flexible and larger plastic bags, this method could therefore be used for detecting leaks in tubes, as well as media fill and infusion bags. Additionally, with optimization our helium leak CCIT method could be suitable for detecting leaks in larger volume polymer containers.

The dye ingress CCIT method

While the helium test is sensitive and works well with flexible bags, there are often cases where leak detection requirements are less specialized, or results are needed faster. In these circumstances, the dye ingress method could be considered as an alternative and has been widely applied as a method for CCIT as it is relatively inexpensive and easy to set up [4], see Figure 8 for the workflow.

In summary, this test involves immersing a container in a blue dye solution in a pressure vessel. The containers are stress tested by exposure to vacuum and/or pressure over specific times to force liquid into any leaks. The container is then inspected visually for dye ingress into any leaks by comparing it with positive and negative dye controls. This dye ingress method for CCIT has several disadvantages, it is non-quantitative and subjective as it requires operator inspection. It is also not as sensitive as the helium leak CCIT, its correlation to microbial ingress CCIT has not been established and specific test method development is needed for each container tested. However, using spectrophotometric techniques for dye detection with fluorescent dyes could solve the issue of this test not being quantitative and eliminates dependency on the subjective observations of different operators as the container inspection can be automated.

For larger drug substance containers, CCIT using the dye ingress method can be challenging due to larger volumes, more head space and the flexibility of the container, therefore significantly more aggressive stress conditions are required, as well as method optimization.

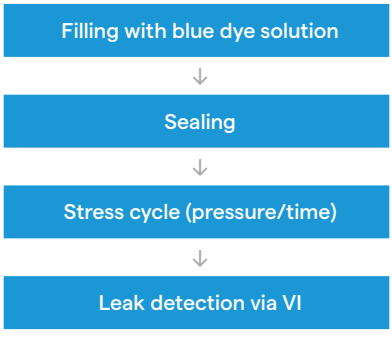


Figure 8
Example of typical workflow for dye ingress CCIT.

Application: Development of a Dye Ingress Method to Quantitatively Assess CCI on Screw-Cap Media Fill Bottles

Case Study 1: CCIT of screw-cap media fill bottles

To develop a quantitative dye ingress CCIT method, we used the experimental workflow summarized in Figure 9. Using glass capillaries, we punctured holes in a range of sizes (20µm, 50µm and 100µm) in screw cap plastic media bottles of different volumes (125mL, 500mL and 2L). To develop a more quantitative CCIT leak ingress method we used a fluorescent dye, which could be measured spectrophotometrically with a fluorescein reader. To determine the best buffer/dye solution for quantitative measurement, we investigated composition and pH parameters using two fluorescent dye and buffer combinations: acetate buffer pH 5.5 + 0.1% (w/v) with fluorescein sodium and histidine buffer pH 6.0 + 0.1% (w/v) with fluorescein sodium. We then used a range of common stress cycle parameters (see Table 1) including USP <381>and ISO 8362-5 CCIT standard methods [4] to induce leaks in DP containers. As a negative control we used a screw-cap bottle without any holes with the cap secured using epoxy glue. All containers were assessed for dye ingress using the methods described in Table 1 and data was collected for analysis.

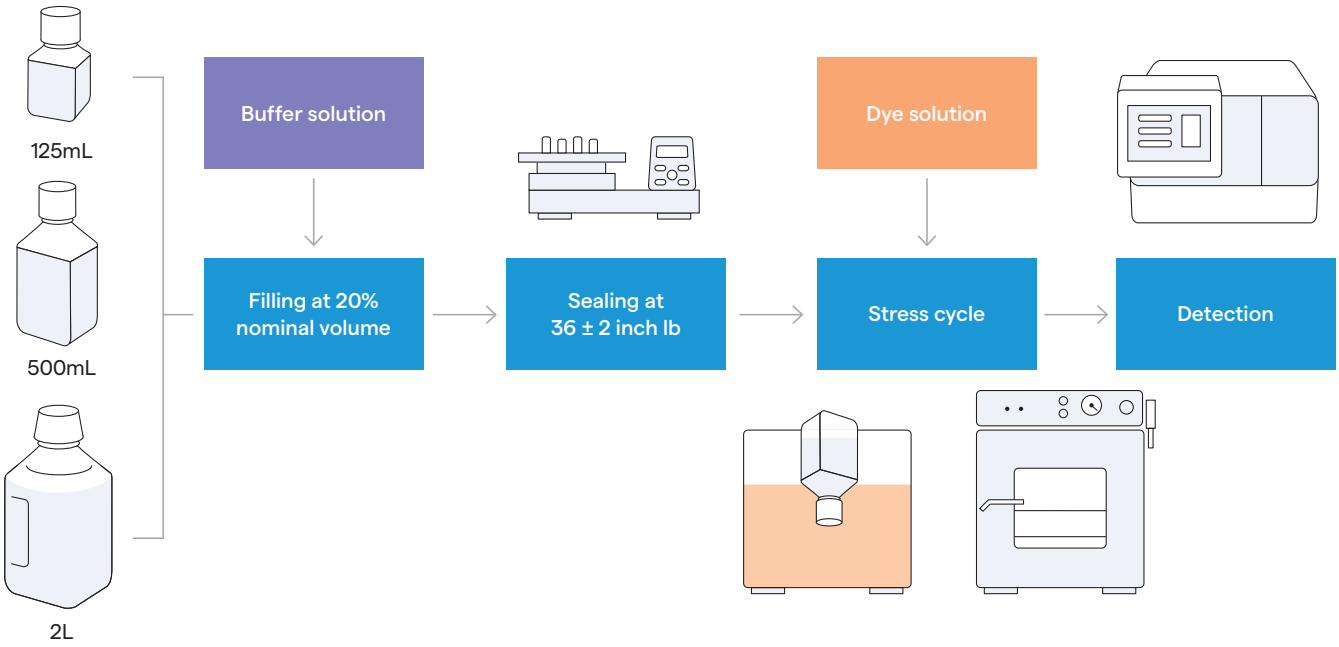


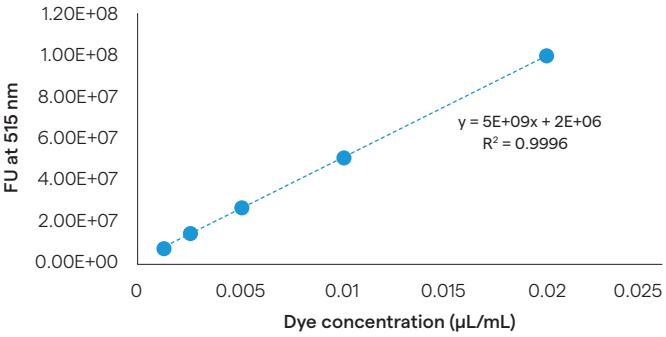
Figure 9
Workflow for developing dye ingress CCIT for quantitative detection.

Table 1
Stress cycle parameters used for dye ingress CCIT of screw-cap media fill bottles

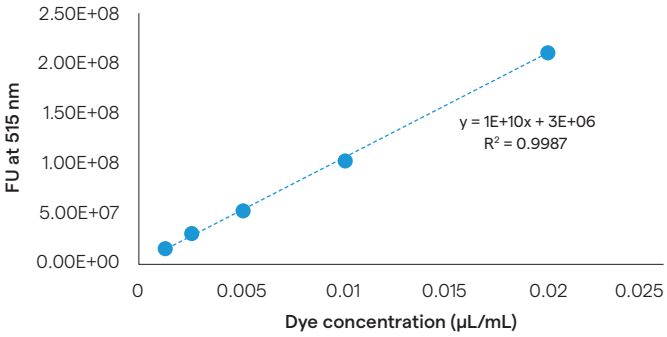
	USP <381> Ph. Eur. 3.2.9	ISO 8362-5	Lonza DPS Method
Vacuum	- 27 kPa (- 270 mbar)	- 25 kPa (- 250 mbar)	(- 300 mbar)
Time at vacuum	10 min	30 min	15h
Time at ambient	30 min	30 min	20h
Detection	Fluorescein reader	Visual inspection and fluorescein reader	Fluorescein reader

Linearity, LoQ and LoD

Absorbance data from the Lonza DPS dye ingress method used was assessed statistically for linearity, limit of quantitation (LoQ) and limit of detection (LoD). The results (Figure 10) showed that this method was sensitive as it could detect < 0.05 µL/ml of dye. Additionally, the absorbance and dye concentration data had good correlation which indicates the Lonza dye ingress method could be used to quantitatively assess leaks in media bottles.



LoQ	LoD
32671 FU	28407 FU



LoQ	LoD
116993 FU	54681 FU

Figure 10

Statistical analysis of Lonza DPS dye ingress method using two different buffer and dye combinations: acetate, pH 5.5 + 0.1% (w/v) fluorescein sodium (left) and histidine, pH 6.0 + 0.1% (w/v) fluorescein sodium (right).

Comparison with compendial test conditions

To validate the Lonza DPS dye ingress method, absorbance and LoD data from the Lonza DPS, USP <381> and ISO 8362-5 dye ingress methods were compared and any that had an absorbance > LoD were considered positive for dye ingress. The results (Table 2) showed that USP <381> test conditions with histidine buffer were not suitable for large volume containers as no leaks were detected in any of the container sizes. With the ISO 8362-5 stress conditions only larger leak sizes (100µm) were detected and there were no positive signals detected for 2L format bottles regardless of the stress conditions used. The same results were obtained for acetate buffer/dye solution (results not shown).

In contrast, the Lonza DPS dye ingress method, showed high sensitivity and allowed detection of 20µm leaks across all container sizes (Table 3). Additionally, a higher signal was measured with higher pH histidine buffer and with larger leak sizes indicating that this could be used as a quantitative test.

Table 2

Dye ingress CCIT results of screw-cap media fill bottles under USP <381> and ISO 8362-5 stress cycle conditions.

Stress cycle conditions	Buffer/ dye solution	125 mL				500 mL				2 L			
		Neg.	20 µm	50 µm	100 µm	Neg.	20 µm	50 µm	100 µm	Neg.	20 µm	50 µm	100 µm
USP/EP	Histidine	–	–	–	–	–	–	–	–	–	–	–	–
ISO	Histidine	–	–	–	++	–	–	–	++	–	–	–	–
Modified ISO	Histidine	–	–	–	++	–	–	–	++	–	–	–	–

Key: (-) < LoD, (+) > LoD, (++) >> LoD, (+++) >>> LoD

Table3
Dye ingress CCIT results of screw-cap media fill bottles under Lonza DPS stress cycle conditions.

Stress cycle conditions	Buffer/ dye solution	125 mL				500 mL				2 L			
		Neg.	20 µm	50 µm	100 µm	Neg.	20 µm	50 µm	100 µm	Neg.	20 µm	50 µm	100 µm
Lonza method	Histidine	–	+	++	+++	–	+	++	+++	–	+	++	+++
	Acetate	–	+	+	++	–	+	+	++	–	+	+	++

Key: (-) < LoD, (+) > LoD, (++) >> LoD, (+++) >>> LoD

Results

These results indicate that by optimizing physical parameters such as buffer/dye solution pH, pressure and time during the stress cycle the Lonza DPS dye ingress CCIT can provide a more sensitive test for measuring 20µm, 50µm and 100µm leak sizes than current compendial standard tests (Table 2 and 3) in media bottles of up to 2L. This method could therefore be used for accurately detecting leaks in larger volume containers.

Conclusions

CCIT is required by regulatory authorities to ensure patient safety. Since many technologies can be applied to CCIT, it is important to understand that there is not one universally accepted CCIT method which will be appropriate or practical for all for all products and container formats [5]. Worldwide and local regulatory requirements offer no clear guidance as to what is required other than that sterility must be maintained until the end of the product's shelf-life.

This array of CCIT options and lack of clear guidance means that choosing the wrong type of testing is all too easy for biopharmaceutical companies. This is why guidance from an experienced Contract Development and Manufacturing Organization (CDMO) that can make recommendations on suitable CCIT methodologies or develop methods that improve compendial standards is needed.

As detailed in this white paper, at Lonza we have for example developed and tested an innovative use of the helium leak CCIT method which is suitable for use with flexible plastic tubes attached to media fill and infusion bags.

Additionally, we have developed a quantitative dye ingress CCIT method which can reliably detect leaks as small as 20µL in larger volume containers (125mL, 500mL and 2L media bottles) and is more sensitive than current compendial standards which are not stringent enough for this application.

In summary, choosing a strategic development partner such as Lonza that has vast experience with recommending appropriate CCIT, and even developing more sensitive CCIT methods, can help save time and costs, and more importantly assure the quality of your drug products to the required safety standards.

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