Selecting the Best Deterministic Method for your CCIT project

Container Closure Integrity Testing (CCIT) is a critical component that must be considered in the manufacturing of a drug product or combination device, such as a pre-filled syringe. The landscape of CCIT has been changing over the past 15+ years due to changes in the regulatory mindset regarding sterility testing and detection limits around CCIT. In 2008, the FDA published a guidance document "Container Closure System Integrity Testing In lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products." This document gave clear indication that CCIT was the preferred avenue for confirming product sterility during stability studies and thus through the lifecycle of the product. While sterility testing is necessary at the release of a product to ensure that the product is sterile upon completion of manufacturing, there are limitations to using this test to indicate the product will remain sterile until the end of the lifecycle. This document did not provide testing specifics for CCIT but stated that during stability testing, sterility testing should be replaced by "properly validated physical or chemical container closure system integrity test (e.g., bubble tests, vacuum/pressure decay, trace gas permeation/leak tests, dye penetration tests, seal force of electrical conductivity and capacitance tests, etc.), or microbial container closure system integrity tests (e.g., microbial challenge or immersion tests)."

This was followed in 2016 by an update of the USP <1207> monograph, in which the monograph went from approximately three pages to more than 30 pages. The updated monograph introduced a couple of new concepts that had not previously been a part of the chapter that were extremely important:

- Maximum Acceptable Leakage Limit (MALL) is the maximum allowed defect size/leak rate that is allowed, which ensures there is no risk to product safety. This rate is set at 6E-6 mbar * L / sec for assurance of a sterile barrier for finished products. This rate may need to be lower depending upon the needs of the product.
- Inherent CCIT is the concept of testing container integrity on empty vials that have been through the validated packing system to show that the MALL can be met with the closure system. This is independent of testing the actual product within the container as the product may influence CCIT and methods for stability should be validated as a product/packing system.
- Because of the addition of a MALL of about 6E-6 mbar * L / sec the use of deterministic methods over probabilistic methods is preferred. The deterministic methods have a greater reliability and lower detection limits than probabilistic methods in most cases. While probabilistic methods are not completely eschewed, it is clear in the chapter that there should be justification for the use of probabilistic methods.

As these requirements go forward, it becomes increasingly important that a manufacturer understand their container closure system, and that a profile for all container systems be maintained. As the expectations move to lower allowed defects for a container, it becomes more important that deterministic methods are used to reach the desired maximum defect levels. While the USP gives a brief outline of some of the different techniques that may be used for CCIT, it does not give specific methods, nor does it require a specific technique to be used for any product. This is because there is not a single technique that can test all product packaging configurations and instrument parameters, as what works for one product may not be acceptable for another. Because of this, it is expected that the verification/validation of any method used for stability studies be performed for each product-container system.

To determine the best technique for a specific product/packaging system, understanding the different deterministic techniques and how they work is critical. Understanding the advantages and flaws in each technique allows the manufacturer to ensure compliance with the expectations of regulatory bodies reviewing CCIT work. A brief description of various techniques and the advantages and disadvantages of each is as follows.

Tracer Gas Detection, Vacuum Mode (Helium Mass Spectrometry)

This is the most sensitive of the techniques for CCIT. It can detect leaks down to and below 6E-6 mbar * L / sec. The samples are prepared by putting helium into the container and then sealing the container. The helium filled sample is then placed into the instrument and placed under a vacuum to determine if helium leaks from the container. The helium flow rate is calculated and reported by the instrument, and values below the rejection limit are integral, while samples higher than the rejection limit contain a defect above the MALL. This technique is extremely useful for inherent CCIT as it is able to demonstrate that the container-closure system can meet the MALL for sterile barriers.

This technique is also useful as samples can be tested at -80C. This is important as it is expected that CCIT will be demonstrated under all conditions in which the package may store and shipped. Many new biologics samples are cryofrozen, and this can be detrimental to the rubber stop-

per. As rubber is cooled, it reaches a transition point where elasticity is lost, and the material begins to contract. When the vials are returned to room temperature, the stopper regains its normal elasticity and can reseal the container. Due to this, a sample in a cryofrozen state may have defects between the container and stopper while they are perfectly intact at room temperature. By testing at -80C, it is possible to determine if there are issues with the mating of the rubber stopper and container that may affect product sterility that would not have otherwise been noticed.

Advantages – Extremely low detection limit (the ability to test inherent (empty container) CCIT to MALL for sterile barrier). With appropriate setup, frozen samples can be tested.

Disadvantages – Destructive testing as helium must be introduced to the container system. Liquid/solids must not be present at leak site, or the defect can be blocked. Liquid vapor from a sample can damage an instrument if not careful. The sample must be able to withstand vacuum pressures, syringes, and flexible packaging may need special tooling for testing. Permeation through semi-porous materials (plastics) may interfere with the ability to detect lowest defect levels.

Limit of Detection – 0.1 micron and lower.

Best Fit – Inherent CCIT for vials and rigid containers.

Vacuum Decay

For vacuum decay, a product is placed inside of a chamber that is designed to fit tightly around the container to reduce the amount of dead space in the chamber. The instrument then pulls a vacuum inside the chamber to a preset point. Should that preset pressure be unable to be reached, the sample is determined to have a gross leak, and further testing is not possible. If the pressure is reached, the instrument stabilises and then begins to measure the increase in the pressure inside the chamber over time. If the pressure in the chamber changes, the only source of the increase is the container indicating a defect. The instrument measures the decay rate and thus indirectly measures the flow rate from the container. Based on values obtained during development and validation of the method (using positive controls and integral samples), the leak rate can be determined and compared to a rejection limit for the product.

Because of the nature of this testing, it can be exceedingly difficult to detect small defects in the presence of a large molecule or high salt samples. As liquid escapes from a container, it meets the incredibly low pressures inside the testing chamber and is instantly vaporised. Upon vaporisation, if there is a large molecule or high salt presence, it is possible to be left behind in the defect thus causing a blockage. This can cause false negative results and thus the technique is not recommended for these materials.

With the correct interment setup, vacuum decay can be used to test flexible packaging. The limitation on the flexible packaging is the ability of the package to withstand vacuum pressure. By using a lower vacuum pressure setting during testing, the flexible packaging can be tested. The drawback of this testing is that most flexible materials will allow gas to permeate, and the permeation can mask smaller defects if not understood.

Advantages – Non-destructive. Flexible packaging possible. Can test liquid, solid or gas product. Fill volume not a concern.

Disadvantages – Large molecule/high salt content can block defects. Each container needs a chamber designed for that container. Syringes possible but must ensure plunger does not move during testing. Vacuum sealed packaging is difficult/impossible to test.

Limit of Detection – About 5 microns.

Best Fit - Small molecule product in vials with crimp top, ampoules, bags, and flexible containers (with proper setup), and bottles able to stand low pressure.

Mass Extraction

This technique is similar to vacuum decay. The sample is placed into a chamber that is then evacuated and brought to vacuum. The difference is that instead of indirectly measuring the flow rate by measuring the decay rate of the vacuum around the container, the mass of the container is measured, and the flow rate is determined in terms of weight / sec. Due to the use of an extremely sensitive balance and the fact that the flow rate is being determined directly from the container, the method can be more sensitive than vacuum decay.

Advantages – Non-destructive. Can test liquid, solid or gas product. Fill volume not a concern. High sensitivity.

Disadvantages - Large molecule/high salt content can block defects. Each container needs a chamber designed for that container. Syringes possible but must ensure plunger does not move during testing.

Limit of Detection – About 1-2 microns.

Best Fit - Small molecule product in vials with crimp top, ampoules, and bottles able to withstand low pressure.

Pressure Decay

There are two different techniques for the use of pressure decay. The most common historically has been to pressurise the inside of a container with gas to a constant pressure. Once the pressure stabilises inside the bag, the loss of pressure is measured over time. The pressure drop is then compared to values determined during development and validation, and if found to be higher than a pre-determined rejection limit, the package is deemed to have a defect.

More recently, instruments like vacuum decay have been designed to test pressure decay. The sample is placed into a high-pressure chamber but instead of pulling a vacuum on the outside of the container, the inside of the chamber is pressurised. The loss of pressure over time is measured and compared to a pre-determined rejection rate to determine if a defect is present. These instruments are often able to use both pressurisation and vacuum of the chamber to overcome the issues with large molecules associated with traditional vacuum decay.

Advantages – Non-destructive (external pressure). Can test liquid, solid or gas product.

Disadvantages -Destructive (internal pressure). Container must be able to withstand high pressure.

Limit of Detection – Internal pressure (20+ micron); External pressure – low micron levels.

Best Fit - Small molecule product in vials with crimp top, ampoules, bottles able to withstand higher pressure, and large Bags.

Electrical Conductivity & Capacitance

This technique, often referred to as High Voltage Leak Detection (HVLD), uses an electrical current to detect defects in samples. This is one of the techniques that can reliably be used with large molecule products as the only sample requirement is that there must be a liquid (of higher conductivity than the container) present at the defect site. The instrument has two probes. One of the probes acts as a source through which an electrical current is applied to the sample. The current passes through the sample to a probe on the other side which measures the voltage of the current passed through the samples. The sample is spinning at a predetermined speed, and when intact, the current is conducted through the material of the container, which would be a low conductivity material (glass or plastic). Because the material is a low conductivity material, the output will be a low voltage at the receptor. As the sample spins, the probes are programmed to move up and down the body of the container. If the current encounters a defect where a liquid is present, the voltage output at the receptor will spike as the material in the container has a higher conductivity than the container. This instrument measures the voltage output over time creating a graph and measuring the peak voltage output during the analysis of the sample. If a spike occurs and is above a pre-determined rejection limit, the sample is deemed to have a defect.

For this technique to work, the sample must have a higher conductivity than the container and must not be flammable for obvious reasons. Also, the volume of sample in the container must be high enough to ensure that the sample solution is in contact with any defect to carry the electrical current. Because of this, the fill volume needs to be about 30% of nominal or higher. As previously stated, the product must have a higher conductivity than the container. This creates issues for containers that have metal components such as a crimp cap or needle. In the case of crimp caps, the most common place for a leak in a vial with a crimp cap is typically at the interface of the rubber stopper to the container. Since this junction is under the crimp cap, defects in this area are not able to be determined. For syringes, one of the most common points to see defects is the point where the needle is inserted into the body of the syringe. Detecting defects at or near this point can be difficult for the same reason as the aluminum crimp caps. The second likely source of defects in a pre-filled syringe is at the stopper to body interface. However, traditional HVLD instruments have two probes on the outside of the container and need liquid at the defect to see a spike in voltage. As the stopper is inside the syringe barrel, a defect at this junction may not be detected. Newer designs have a third probe that enters the syringe barrel to detect leaks at this junction, but detection can still be problematic.

Advantages – Non-destructive. Large molecules not an issue.

Disadvantages – Crimp caps and needles can hide defects. Output not proportional to defect size.

Limit of Detection – ~5 microns.

Best Fit - Ampoules, glass containers.

Laser Headspace (Oxygen)

In oxygen headspace, a laser beam is passed through headspace of a container. If oxygen is present, there is absorbance of the beam. Because a laser is transmitted through the headspace of the container, it is also necessary that the container be transparent at the laser's frequency (glass, some plastics) and that sufficient headspace be present in the sample (3+ mm usually sufficient). The absorbance is quantified against a calibration curve created by running oxygen standards at varying concentrations. These standards are created using the same containers (reduce variability) that are used for the container closure system and adding various amounts of oxygen. This allows the direct measurement of oxygen in the container. This data can be used at the beginning of a project to ensure low oxygen filling lines are operating properly and that the containers are low in oxygen. This can also be used in stability studies to determine if samples are increasing in oxygen content over time. For stability studies, the samples are measured at the initial point to get an oxygen value prior to being placed into the correct stability conditions. At the desired timepoint, the same samples are pulled and retested. If the oxygen has increased above a set level, then it is determined that a defect is present. For products where the oxygen content can cause degradation of the product or where vacuum conditions are required for storage, the rejection limit can be set to state that any oxygen above a certain level is a failure. The key component for oxygen headspace in stability is time. During development with nitrogen to get low levels of oxygen. The defects are allowed to sit at ambient conditions for a period time (72 hours) and then retested to show whether oxygen levels have increased.

Since oxygen is present in the normal packaging environment at a level of about 21%, this technique is limited in use. The samples must have been packaged under vacuum or in a modified atmosphere environment (Nitrogen, Helium) for the technique to be used. Another drawback is that even in the best sealed mated closure systems there will be permeation of oxygen into the container. For longer stability studies there could be an increase of oxygen levels of up to 5% (about 1% per year for a 5-year study). It is imperative to understand this natural increase so that a limit can be set that demonstrates a defect and not the natural ingress of oxygen over time.

As the technique is measuring ingress of oxygen gas over time, it can be extremely sensitive as oxygen is able to enter the containers through very small defects. This technique can be used for inherent CCIT to determine if the container is able to meet the MALL per USP <1207> as defects below 0.2 microns are able to be detected with the correct setup.

Advantages – Fast testing. Low detection limits. Non-destructive.

Disadvantages – Modified headspace necessary. Must have headspace present in package.

Limit of Detection – ~0.2 microns or lower.

Best Fit – Rigid containers of glass and some plastics. Modified headspace studies. Inherent CCIT.

Laser Headspace (Carbon Dioxide)

This newer technique is nearly identical to oxygen headspace. The difference is that instead of looking for oxygen in the headspace of a container, the analyte of interest is carbon dioxide. While this may seem like a minor change, it opens the headspace technique to a dramatic degree. Carbon Dioxide makes up a very small percentage of air in normal surroundings (0.04%) unlike oxygen. The fact that carbon dioxide is not expected to be inside the container allows this technique to be used on containers that are packaged in any environment. This technique employs the same concepts as dye ingress, but instead of using a liquid as the substance for ingress, gas is used. And instead of getting a visual observation for the result, a numeric value can be obtained.

The first step is to place the samples into a chamber very similarly to the ones used for dye ingress. The air in the chamber is replaced with carbon dioxide, and the samples are allowed to sit in the chamber filled with carbon dioxide for a pre-determined amount of time. The samples are then removed from the chamber and tested to determine carbon dioxide levels. If the carbon dioxide levels in the container are above a pre-determined level, then a defect is present.

Because the liquid inside the container is not subjected to vacuum pressures as in many of the deterministic techniques, this method is able to test large molecule products down to very low levels. This technique is also extremely useful for cryo-stored materials. As previously mentioned under the helium technique, the rubber stoppers of a container can become rigid and inflexible when frozen at very cold temperatures. By exposing the samples to carbon dioxide at these temperatures, it is possible to determine if the freezing/thawing process is a problem for the container. The carbon dioxide will enter the container if there is a defect formed by the freezing of the stopper and will be trapped in the container once the rubber stopper regains elasticity upon returning to room temperature. This makes this technique a viable measurement of shipping studies especially when samples are shipped on dry ice.

This is another technique that can be used for inherent CCIT as it is possible to determine if defects on the order of 0.2 microns are present and can be used to test samples that are stored in frozen conditions to determine if the freezing affects the sealing capability of the container.

Advantages – Non-destructive. No special headspace required. Shipping studies.

Disadvantages – Must have headspace in container.

Limit of Detection – 0.2 microns and below.

Best Fit - Vials and syringes with at least 2-3 mm headspace and large molecule products.

This is not meant to be an exhaustive list of the current methodologies available for CCIT. This would be difficult as there are new techniques being established, and there are many types of packaging difficulties for the techniques listed above. Due of this, probabilistic methods such as dye ingress are still the best choice for some items. However, when this is the case, it is becoming more apparent from the regulatory bodies that justification for why dye ingress was used is expected.

It should be apparent that the choice of technique used is critical to successful CCIT. It is imperative to understand the advantages and disadvantages of the technique chosen for testing. It is also important to understand that CCIT is not a once and done test, and an overall CCIT profile of a package-product system is necessary. In December 2025, a new USP monograph (<382>) is scheduled to become effective. This monograph further reinforces the idea that a package system being used for sterile products must be able to meet the MALL of 6E-6 mbar * L / sec that was established in USP <1207>. It is also further emphasised that inherent CCIT is a requirement for packaging systems. Once a package system is deemed to be able to meet these requirements under the validated packing of the product, then the use of methods that may not be able to meet the MALL becomes acceptable. This is because once the product is added, the ability to detect small defects becomes much much more difficult, if not impossible, in some cases In many techniques, it was stated that the rejection limits were based on pre-determined values. Just as no one technique is able to test all packaging-product configurations. There is not a single method for the technique that can be expected to give the same results for all product-packaging systems. There are many factors that can affect the output received from an instrument, and it is imperative to understand and control these variables to determine if a package is integral in ensuring the safety of the product. This is why there are no specific instrumental conditions or rejection limits listed for any of the techniques. The development and validation of an individual product-packaging system is an important part of the process to ensure that the packaging is integral. A contract laboratory that has access, knowledge, and experience can help guide a manufacturer through the process to ensure compliance with regulations and safety of the end product.