Container-Closure Integrity

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“The Aseptic Core” discusses scientific and regulatory aspects of aseptic processing, with an emphasis on aseptic formulation and filling. This column has been developed with the intention of providing practical advice to professionals involved in the qualification of aseptic processes and the myriad support processes involved. The primary objective for this column: Useful information.

Reader comments, questions, and suggestions are needed to help us meet our objective for this column. Please email your suggestions to shaigney@advanstar.com.

KEY POINTS
The following key points are discussed:
• Container-closure integrity is a critical property of a pharmaceutical product. This attribute is especially important for sterile products.
• The US Food and Drug Administration guidance has categorized container-closure risks for common pharmaceutical products including degree of concern associated with route of administration (highest-low) and likelihood of packaging component-dosage form interaction (high-low).
• Sterile product container closure systems are described for most common sterile products including ampoules, sterile vials, and other systems.
• Multiple integrity test methods including microbial immersion test, microbial aerosol challenge, bubble test, and other methods are described.
• Validation considerations for integrity tests are discussed.

INTRODUCTION
One of the main purposes of a container closure system is to protect its contents from the surrounding environment. In the case of sterile products, this means maintaining the sterility of the product. The container-closure system may also need to protect the product from other potentially harmful agents such as light, moisture, and oxygen. This paper discusses container-closure integrity—maintaining the sterility of the product plus protecting the product from moisture and oxygen. It further discusses various test methods used to test for container-closure integrity.

CONTAINER-CLOSURE SYSTEMS
A container-closure system typically consists of a container for the product plus a means of sealing the product container (closure). Some systems, such as flame-sealed ampoules, may not have a separate closure. Other systems, such as dental cartridges and syringes, may have more than one closure (tip cap and plunger). The US Food and Drug Administration defines a container-closure system as the following:

A container closure system refers to the sum of packaging components that together contain and protect the dosage form. This includes primary packaging components and secondary packaging components, if the latter are intended to provide additional protection to the drug product. A packaging system is equivalent to a container closure system (1).

Figure 1 shows some typical types of container-closure systems used for parenteral products. This is far from a comprehensive list. There are many other types of systems. These are the most common types.

As noted in Table I, adapted from the FDA guidance document on container-closure systems, sterile injections and injectable suspensions represent some of the highest risks to patients of any dosage forms, both from the degree of concern associated with the route of administration (injection) and from the likelihood of interaction between one of the packaging compo-
nents. Sterile powders and powders for injection (i.e., lyophilized products) are in the highest degree of concern associated with the route of administration, and have a medium likelihood of interaction between the packaging components and the dosage form. Because of the risk to the patient, the integrity of the container-closure system is a critical quality attribute.

**Table I: Container closure risks for common drug product classes, adapted from FDA guidance (1).**

<table>
<thead>
<tr>
<th>Degree of concern associated with the route of administration</th>
<th>Likelihood of packaging component—dosage form interaction</th>
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<tbody>
<tr>
<td>High</td>
<td>Medium</td>
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<tr>
<td>Highest</td>
<td>Inhalation aerosols and solutions; injections and injectable suspensions</td>
</tr>
<tr>
<td>High</td>
<td>Ophthalmic solutions and suspensions; transdermal ointments and patches; nasal aerosols and sprays</td>
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<tr>
<td>Low</td>
<td>Topical solutions and suspensions; topical and lingual aerosols; oral solutions and suspensions</td>
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** Figures:**

**Figure 1:** Typical container-closure systems.

**Figure 2:** Inhalation aerosols and solutions; injections and injectable suspensions.

**Figure 3:** Sterile powders and powders for injection; inhalation powders.

**Figure 4:** Ophthalmic solutions and suspensions; transdermal ointments and patches; nasal aerosols and sprays.

**Figure 5:** Topical solutions and suspensions; topical and lingual aerosols; oral solutions and suspensions.

**Figure 6:** Topical powders; oral powders.

**Figure 7:** Oral tablets and oral (hard and soft gelatin) capsules.

**Ampoules**

Ampoules are typically formed from glass tubing or from plastic using blow-fill-seal technology. Glass ampoules contain an open top prior to filling (see Figure 2). After filling, the ampoules are heat-sealed using a gas flame. Ampoules may be purged with an inert gas such as nitrogen before filling. Integrity test methods for ampoules...
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typically include high voltage leak detection (HVLD) or dye ingress (test methods are discussed below).

Sterile Vials
Sterile vials may be made from glass tubing (i.e., tubing, or tubular, vials), blow molded glass, or from plastics such as Cyclic Olefin Copolymer (COC) resins. They are sealed with rubber stoppers and an aluminum or aluminum and plastic cap that is crimped over the stopper and vial neck to ensure integrity. See Figure 3 for a pictorial of a typical vial filling and lyophilization operation.

Sterile vials typically have three sealing surfaces:
- Flange seal, where the flange of the stopper contacts the top sealing surface of the vial
- Plug seal, where the stopper legs or plug contacts the inner neck of the vial
- Transitional seal, where the stopper plug transitions into the stopper flange.

The plug seal and transitional seal have a high degree of importance between the time the stopper is seated and the time the cap is applied. The flange seal becomes the main seal after the cap is crimped to the stopper, creating a residual seal force that compresses the stopper against the vial.

Injection vials for lyophilized products typically contain a blowback feature that helps to maintain the position of the partially seated stopper by engaging with tabs or rings on the stopper. The two main types of blowback seal are the US blowback ring (Figure 4), which is an indentation in the plug-sealing surface of the vials, and the European style blowback, in which the vial neck is widened below the sealing surface (Figure 5). Both blowback styles are suitable, but the stopper dimensions and glass finish should be matched to prevent container-closure issues.

Figure 2: Open and closed ampoules.

Figure 3: Vial filling and lyophilization.
Syringes and Cartridges
Syringes and cartridges share many features that consist of a barrel, a plunger, and a tip seal. Syringes add finger grips and a plunger. Cartridges do not have these features, as they are designed to fit into an injection device. See Figure 6 for illustrations of typical syringes and cartridges. Syringes and cartridges can be made of glass or plastic. Syringe and cartridge seals depend on an interference fit between the syringe or cartridge barrel and the piston, plus the tip seal, which may be a protective cap, crimp seal, or Luer-Lock fitting.

Flexible IV Bags
Flexible heat-sealed bags are commonly used for intravenous therapies. These are heat-sealed pouches, typically with two septa attached, allowing for attachment of IV tubing and for injection of drugs into the IV solution.

CONTAINER-CLOSURE INTEGRITY TESTS
There are many types of container-closure integrity tests used in the pharmaceutical industry. This paper focuses on major tests as defined in PDA Technical Report Number 27, Pharmaceutical Package Integrity (2). These tests include the following:

- Microbial immersion test
- Microbial aerosol challenge
- Bubble test
- Liquid tracer test
- Dye leak test
- Helium leak detection
- High voltage leak detection
- Corona discharge test
- Pressure/vacuum decay
- Vacuum retention
- Headspace gas analysis.

Microbial Immersion Test
This test is suitable for any container-closure system that can withstand immersion and pressure changes. In the microbial immersion test, the test article is immersed in a broth containing the test organism. Brevundimonas diminuta, Serratiamarcescens, Escherichia coli and other organisms have been used for this test. This test may be static, in which no pressure or vacuum is applied, or dynamic, in which pressure and vacuum are applied, typically to simulate air transport. Key test factors include: bacterial size and motility, differential pressure, challenge media, exposure time, and viable count in the challenge media.

Microbial Aerosol Challenge
This test is used for packaging, which relies on a tortuous path to protect product sterility. In this test, bacteria or bacterial endospores are aerosolized in a sealed chamber containing the product sample. The product is then incubated if it supports bacterial growth, or sterile filtered and incubated if it does not support bacterial growth. Key test factors include: concentration of the microbial aerosol, size of the challenge organism, resistance of the challenge organism to desiccation, uniform air flow in the chamber, chamber temperature and humidity, exposure time, and differential pressure.
Bubble Test
In this test, the item under test is pressurized to about 3 psig and immersed in a bath containing water or water and surfactant (e.g., polysorbate 80). This test can detect leaks as small as 10⁻⁵ mbar·L/sec. Key test factors include differential pressure, test time, immersion fluid surface tension, visible inspection conditions (e.g., light intensity, magnification, and background), and visual inspector training and experience.

Liquid Tracer Test
This test is similar to the dye leak test in that it measures ingress of a chemical tracer into the container-closure system. Literature has reported the use of copper ions and chloride ions, radioactive tracers, and other substances as liquid tracers (2). For this test, the container-closure system is immersed in the tracer solution, a differential pressure is applied, and the product inside the container-closure system is tested for the presence of the tracer material. Key test factors include differential pressure, exposure time, sensitivity of the assay for the tracer, tracer liquid viscosity, surface tension, tracer liquid hydrophilicity/lipophilicity and temperature, compatibility of the tracer liquid with the product, and stability of the tracer liquid.

Dye Leak Test
The dye leak test is the most common variant of the liquid tracer test. In this test, the container is immersed in a methylene blue solution and pressure and vacuum are applied to the container. The container is then inspected visually or via spectrophotometry (preferred method) for dye penetration. Key factors include differential pressure, compatibility of the dye with the product, liquid viscosity and surface tension, inspector training and experience (for visual inspection) or assay sensitivity (for spectrophotometry).

Helium Leak Detection
The helium leak test is the most sensitive leak detection method for container-closure systems. In this test, the container-closure system is placed in a test fixture and helium is injected into the inside of the container-closure system. Alternately, the container-closure system can be pre-filled with helium before sealing. The test fixture is connected to a mass spectrometer that evacuates the test fixture and detects helium leakage from the container-closure system into the test fixture. Calibrated leakage standards are run as controls for each test. Advantages of the helium leak test include test sensitivity and repeatability, and the ability to use NIST traceable standards for leakage.

Disadvantages include the destructive nature of the test, the extensive sample preparation, and the cost of the leak testing equipment. Key factors include test duration, package temperature, background helium in the vacuum system, concentration of helium in the container closure system under test, and other factors.

High Voltage Leak Detection
High voltage leak detection is used for leak detection in liquid products, especially in ampoules. In this test, a high voltage/high frequency charge is
applied across the container-closure system. A leak will cause an increase in current across the high voltage electrodes, triggering the reject mechanism for the leak detector. Advantages include automation of the integrity test and the rapidity of the test procedure. This allows high voltage leak detection to be used for online inspection. Disadvantages are that the high voltage/high voltage field could potentially affect potency and stability of some protein-based therapeutics. Key factors include voltage level, probe positioning, container-closure system geometry and wall thickness, and product formulation.

Corona Discharge Test
This test is used for leak detection in lyophilized products that have been evacuated, or other container systems that have been filled under vacuum. The corona discharge test may be performed manually or automatically. For the manual test, a high voltage/high frequency electrode is applied to the outside of the vial. The operator inspects the vial for the presence of a corona discharge (glow) caused by the ionization of the gas molecules in the headspace. If the glow does not appear, the operator can adjust the voltage and retest the vial. If the retest fails, the vial is rejected. The corona discharge test can be automated—in this case the inspection system applies the high voltage/high frequency field across the vial headspace while measuring the current across the electrodes. If no current is detected, the vial is rejected. Machine setup is performed using NIST traceable pressure standards prepared from the container-closure system under test. The higher pressure standard is used to set up the 100% rejection level; the lower pressure standard is used to set up the 100% acceptance level. Factors affecting the corona discharge test include test voltage, vacuum level (too low a vacuum level may affect the efficiency of the test because of the absence of gas molecules to ionize), headspace volume, and container-closure system geometry.

Pressure/Vacuum Decay
For this test, the container-closure system is placed in a test fixture that is either pressurized or evacuated. The test chamber is allowed to stabilize, and then the change in pressure or vacuum is measured over time. Pressure or vacuum can be measured directly or by differential pressure between the test chamber and a reference chamber. Advantages of this test are that it is relatively rapid and sensitive, and can be performed as an online test. Disadvantages are that the test requires test fixtures specific to each container-closure system. Key test parameters include temperature, package geometry, test fixture geometry, volume of package headspace, water vapor pressure inside the package, stabilization time, and test time.

Vacuum Retention
Vacuum retention is used for evacuated vials, typically lyophilized vials or oxygen-sensitive liquid products. Traditionally, vacuum levels were measured using a capacitance manometer attached to a needle that was used to pierce the package, making this a destructive test. Vacuum levels can now be detected using frequency modulation spectroscopy (FMS), which measures vacuum levels by the width of the water or oxygen peak inside the package. This method has the advantage of being non-destructive, allowing 100% inspection of headspace vacuum and allowing the same package to be measured multiple times. Disadvantages of the vacuum retention method are that it requires a minimum oxygen or moisture content to be able to directly measure vacuum inside the vial. Most systems do not accurately measure pressures below 20 torr. Another disadvantage is that extended hold times may be needed to detect slow leakers. Key test parameters include headspace moisture or oxygen content, initial vacuum level, temperature of packaging, headspace volume of packaging, time allowed between package sealing, and vacuum measurement.

Headspace Gas Analysis
Headspace gas analysis is typically performed using non-contact methods such as frequency modulation spectroscopy. This method may be used for lyophilized products or oxygen-sensitive liquid products. Headspace gas analysis is a relatively rapid analysis method—on the order of a few seconds—allowing 100% inspection of oxygen sensitive products or products packaged under vacuum. Key parameters for headspace gas analysis include headspace volume, package temperature, headspace pressure and vacuum, and sensitivity of the headspace analysis instrumentation.

VALIDATION IMPLICATIONS
All test methods need to be validated or consistently verified for accuracy. This is typically done by introducing a number of defects of known size or leak rate as controls to establish the linear range and repeatability of the leak test. Calibrated leaks can be established by using laser-drilled holes, calibrated
orifices, or through introducing wires of a know size between the closure and the sealing surface (3). The detection limit of the leak test can be determined per ICH Q2 (R1) (4).

Correlation between the leak test parameters to the microbial ingress test may be done via direct or indirect methods. Indirect comparison is performed by comparing the leak test to another test that is well defined and has a known correlation to microbial leak rates (e.g., comparing vacuum decay test to helium leak test). Direct comparison is performed by performing microbial ingress testing and the physical leak test on the same items or items with the same defect sizes to establish a direct correlation between the leak test values and microbial ingress.

Container-closure integrity testing should be a component of stability testing for new and existing products. The FDA Guidance on Container-Closure Integrity Testing in Lieu of Sterility Testing encourages companies to use container-closure integrity testing to replace sterility testing in stability protocols (5). A non-destructive test is especially useful for stability testing, as it allows the same container to be tested multiple times over the stability period. This reduces the number of stability samples and allows more meaningful profiles of container-closure integrity.

CONCLUSIONS
This discussion has provided an overview of sterile product container-closure systems and the respective integrity tests used for testing. Container-closure integrity is a critical attribute of a sterile product. Many types of container-closure integrity tests are used in the pharmaceutical industry. All test methods need to be validated or consistently verified for accuracy. Test methods are correlated to microbial ingress tests.

REFERENCES
4. ICH, Q2 (R1). Validation of Analytical Procedures: Text and Methodology.

GENERAL REFERENCE
USP <381> Elastomeric Closures for Injection. JVT