⟨1207.2⟩ PACKAGE INTEGRITY LEAK TEST TECHNOLOGIES

1. INTRODUCTION

The purpose of this chapter is to provide information guiding the selection and proper use of leak test technologies (also called methodologies, approaches, or methods). The leak test technologies described in this chapter were selected on the basis of relevant research study data published in peer-reviewed journals and/or precision and bias study data generated in support of recognized test method standards. When referencing standard test methods (e.g., ASTM), the reader is advised to refer to the most recent versions. In some cases, the scope of referenced standard test methods does not include the package types of the scope in *Package Integrity Evaluation—Sterile Products* ⟨1207⟩. In all cases, methods and literature studies are cited to provide benchmark information useful for pharmaceutical package leak test method development and validation.

The technologies described in this chapter are not prescriptive methods but represent testing concepts that may be applied when leak testing sterile product–packages. Test technologies vary in terms of their potential detection limits, reliability, and applications; therefore, none are universally appropriate for leak testing all product–packages. This chapter provides information to allow a thorough comparison of testing approaches so that the most appropriate technology for a given situation can be identified.

After a methodology has been selected for use, the test equipment operation and performance is qualified. Test method parameters are optimized during method development and confirmed during validation. Thus, a final leak test method is specific to a particular container–closure or product–package system.

The leak test methods included are divided into two categories: deterministic and probabilistic. Deterministic leak test methods (*Table 1*) are preferred over probabilistic methods when other key method selection criteria permit. Probabilistic leak test methods (*Table 2*) are best used when the product–package system proves incompatible with deterministic methods, or when method outcome requirements demand a particular probabilistic testing approach.

In this chapter's *Table 1* and *Table 2*, the "leak size detection limit" provided for each methodology refers to leakage rates/leak sizes listed by row in *Package Integrity Testing in the Product Life Cycle—Test Method Selection and Validation* ⟨1207.1⟩, *Table 1*. The reported leak detection limits were chosen on the basis of literature sources, as well as commonly accepted experience. This information is intended to aid in the selection of the test technology but should not be used as a definitive statement of test method performance for any specific leak test method applied to any given product–package system. Instead, leak detection limit and range should be established during leak test method development and validation for the respective product–package or container–closure system. For instance, an approach cited as capable of detecting row 6 leaks may be validated by the user to detect leaks as small as those in row 3. Conversely, a method described as capable of detecting leaks in row 3 may be determined by the user to detect leaks only as small as row 5.

This battery of testing technologies and the information provided are intended to aid, not limit, the selection, development, validation, and use of leak test methods. Unlisted methodologies shown by the user to meet the qualification and validation requirements for a satisfactory leak test may be used. In addition, listed technologies may demonstrate expanded testing capabilities beyond those currently identified.

Table 1. Deterministic Leak Test Technologies[a]

Deterministic Leak Test Technologies	Package Content Requirements	Package Requirements	Leak Detection Limit[b]	Measurement Outcome and Data Analysis	Effect of Method on Package	Test Time Order of Magnitude
Electrical conductivity and capacitance (high-voltage leak detection)	Liquid (with no combustion risk) must be more electrically conductive than package. Product must be present at leak site.	Less electrically conductive than liquid product.	**Row 3** Varies with product–package, instrument, test sample fixtures, and method parameters.	Quantitative measure of electrical current passing through the test sample: provides an indirect determination of leak presence and leak location as shown by a drop in test sample electrical resistivity, with a resultant increase in voltage reading above a predetermined pass/fail limit.	Nondestructive, although impact of test exposure on product stability is recommended	Seconds

Table 1. Deterministic Leak Test Technologies[a] *(continued)*

Deterministic Leak Test Technologies	Package Content Requirements	Package Requirements	Leak Detection Limit[b]	Measurement Outcome and Data Analysis	Effect of Method on Package	Test Time Order of Magnitude
Laser-based gas headspace analysis	Gas volume, path length, and content must be compatible with instrument's detection capability.	Allows transmission of near-IR light.	**Row 1** Varies as a function of time span between analyses.	Quantitative measure of gas headspace content of the test sample by laser-based gas analysis, for a product requiring a headspace low in oxygen, carbon dioxide, or water vapor concentration; and/or low in absolute pressure. Whole test sample leakage rate is determined by compiling readings as a function of time.	Nondestructive	Seconds
Mass extraction	Gas or liquid must be present at leak site. Presence of liquid at leak site requires test pressures below vapor pressure. Product must not clog leak path.	Rigid, or flexible with package restraint mechanism.	**Row 3** Varies with product–package, instrument, test fixtures/chamber, and method parameters.	Quantitative measure of mass flow rate resulting from test sample headspace escape or liquid product volatilization within an evacuated test chamber housing the test sample. Quantitative pressure readings early in the test cycle indicate larger leak presence. Whole test sample leakage rate is determined by comparing the test sample mass flow results to results using leak rate standards and positive controls.	Nondestructive	Seconds to minutes
Pressure decay	Gas must be present at leak site. Product (especially liquids or semi-solids) must not cover potential leak sites.	Compatible with pressure detection mode. Rigid, or flexible with package restraint mechanism.	**Row 3** Varies with product–package, instrument, and method parameters.	Quantitative measure of pressure drop within a pressurized test sample. Pressure drop readings are a measure of gas escape through leak paths. Whole test sample leakage rate is determined by comparing pressure decay results to results using leak rate standards and positive controls.	Nondestructive, unless the means used to access test sample interior compromises test sample barrier.	Minutes to days, depending on package volume and required leak limit of detection
Tracer gas detection, vacuum mode	Tracer gas must be added to package. Tracer gas must have access to package surfaces being tested for leaks.	Able to tolerate high-vacuum test conditions Rigid, or flexible with package restraint mechanism Limited tracer gas permeability	**Row 1** Varies with instrument capability and test sample fixtures.	Quantitative measure by spectroscopic analysis of tracer gas leak rate emitted from a tracer-flooded test sample positioned in an evacuated test chamber. Whole test sample leakage rate is calculated by normalizing the measured tracer leak rate by tracer concentration in the test sample.	Nondestructive, unless tracer gas introduction into the package compromises test sample barrier.	Seconds to minutes
Vacuum decay	Gas or liquid must be present at leak site. Presence of liquid at leak site requires test pressures below vapor pressure. Product must not clog leak path.	Rigid, or flexible with package restraint mechanism	**Row 3** Varies with product–package, instrument, test sample chamber, and method parameters.	Quantitative measure of pressure rise (vacuum decay) within an evacuated test chamber housing the test sample; vacuum decay readings are a measure of headspace escape from the test sample, or liquid product volatilization. Whole test sample leakage rate is determined by comparing vacuum decay results for the test sample to results of tests performed using leak rate standards and positive controls.	Nondestructive	Seconds to minutes

[a] All methods apply to nonporous, rigid and flexible packages as per the scope of 〈1207〉.
[b] The leak detection limit cited for each technology refers to *Package Integrity Evaluation—Sterile Products* 〈1207〉, *Table 1* and is provided for information only. This information is intended to assist in early methodology selection. The validated leak detection limit for a product–package test method may deviate from these values.

Table 2. Probabilistic Leak Test Technologies[a]

Probabilistic Leak Test Technologies	Package Content Requirements	Package Requirements	Leak Detection Limit[b]	Measurement Outcome and Data Analysis	Effect of Method on Package	Test Time Order of Magnitude
Bubble emission	Gas must be present at leak site. Product (especially liquids or semi-solids) must not cover package surfaces to be leak tested.	Rigid, or flexible with package restraint mechanism.	**Row 4** Varies with product–package, test sample fixtures and positioning, method parameters, and analyst technique and skill.	Qualitative measure by visual inspection of bubble emission caused by escape of test sample headspace while sample is submerged and exposed to differential pressure conditions. Alternatively, sample surfaces may be exposed to surfactant. Continuous bubble emission indicates leak presence, location, and relative size.	Destructive	Minutes
Microbial challenge, immersion exposure	Growth-supportive media or product. Presence of liquid at the leak site required for method reliability.	Able to tolerate pressure and immersion challenge. Rigid, or flexible with package restraint mechanism.	**Row 4** Varies with container–closure, test sample fixtures and positioning, challenge condition severity, and inherent biological variability.	Qualitative measure by visual inspection of microorganism growth inside test samples filled with growth-supportive media or product, post immersion in heavily contaminated challenge media while exposed to differential pressure conditions, followed by incubation to encourage microbial growth. Growth in the test sample indicates the presence of test sample leak site(s) capable of allowing passive or active entry of microbes.	Destructive	Weeks
Tracer gas detection, sniffer mode	Tracer gas must be added to package. Tracer gas must have access to package surfaces to be tested for leaks.	Leak site accessible to probe. Limited tracer gas permeability.	**Row 2** Varies with test sample, method parameters, test sample fixtures, and analyst technique and skill. Smaller leak detection may be possible under optimum test conditions.	Quantitative measure by spectroscopic analysis of tracer gas near the outer surfaces of the tracer-flooded test sample, sampled using a sniffer probe. Tracer presence above a pass/fail limit indicates leak presence and location.	Nondestructive, unless tracer gas introduction to the package interior compromises test sample barrier.	Seconds to minutes
Tracer liquid	Contents must be compatible with liquid tracer. Product must not clog leak path.	Rigid, or flexible with package restraint mechanism. Able to tolerate liquid immersion. Compatible with liquid tracer detection mode.	**Row 4** Varies with container–closure, test sample fixtures and positioning, challenge condition severity, and tracer liquid content. Smaller leak detection may be possible under optimal test conditions employing chemical analysis tracer detection.	Measure of tracer in test sample previously submerged in tracer-charged liquid while exposed to differential pressure conditions. Alternatively, tracer-charged test samples may be submerged in tracer-free collection fluid. Tracer migration measurement may be quantitative (by chemical analysis; preferred approach for small leak detection) or qualitative (by visual inspection). Tracer presence indicates leak site(s) capable of allowing tracer passage. Tracer magnitude may indicate relative leak size (assuming a single-leak pathway).	Destructive	Minutes to hours

[a] All methods apply to nonporous, rigid and flexible packages as per the scope of ⟨1207⟩.

[b] The leak detection limit cited for each technology refers to *Package Integrity Evaluation—Sterile Products* ⟨1207⟩, *Table 1* and is provided for information only. This information is intended to assist in early methodology selection. The validated leak determination limit for a product–package specific test method may deviate from these values.

2. DETERMINISTIC LEAK TEST TECHNOLOGIES

2.1 Electrical Conductivity and Capacitance (High-Voltage Leak Detection)

2.1.1 DESCRIPTION

The electrical conductivity and capacitance leak test (high-voltage leak detection, or HVLD) is an approach for detecting the presence, and potentially the location, of a leak(s) in the wall of a nonporous, rigid or flexible package containing liquid or semi-liquid product. Test analysis is based on quantitative electrical conductance measurements (*1–5*). HVLD leak tests are generally nondestructive to the package and to the product, although an evaluation of HVLD exposure impact on product physicochemical stability is advised.

The test is performed by first positioning the test sample (containing liquid product) onto an electrically grounded instrument test fixture. Alternatively, the test sample may be placed onto a transport system that will carry the test sample through an electrically grounded testing zone. Upon test start, an electrode uniquely designed for the product–package type under test exposes all or part of the test sample to a high-frequency, high-voltage, low-amperage current. The presence of a leak path in the proximity of an electrically conductive, liquid-formulation product results in a drop in the electrical resistance of the test sample, as shown by a spike in current passing through the test sample above a predetermined pass/fail limit established using negative controls.

2.1.2 APPLICATION

Rigid or flexible packages of nonporous components containing liquid or semi-liquid product may be tested:

- Package components must be relatively electrically nonconductive.
- Product must be electrically conductive, relative to the package.
- Product must not be flammable (i.e., not a combustion risk).
- Product must be near or at the leak inspection location at the time of the leak test.
- Solidified, electrically conductive product that blocks leak paths may be detected.
- Metal caps used to seal stoppered vial or cartridge packages conduct current, improving the likelihood of finding leaks under the cap.

HVLD tests are rapid, requiring no more than several seconds for a full scan of the test sample, thus making them appropriate for off-line testing, or as an on-line, 100% product inspection test method. This technology is useful for any product life cycle phase.

2.1.3 TEST EQUIPMENT

HVLD instrumentation comes equipped with tooling and/or a test sample transport system for proper test sample and probe/ ground positioning, an internal high-voltage transformer, electrode voltage and ground potentiometer adjustment capabilities, and a test result output display. The design and materials of construction used for the electrode probe and electrical ground are product–package specific.

2.1.4 TEST PARAMETERS

The following are test parameters for the electrical conductivity and capacitance leak test, also known as HVLD:

- Conductivity of test sample product relative to test sample package: a greater difference will improve leak detection sensitivity
- Test voltage set point: voltage is set high enough to ensure leak detection, but not so high that current will arc, falsely rejecting the test sample
- Test sensitivity set point (potentiometer or gain set point): sensitivity should be maximized to ensure leak detection without triggering a false reject result
- Package content proximity to potential leak paths: leak detection sensitivity is directly related to the proximity of product to the leak path
- Electrode probe position relative to potential leak paths: probe proximity to the leak is directly related to the test method sensitivity
- Speed at which the electrode passes over the test sample surface: although the test is very rapid, test speeds too rapid may cause leaks to be missed
- Moisture presence on the package: test sample surface condensation can potentially trigger a false reject reading

2.2 Laser-Based Gas Headspace Analysis

2.2.1 DESCRIPTION

Gas headspace analysis via laser-based techniques provides a quantitative, nondestructive measure of oxygen content, water vapor content, and low internal pressure in the headspace of a nonporous, rigid or nonrigid package (*6–8*). Some instruments are capable of measuring headspace carbon dioxide concentration as well.

The test is performed by first placing the test sample in a fixture designed for precise test sample positioning. Upon test start, frequency-modulated spectroscopy is used to cause a near-infrared (IR) diode laser light to pass through the gas headspace region of the sealed test sample. Light is absorbed as a function of gas concentration and pressure. The absorption information is processed using phase-sensitive detection techniques; a mixer demodulates the signal. The output voltage, which is proportional to the absorption line shape, is digitally converted and further analyzed by a microprocessor, yielding test sample signal results. Final test sample readings are automatically generated based on a comparison of test sample signals to a calibration curve. This curve is pre-established by using control packages flooded with traceable gas reference standards. Gas headspace analysis, as a function of time, provides a quantitative measure of the total leakage rate of the test sample. Leakage rates are judged acceptable or unacceptable on the basis of predetermined limits, calculated to ensure proper gas headspace content maintenance over the product life cycle.

2.2.2 APPLICATION

Rigid or flexible packages made of nonporous components (transparent or semi-transparent material, either amber or colorless) that allow transmission of near-IR diode laser light may be tested. Test samples require a minimum headspace volume and headspace path length. The requirements vary on the basis of the gas moiety to be tested and may be specific to the instrument as well as to the construction and design of the package materials.

Test samples that may be analyzed fall into these categories:

- Products that require low-oxygen or low-carbon-dioxide headspace content

- Products that require low water vapor content (e.g., lyophilized or powdered products)
- Products that require low internal package pressure (e.g., lyophilized products)

Package integrity, or absence of leakage, is confirmed by replicating tests on a given test sample as a function of time. Longer time periods between tests are needed to detect smaller leaks. Mathematical models appropriate to leak flow dynamics may be used to predict the time required for detecting leaks of various sizes or rates.

Headspace analysis at a single time point provides the headspace content result, which may or may not be indicative of package integrity.

- A test result not meeting specification could be due to package leakage, or could result from improper package filling or assembly processes that caused the package headspace to be out of specification.
- A test result that meets specification may confirm package integrity if enough time has elapsed since product–package preparation for measurable leakage to have occurred, assuming that the initial preparation of the test sample met manufacturing standards.

Methods of laser-based gas analysis may be used during any phase of the product life cycle. Tests are rapid and are appropriate for off-line testing using lab-scale equipment (typical measurement time, 2 s) or as an on-line, 100% product inspection method (typical measurement time, 0.2 s).

2.2.3 TEST EQUIPMENT

Test instrumentation for laser-based gas headspace analysis is capable of accurate and reproducible near-IR diode laser light emission, light detection, and signal analysis. Tooling specific to product–package test samples is used to properly position test samples, ensuring reproducible laser-light transmission and detection. Standards with components identical to the packages under test, in terms of both the materials of construction and the dimensions (at the point where light is to be transmitted), are required. These standards also need to contain headspace content that is representative of the gas mixture under test (i.e., oxygen, carbon dioxide, water vapor, pressure).

2.2.4 TEST PARAMETERS

The following are test parameters for laser-based gas headspace analysis:

- Test sample position with respect to laser beam transmission and detection points: imprecise test sample handling, and/or dimensional irregularity of the package, can increase measurement standard deviation
- Test sample headspace volume: the volume must meet minimum requirements for test instrumentation
- Test sample headspace absolute pressure: headspace absolute pressure will influence the detection limit and range for all test types
- Test sample speed: increase in testing speed will increase standard deviation of measurement
- Test sample temperature: temperature can influence moisture and pressure test results (e.g., lower temperatures are associated with lower internal pressure of the test sample) (*9*)
- Test sample outer surface moisture: presence of moisture may hamper test performance
- Time allotted between the replicate tests: performing replicate tests over a period of time allows calculation of continuous package leakage

2.3 Mass Extraction

2.3.1 DESCRIPTION

The mass extraction test is a nondestructive, quantitative measurement approach for detecting leakage in nonporous, rigid or flexible packages (*10*). Leakage of package headspace gases and/or leakage below the product fill level may be detected, given appropriately designed equipment and test parameters.

The test is performed by first placing the test sample inside a test chamber that is pneumatically connected to a mass extraction leak test system equipped with a vacuum generator package. The test chamber is uniquely designed to contain the test package, which is fitted with appropriate tooling to limit movement or expansion of moveable or flexible components, respectively.

Upon test start, the chamber is quickly evacuated for a predetermined time to reach a predetermined vacuum level. A series of such evacuation cycles may be performed, each intended to identify smaller leakage rates. After each cycle, the test system is isolated from the vacuum source and measurements of absolute pressure, pressure decay rate, and/or gas mass flow rate are captured. Readings greater than predetermined limits that were established using negative controls are indicative of container leakage, triggering test cycle abort.

For those test samples passing all previous larger leak vacuum cycles, a final vacuum is drawn. The test system is then isolated from the vacuum source. With all flow from the test chamber directed through the mass flow sensor, the mass flow rate is measured. Mass flow above a predetermined limit established using negative controls is indicative of container leakage.

2.3.2 APPLICATION

Nonporous, rigid or flexible packages may be tested. Packages containing gas, liquid, and/or solid materials can be tested:

- Flexible packages or packages with nonfixed components require tooling to restrict package expansion or movement, respectively, when exposed to test vacuum conditions. Tooling minimizes the seal stress of flexible packages and maintains consistent package volume and differential pressure conditions across the leak path.
- Gas headspace must be at atmospheric pressure or at a pressure notably greater than test vacuum conditions.

- Package surfaces below the product-fill level may be leak tested for those solid-formulation products that do not block leak-site gas flow and for those liquid products that volatilize at test vacuum but do not solidify and so block leak paths.
- Packages ranging in volume from a few milliliters to several liters may be tested.

Tests require anywhere from several seconds to a few minutes to perform. Longer test times are necessary for testing larger-volume packages. Lengthening test cycles also allows for detection of smaller leaks.

Mass extraction leak tests are useful in any phase of the product life cycle. Tests may be performed in a laboratory setting or off-line in the production environment. Longer laboratory or off-line test cycle times are generally capable of detecting smaller leaks. Higher speed on-line tests are restricted to larger leak detection.

2.3.3 TEST EQUIPMENT

Mass extraction test instrumentation consists of a system of conduits and valves that pneumatically connect a test chamber with a test system pressure sensor, micro-flow mass sensor, and vacuum generator package, including an external vacuum source. The instrument includes appropriate timers, electronic controls, and monitors. A fixed-size orifice is included for periodic system performance verification. The test chamber is uniquely designed to contain the test package, which is fitted with appropriate tooling to limit movement or expansion of moveable or flexible components, respectively.

2.3.4 TEST PARAMETERS

The following are test parameters for mass extraction:

- Pressures
  - Test system pressure reading after initial gross leak check. At evacuation stage, pressure is a function of test system volume, time allotted for evacuation, and the vacuum source pressure level.
  - Test system pressure reading after the secondary evacuation stage(s): pressure above a predetermined limit is due to test package leakage. The pressure level above baseline is a function of leak size, available headspace volume, and/or volatile liquid in the test sample.
  - The final absolute pressure of the test cycle must be lower than the headspace pressure of the test package for detection of gas headspace leaks, and/or lower than the volatilization pressure of liquid product formulation for leaks located below the liquid-product fill level.
- Mass flow
  - Mass flow rate reading after secondary evacuation stage(s).
  - The mass extracted from the test sample is monitored for larger leak detection after the first secondary evacuation stage; mass extracted from the test sample is monitored for the smallest leak detection after the last evacuation stage.
  - Background flow level (i.e., the baseline flow or noise level) is the flow rate for packages without leaks. Baseline flow is a function of test package and system outgassing, test system volume, and the time allowed for evacuation.
  - The mass flow rate at steady-state conditions, when extracted from the test chamber, is equal to test sample leakage into the test chamber, assuming that outgassing from the sample and external leakage from the test system are insignificant. Leakage is identified once the mass flow rate notably exceeds the rate of negative controls.
- Times
  - Time allotted for system evacuation for gross leak detection: enough time is allotted to draw off most of the test chamber gases, without exhausting the headspace gases of the test package, or without drawing off the liquid contents from grossly leaking packages.
  - Time allotted for large-leak check through the mass flow sensor: a brief time is required for detection of large- and medium-sized defects.
  - Time allotted for system evacuation for small leak detection: enough time should be allowed to establish the desired vacuum equilibrium of the test chamber. Insufficient time will not adequately draw off gases sorbed onto package surfaces or entrapped between components.
  - Time allotted for mass flow to stabilize: after the secondary evacuation stage(s), monitor the flow as the flow rate approaches steady state. Enough time is allotted so that flow from the smallest allowed defect is statistically greater than baseline (no-leak) flow.
  - Time allotted for the final leak test by mass flow: enough time should be allotted so that the mass flow rate exceeds baseline readings for negative controls.

2.4 Pressure Decay

2.4.1 DESCRIPTION

The pressure decay test is a quantitative measurement approach for detecting leakage in nonporous, rigid or flexible packages. The test is destructive if the introduction of pressurized gas creates a break in the package wall or seal. The test is nondestructive if the introduction of gas into the test sample does not compromise the package barrier. Pressure decay testing is intended for integrity testing of the gas headspace region of the test sample.

To perform the test, a dry air or inert gas pressure source is attached to the test sample that is fitted with an internal pressure monitoring device. The test sample is pressurized to a predetermined pressure, after which the pressure source is isolated from the test sample. The decay in pressure is monitored for a predetermined time. Pressure decay that exceeds a predetermined limit established using negative controls indicates container leakage.

The referenced ASTM F2095 method (*11*) is intended for testing flexible packages (pouches and foil-sealed trays). Seals or surfaces being tested cannot be in contact with product such as water, oils, or other liquids. ASTM method A describes testing packages without use of a restraint mechanism. The method requires that the package reach a stable volume configuration (i.e., it stops stretching) to take a measurement. ASTM method B requires that the test sample is kept between restraining plates during the test to limit the volume of the pressurized package.

2.4.2 APPLICATION

Nonporous, rigid or flexible packages may be tested:

- Package surfaces that can be tested are those unobstructed by product (e.g., oils, water, or other liquids); small leaks below the liquid-fill level would not be detected by this method.
- Flexible packages or packages with nonfixed components require tooling to restrict package expansion or movement, respectively, when exposed to test pressure conditions. Tooling minimizes the seal stress of the flexible package and maintains consistent package volume and differential pressure conditions across the leak path.
- Pressure decay can be used for testing packages anywhere from a few milliliters in volume to large, bulk-storage vessels.

Tests require anywhere from a few seconds to a several hours to perform. Longer test times are necessary for testing larger-volume containers. Lengthening test cycles also allows for detection of smaller leaks.

Pressure decay tests are useful in any phase of the product life cycle.

Tests may be performed in a laboratory setting or off-line in the production environment. Laboratory or off-line test equipment that allows for longer test times is generally capable of detecting smaller leaks. Higher-speed, on-line pressure decay equipment may be used to check for defects in open packages before package filling and closure.

2.4.3 TEST EQUIPMENT

Pressure decay test instrumentation includes conduits to connect the test sample with test system pressure transducers (absolute, differential, or a combination of both) and a pressure source (*12*). Instrumentation includes appropriate timers, electronic controls, and monitors. Greatest test method sensitivity and reproducibility are achieved when the instrument is kept in a temperature-controlled environment; test samples (especially larger-volume samples) are kept at a controlled, constant temperature during test; and dry pressurizing gas at constant temperature is used. It is optional to use tooling uniquely designed to limit movement or expansion of moveable or flexible components, respectively, thereby keeping test sample volume constant and limiting seal stress.

2.4.4 TEST PARAMETERS

The following are test parameters for pressure decay:

- Test sample internal pressure after pressurization:
  - The initial pressure reached after sample pressurization is a function of the test system volume, time allotted for pressurization, pressure source capacity, and temperature of the test sample headspace.
  - Higher pressure creates the potential for more rapid and sensitive leak testing. However, the selection of maximum test pressure should take into consideration personnel safety risks and potential damage to equipment and package.
- Pressure decay baseline: the baseline pressure decay (i.e., noise level) is the pressure drop that occurs for packages without leaks.
  - Baseline pressure drop is a function of test package volume, temperature conditions, and the length of time allowed for pressure to rise.
  - Baseline pressure drop requiring longer time periods is affected by gas sorption onto test package surfaces, gas moisture content (dry gas should be used), and gas temperature. Techniques to limit baseline pressure drop include the use of dry gases, and keeping the test container and the pressurized gas at a constant temperature.
- Pressure decay due to test package leakage: the extent of pressure decay above baseline is a function of leak size, available headspace volume in the test sample, the initial pressure inside the test sample, temperature control, and the time allotted for pressure to rise.
- Times
  - Time allotted for test sample pressurization: enough time is allotted to establish the desired pressure inside the test sample.
  - Time allotted after pressurization for pressure decay: enough time should be allotted so that the pressure decay from the smallest leaks can be detected (i.e., baseline decay is exceeded).
  - A time lag may be incorporated before monitoring for pressure decay to allow for gas equilibrium within the container and test system.
- Temperatures
  - Temperature of the pressurized gas can significantly affect test method sensitivity and reliability, especially when testing larger-volume containers.
  - An increase in gas temperature causes a rise in pressure, and conversely, a rise in gas pressure triggers an increase in gas temperature. Therefore, upon initial test package pressurization, the gas temperature will spike, causing a further spike in pressure. The subsequent drops in temperature and pressure during system equilibrium may be mistaken for leakage.

- Effects of temperature variation may be minimized by: 1) allowing the temperature in the pressurized system to come to equilibrium before starting the pressure decay test, 2) minimizing temperature variation outside the test system, 3) minimizing test duration, and/or 4) applying a mathematical correction to the pressure readings.

2.5 Tracer Gas Detection, Vacuum Mode

2.5.1 DESCRIPTION

The leak detection method for tracer gas detects leakage from nonporous, rigid or flexible packages. The method can be destructive or nondestructive, depending on the test approach used. The test requires the presence of tracer gas inside the test sample package. Helium is the most commonly used tracer gas, and hydrogen is also used. The leakage rate of tracer gas is quantitatively measured using a spectrometric analytical instrument specific for the tracer gas. Instruments are designed to check for tracer gas leaking out of a test package, either by means of a test chamber that can be evacuated to draw gas out of test sample leaks (the vacuum mode) or by use of a vacuum wand (the sniffer mode) for scanning the outer surfaces of the test package (see *Tracer Gas Detection, Sniffer Mode*). The vacuum-mode tracer gas test is used more commonly than the sniffer mode for integrity testing sterile pharmaceutical product–packages. The vacuum mode is both quantitative and deterministic and is used to capture and quantify leakage from an entire test package, or it can be used to test for leakage along a test package surface or seal, given proper sample fixtures. Both vacuum and sniffer testing modes using helium as the tracer gas are described in ASTM F2391 (*13*).

To perform the vacuum mode test, test samples that have been fully or partially flooded with tracer gas are placed inside an evacuation chamber that is pneumatically connected to the tracer gas analysis instrument. Alternatively, the test sample may be fitted in such a manner that only the surface or seal of interest is exposed to the instrument, allowing for targeted leak detection at one specific seal or surface. In some cases, test samples that cannot withstand the high-vacuum test conditions may be tested with the use of tooling to restrict package expansion or movement.

At test start, the instrument's vacuum pump evacuates the test chamber or fixture, drawing leaking tracer gas through the analyzer. The absolute leak rate of the test sample is calculated by normalizing test results by the partial pressure of the tracer gas within the test sample at the time of test. For accurate results, tracer gas concentration within the sample must be uniform and consistent at the time of test; also, there should be minimal tracer gas permeation out of the test sample that can mask test sample leakage. Calibration tracer gas reference standards can be used for understanding the relationship between true leak rates and measured leak rates under actual test conditions.

The vacuum-mode tracer gas leak test is a nondestructive test, unless tracer gas introduction into the test sample requires package wall compromise (e.g., piercing), or if the presence of tracer gas is detrimental to the package contents.

2.5.2 APPLICATION

Rigid or flexible packages made of nonporous components:

- Flexible packages or packages with nonfixed components may require tooling to restrict package expansion or movement, respectively.
- Tracer gas permeation through the package material must not be so great that the leakage rate of concern is masked.
- A wide range of package sizes may be tested.

Leak paths must be clear of liquid or solid materials that could potentially block tracer gas flow.

Caution is advised when testing liquid-filled packages, because vapors or liquid drawn into the test system can seriously damage instrumentation.

Detection capabilities range from large leaks to the smallest leaks.

- Method capability is related to the size of the unobstructed leak path.
- Large leaks in the smallest packages may be missed because of the rapid loss of tracer gas (e.g., during the evacuation phase of the vacuum mode test).
- Significant tracer gas permeation through the package itself can interfere with the test by swamping leakage rate.

The test is nondestructive if the tracer gas is introduced into the package at the time of package assembly or closure, but the inclusion of tracer gas may prevent introduction of these packages into commercial or clinical markets. The test is destructive if the introduction of tracer gas compromises assembled package integrity (e.g., package puncture). Following test sample preparation, the actual leak test generally takes less than 1 min.

Tracer gas leak test methods may find application in any product life cycle phase. They are generally used in a laboratory environment. Tracer gas methods can also be used in production as an off-line testing approach; they can be used on-line if tracer gas is introduced into the test samples before final package closure.

2.5.3 TEST EQUIPMENT

Analytical instrumentation specific for tracer gas detection (e.g., mass spectrometer for helium detection) is required, typically equipped with an internal leak rate standard for instrument calibration at time of use. Additional equipment needed includes external calibration reference standards of tracer gas, to be used for comparisons of true leak rate versus measured leak rate under actual test conditions; a tracer gas source with a means for introducing tracer gas into the test sample; a test chamber or fixture to pneumatically connect the test sample with the instrument; tooling to restrict package expansion or movement, as appropriate; a means for accessing and analyzing the test sample headspace for tracer gas partial pressure after execution of the vacuum-mode leak test; and ventilation to remove tracer gas from the test area, or a tracer gas recapture system.

2.5.4 TEST PARAMETERS

The following are test parameters for tracer gas detection, vacuum mode:

- Tracer gas partial pressure within the test sample at the time of test:
  - When added by soaking the intact package, the concentration of the tracer gas is dependent on the time allotted, the positive pressure applied, and the tracer gas leak rate, plus the rate of permeation into the package
  - When added before package closure, tracer gas concentration is dependent on the gas flooding rate and time, the tooling and/or enclosure used to concentrate gas inside the package, and the efficiency of package closure for preventing escape of tracer gas
  - When added after package assembly, tracer gas concentration is dependent on the tooling used to pierce, flush, and vent the package; the gas flooding rate and time; and the sealant material applied to reseal the puncture site
- Differential pressure applied to the test sample during the vacuum mode will drive tracer gas out of the test sample, increasing method sensitivity
- Time allotted to allow for leakage to reach steady state should not exceed tracer gas permeation lag time

2.6 Vacuum Decay

2.6.1 DESCRIPTION

The vacuum decay test is a nondestructive, quantitative measurement approach for detecting leakage in nonporous, rigid or flexible packages. Leakage in the package headspace gas region and/or below the product-fill level may be detected given appropriately designed test parameters and if product properties allow (as detailed below).

To perform the test, the test sample is placed in a closely fitting evacuation test chamber pneumatically connected to the leak test system, which is equipped with an external vacuum source. The test chamber is uniquely designed to contain the test package. Test samples with moveable or flexible components require appropriate tooling to limit the movement or expansion of such components, respectively.

Upon test start, the test chamber plus test system dead space are evacuated for a predetermined period of time. The targeted vacuum level chosen for the test is predetermined on the basis of the test sample type, size, and content. The vacuum source is then isolated from the test system. After a short time has elapsed to allow for system equilibration, the rise in dead space pressure (i.e., vacuum decay) is monitored for a predetermined length of time using absolute and/or differential pressure transducers. A pressure increase that exceeds a predetermined pass/fail limit established using negative controls indicates container leakage. ASTM F2338 may be referenced (*14*).

2.6.2 APPLICATION

Nonporous, rigid or flexible packages may be tested. Packages containing gas, liquid, and/or solid materials can be tested:

- Flexible packages or packages with nonfixed components require tooling to restrict package expansion or movement, respectively, when exposed to test vacuum conditions. Tooling minimizes flexible package seal stress and maintains consistent package volume and differential pressure conditions across the leak path.
- Product–package gas headspace must be at atmospheric pressure or at a pressure notably greater than test vacuum conditions.
- Package surfaces below the product-fill level may be tested for leaks for those solid dosage formulation products that do not block leak-site gas flow or for those liquid dosage form products that volatilize at test vacuum without solidifying and blocking leak paths.
- Packages ranging in volume from a few milliliters to several liters may be tested.

Vacuum-decay leak tests are useful in all phases of the product life cycle. Tests require anywhere from a few seconds to a few minutes to perform. Longer test times are necessary for testing larger-volume packages or for detection of the smallest leaks. Longer test times are more appropriately performed in a laboratory setting or off-line in the production environment. Higher-speed on-line equipment is generally used for detecting larger leaks.

2.6.3 TEST EQUIPMENT

Vacuum-decay leak test instrumentation consists of a system of conduits and valves that pneumatically connect a test chamber with the test system pressure sensors and an external vacuum source. The instrument includes appropriate timers, electronic controls, and monitors. An external gas flow meter allows for periodic system performance verification. The test chamber is uniquely designed to closely contain the test package and may be fitted with tooling to limit movement or expansion of moveable or flexible package components, as appropriate.

2.6.4 TEST PARAMETERS

The following are test parameters for vacuum decay:

- Pressures
  - Test chamber pressure after evacuation: the initial pressure reached during test chamber evacuation is a function of test system volume, time allotted for evacuation, and vacuum pump capacity. The absolute pressure of the test system must be lower than the headspace pressure of the test package for detection of gas headspace leaks, and/or lower than the volatilization pressure of the liquid-product formulation for leaks located below the liquid-product fill level.

- Pressure rise baseline: the baseline pressure rise (i.e., noise level) is the pressure increase that occurs for packages without leaks. Baseline pressure rise is a function of test package and system outgassing, test system volume, and the time allowed for pressure rise.
- Pressure rise due to test package leakage: the extent of pressure rise above baseline is a function of leak size, test chamber vacuum level at test start, available headspace volume or volatile liquid in the test sample, and the time allotted for pressure rise.

- Times
  - Time allotted for system evacuation: enough time should be allowed for establishing the desired vacuum pressure level of the test chamber, plus draw off gases sorbed onto package surfaces or entrapped between components. Excessive time will evacuate headspace from largely leaking packages, risking that there will be insufficient headspace gas for leak detection. Times should not be so great that leaking package headspace gases are exhausted or the liquid product floods and contaminates the test system.
  - Time allotted after evacuation for pressure rise (vacuum decay): enough time should be allotted so that the pressure rise from the smallest leaks to be detected exceeds baseline. A time lag may be incorporated after evacuation and before vacuum decay monitoring to allow for gas equilibrium within the container and test system.

3. PROBABILISTIC LEAK TEST TECHNOLOGIES

3.1 Bubble Emission

3.1.1 DESCRIPTION

The bubble emission leak test is a destructive, qualitative measurement approach for detecting and locating leaks in nonporous, rigid or flexible packages containing headspace gas.

The test is performed in one of two ways. The first is an internal pressurization method referenced in ASTM F2096 (*15*) in which a positive pressure air source with pressure monitor is inserted into the test sample. The test sample is then submerged in water, and air pressure is applied to a predetermined level, for a predetermined time period. The second approach is referenced in ASTM D3078 (*16*). The intact test sample is submerged in water or other suitable submersion fluid contained in a vacuum chamber. Vacuum is established to a predetermined level, for a predetermined time period.

With both approaches, leakage can be observed as a continuous stream of bubbles emitted from the leak site. Bubble diameter and emission rate may provide some indication of relative leak size. An alternative to test sample submersion is coating the test sample with surfactant, in which case any leakage is seen as foaming or bubbling at the leak site. The surface tension of the submersion fluid or surfactant allows for smaller bubble formation, potentially improving test sensitivity. Use of submersion fluid with low gas solubility may also improve test sensitivity.

The bubble emission test is categorized as a probabilistic leak test method. Although this method relies on the predictable flow of gas through leak paths, escaping gas can become entrapped within or between package components; false-leak outgassing events may occur; gas emitted from small leaks may solubilize in the immersion fluid before bubble formation; and test sample set up may be inadequate to ensure sufficient differential pressure conditions and appropriate bubble visibility. The use of negative and positive controls along with test samples provides evidence of test method limit of detection.

3.1.2 APPLICATION

Nonporous, rigid or flexible packages with gas headspace may be tested by bubble leak methods:

- Packages must be able to tolerate wetting or submersion
- Flexible packages or packages with nonfixed components generally require tooling to restrict package expansion or movement, respectively, when exposed to vacuum conditions. Tooling minimizes the seal stress of the flexible package and maintains consistent package volume and differential pressure conditions across the leak path. However, tooling may block leak paths or hinder bubble emission visibility
- Only leak sites that are present in the gas headspace region of the package can be detected
- This test is generally used for testing smaller-volume packages that are less than a few liters in size

Bubble tests are applicable in any product life cycle phase. Bubble tests require several minutes or longer for test sample analysis and subsequent cleaning and/or drying. Bubble tests are most commonly used in laboratory settings as part of a research investigation to verify leak presence and location. They can also be used as an off-line production leak test. Bubble tests are also used for integrity testing of aerosol–package products in a research or production setting. In this application, the test may be considered nondestructive to the product–package test sample.

3.1.3 TEST EQUIPMENT

Bubble tests require a pressure or vacuum source (as appropriate) equipped with pressure monitors and controls; tooling to restrict expansion of flexible packages or movement of nonfixed components; submersion fluid or surfactant to be applied to the package surface; submersion vessel equipped for external vacuum or internal pressure test mode; and visual inspection aids such as lighting, magnification, and/or background, as needed.

3.1.4 TEST PARAMETERS

The following are test parameters for bubble emission:

- Differential pressure: greater differential pressure evokes more rapid bubble emission. Differential pressure should not be so great that package seals are compromised or that gas escapes through large leaks so rapidly that it may be confused with package surface outgassing
- Time allotted during differential pressure application: longer test times allow for smaller leak detection
- Times allotted for inspection (pacing) and for inspection breaks to lessen operator fatigue
- Package positioning during inspection
- Package mode of restraint for moveable or flexible components
- Submersion fluid (or surfactant) surface tension: lower surface tension improves method sensitivity
- Inspection environment parameters: lighting intensity and angle, degree of magnification, background color

3.2 Microbial Challenge, Immersion Exposure

3.2.1 DESCRIPTION

The immersion exposure microbial challenge is a destructive, qualitative measurement approach for confirming leaks in nonporous, rigid or flexible packages.

The test is performed by first filling test samples with sterile, growth-supporting media, followed by incubation and visual inspection of samples to ensure sample sterility before microbial challenge. Samples are then immersed in a concentrated bacterial suspension for a predetermined time. Samples can be exposed during immersion to a predetermined vacuum for a predetermined time, followed by release of vacuum while the packages remain immersed at ambient pressure for a predetermined time. Samples are then incubated under growth-promoting conditions, followed by examination of package contents for evidence of microbial growth by visual inspection or other appropriate analytical means. Alternative approaches can include exposure of immersed test samples to positive pressure conditions, or to multiple cycles of vacuum and/or pressure conditions. Test sample leakage is evidenced by visible growth of the challenge microorganism(s) inside test samples. Immersion microbial challenge tests rely on the presence of a liquid carrier in the leak path that sweeps microorganisms into the package or provides a means whereby microorganisms can actively migrate and/or grow into the test sample. The use of negative and positive controls along with test samples provides evidence of test method limit of detection.

The microbial challenge by immersion test is categorized as a probabilistic leak test because of the multiple events that must occur sequentially and/or simultaneously for leak detection to take place. All such events are difficult to predict or control, especially for detection of smaller leaks. For example, the microorganisms must be physically present at the leak site. The necessary presence of liquid in the leak path, and/or flowing through the leak path, is influenced by the package materials of construction, leak path tortuosity and topography, media surface tension, and leak path blockage by product, extraneous debris, or air locks. Microorganisms must not be hindered from entering the package by getting trapped in a tortuous leak path, and enough microorganisms must enter the package to allow for sufficient growth that can be detected visually after test sample incubation.

3.2.2 APPLICATION

Microbial challenge tests by immersion are most useful when an appropriate and validated physicochemical leak test method does not exist, or when the test outcome demands direct evidence of the prevention of microbial entry.

Nonporous packages of rigid or flexible components may be tested by immersion microbial challenge methods:

- Packages must be able to tolerate submersion.
- Flexible packages or packages with nonfixed components may require tooling to restrict package expansion or movement, respectively. Tooling minimizes stress on flexible package seals and maintains consistent differential pressure conditions across the package seal.

Immersion microbial challenge tests are performed in a laboratory environment, not as an on-line test of the finished product.

A test requires several days to prepare; the test samples are pre-incubated before microbial challenge to ensure initial package content sterility. The challenge itself, plus post-challenge sample decontamination, may take several hours. Final incubation followed by sample inspection may take 1–2 weeks. The immersion microbial challenge test is primarily used in product–package development and validation studies.

3.2.3 TEST EQUIPMENT

The test chamber for conducting the immersion microbial challenge test is designed and equipped to maintain suspension uniformity and appropriate temperature while exerting the required differential pressure condition. Fixtures for restraining and/or positioning packages during immersion are also required. Small, motile microorganisms are preferred for the challenge; examples include *Brevundimonas diminuta* and *Serratia marcescens*. The immersion challenge media should support challenge microorganism growth to the desired concentration. Soybean-casein digest medium is one commonly used medium.

The media filled into test samples may match the immersion challenge media formulation. Alternatively, product that has been shown to support microbial growth may be used. Media should allow sufficient growth of the particular challenge organism so that package contamination can be detected in the positive controls. Verification of growth promotion in the immersion media and the test sample media should be performed each time the immersion challenge test is conducted (see *Sterility Tests* ⟨71⟩, *Growth Promotion Test of Aerobes, Anaerobes, and Fungi*). Use of an incubation chamber is required for test samples, both before and after exposure. A means for determining microbial growth inside test packages is required.

3.2.4 TEST PARAMETERS

The following are test parameters for microbial challenge, immersion exposure:

- Microorganism concentration: in the immersion challenge media, microorganism concentration should meet appropriate predetermined levels throughout the challenge test (a minimum concentration of 10^5 CFU/mL is commonly used).
- Media fill volume: the fill volume of media in test samples should be sufficient to ensure a liquid path at each potential leak site. Exposure of package seals and leak sites to the media may also be accomplished by test sample positioning during the immersion challenge. It is also necessary to have sufficient volume and correct composition of the package headspace to encourage growth of the selected challenge microorganism. Note that when the composition of the package headspace does not include oxygen, other test conditions (e.g., anaerobic) may be applicable.
- Vacuum/pressure conditions: exposing immersed test samples to differential pressure conditions is an important method parameter that serves multiple purposes:
  - Differential pressure helps eliminate trapped air and ensures the presence of liquid media between package components and at leak sites.
  - Differential pressure simulates the pressure changes incurred during air or land freight transport of the product. Absolute pressure conditions that correspond to various altitudes anticipated during land and/or air freight transport are provided in ASTM D6653/D6653M (*17*). Differential pressure conditions of the test may be modified on the basis of knowledge of the product–package shipping environment.
  - Differential pressure exposure can simulate conditions experienced by the product during some sterilization treatments.
- Test times: longer exposure times improve the likelihood of microbial ingress into defective test samples and positive controls. However, a possible decrease in the ability of media to support growth over time must be considered.
  - Time allotted for immersion exposure during differential pressure exposure (vacuum and/or pressure)
  - Time allotted for immersion exposure at ambient pressure conditions
- Temperature during challenge: temperatures sufficient to support microbial growth are recommended. Temperature cycling can also be used as a tactic to eliminate airlocks and promote the presence of liquid media at package seal sites.
- Pre- and post-challenge test incubation temperature and times: the temperature selected should allow sufficient microbial growth. Incubation times should be sufficient to ensure visualization of growth; these times are determined on the basis of positive controls and samples from growth-promotion studies.
- Parameters for detecting post-incubation microbial growth (e.g., lighting and background color for visual inspection, handling procedure, and pacing).

3.3 Tracer Gas Detection, Sniffer Mode

3.3.1 DESCRIPTION

The following information is specific to the tracer gas detection performed in the sniffer mode. (For additional information, see *Tracer Gas Detection, Vacuum Mode*.)

Tracer gas detection using a sniffer attachment is used to detect leak presence and location in nonporous, rigid or flexible packages. This is a nondestructive leak test, unless tracer gas introduction into the test sample requires package wall compromise (e.g., piercing) or if tracer gas presence is detrimental to package contents. The sniffer mode test using helium as the tracer gas is described in ASTM F2391, Procedure A (*13*). Briefly, test samples are flooded completely or partially with the tracer gas via one of several options. These options may include piercing a closed test sample to introduce pressurized tracer gas (sealant is applied to close the puncture site); flooding the test sample before package closure; or "soaking" a closed test sample by pressurizing with tracer gas (most applicable to larger leak detection). Test samples are checked for leakage by scanning the outer package surfaces using a vacuum wand that is pneumatically connected to the tracer gas analytical test instrument (e.g., a mass spectrometer for helium detection). Calibration reference standards of tracer gas can be used for understanding the relationship between true leak rates and measured leak rates under actual test conditions. The use of negative and positive controls along with the test samples provides evidence of test method limit of detection.

The sniffer mode of tracer gas leak testing is a probabilistic leak test method. This is because the presence of concentrated tracer gas near the test sample surface is not a well-defined or predictable event, and the sniffer scanning procedure is prone to variability related to human technique. The sniffer mode is generally chosen when the leak location is to be identified.

3.3.2 APPLICATION

Rigid or flexible packages made of nonporous components may be tested:

- Flexible packages or packages with nonfixed components may require tooling or manual manipulation to force tracer gas through leak paths.
- Tracer gas permeation through the package material must not be so great that the leakage rate of concern is masked.
- A wide range of package sizes may be tested, ranging from small packages to large multi-liter vessels.

Leak paths must be clear of liquid or solid materials that could potentially block tracer gas flow. The leak size detection capability is related to an unobstructed leak path.

The sniffer probe must not be allowed to draw liquid or hazardous vapors into the test system, as this would risk serious instrument damage.

Tracer gas leak test methods require time for the introduction of tracer gas into the test package, and up to several minutes to scan the package. Large leaks in the smallest packages may be missed because of the rapid loss of tracer gas.

Methods of tracer gas leak tests in the sniffer mode are generally used in the laboratory environment for locating package leaks. Tracer-gas sniffer mode tests are useful in any product life cycle phase.

3.3.3 TEST EQUIPMENT

Test equipment for tracer gas leak detection is described in *Tracer Gas Detection, Vacuum Mode*. However, a sniffer probe with an enclosure and/or tooling for concentrating tracer gas passing through smaller leaks is used, rather than the test chamber or test fixtures described in *Tracer Gas Detection, Vacuum Mode*.

3.3.4 TEST PARAMETERS

The following are test parameters for tracer gas detection, sniffer mode:

- Differential pressure applied to the test sample will drive tracer gas out of the test sample, increasing the method's sensitivity. For flexible packages, differential pressure can be exerted by package compression.
- Tracer gas partial pressure within the package at the time of the test is discussed, with instructions, in the *Tracer Gas Detection, Vacuum Mode*.
- Aspects of the sniffer mode vacuum wand, such as sweeping speed and distance from the package surface, as well as the tooling or enclosure used for concentrating leaking gas.

3.4 Tracer Liquid

3.4.1 DESCRIPTION

The tracer liquid test method is a destructive approach for detecting and potentially locating leaks in nonporous, rigid or flexible packages. Tracer liquid tests provide an indication of leak presence and may provide a measure of relative leak size. Tracer liquid tests using liquid submersion work by the diffusive flow of the tracer element through a liquid-filled leak path and/or the effusive flow of tracer solution through the leak path.

The liquid submersion test uses one of two basic approaches. In the first approach, test samples are submerged in a tracer-element solution formulation contained in an evacuation chamber. Examples of tracer elements include dyes, radionuclides, or metallic ions. In the second approach, test samples containing tracer formulation are submerged in tracer-free liquid contained in an evacuation chamber. For both approaches, test samples may be fitted with tooling to ensure proper positioning and to restrict flexible or moveable components. The submerged test samples are subjected to vacuum at a predetermined pressure level for a predetermined time. After vacuum release, test samples remain submerged for a predetermined time. Additional test options include the use of positive pressure exposure or multiple cycles of differential pressure conditions to encourage effusive flow of the tracer element through the leak path.

In the first approach, after the challenge is complete, test sample outer surfaces are cleaned, and the contents are checked for evidence of tracer ingress (*18*). In the second approach, after the challenge is complete, the immersion fluid is checked for evidence of tracer liquid egress out of the test sample. In both cases, measurement of tracer liquid migration may be performed in a quantitative manner by using chemical analysis techniques (preferred for small leak detection). Alternatively, the presence of leakage may be determined qualitatively by visual inspection if the tracer element can be discerned visually. The use of negative and positive controls along with test samples provides evidence of test method limit of detection.

Liquid tracer tests are categorized as probabilistic methods. Successful liquid tracer detection relies on a combination of tracer solution wicking, tracer solution effusion, and tracer element diffusion through a liquid-filled leak path and are events that are difficult to predict or control, especially for detection of smaller leaks. These events are influenced by numerous factors, including the package materials of construction, leak path tortuosity and topography, tracer liquid surface tension, and leak path blockage by product, extraneous debris, and air locks.

3.4.2 APPLICATION

Rigid or flexible packages of nonporous components may be tested using tracer liquid submersion methods:

- Packages must be able to tolerate wetting or submersion.
- Flexible packages or packages with nonfixed components may require tooling to restrict package expansion or movement, respectively.
  - Testing by submerging a test sample in tracer liquid is used when the test sample allows for visual examination of the tracer (e.g., dye) ingress, or when the tracer element is to be contained within the test sample after testing.
  - Testing by submerging tracer-filled test samples in tracer-free liquid may be used when the sample interior cannot be visually examined or when the tracer element is best captured for analysis outside the test sample.

Tests may require up to 1 h or longer for test sample exposure, cleaning, and inspection or analysis. Tracer liquid tests are primarily used for laboratory testing or off-line product sample testing. Tracer liquid submersion tests can be used in any product life cycle phase.

3.4.3 TEST EQUIPMENT

A test vessel equipped for challenge conditions of vacuum and/or positive pressure is required and is pneumatically connected to a pressure and/or vacuum source, as appropriate, and equipped with pressure monitors and controls (*19*). The tracer liquid formulation should be physicochemically compatible with the test sample components and the tracer-free solution to be filled into the test samples or used as the immersion bath, to ensure optimal tracer solution functionality. Formulation considerations include tracer element type and concentration; surfactant use, type, and concentration; and solvent system. Examples of tracer liquid incompatibilities apparent upon contact with the test product include tracer dye fading, tracer element precipitation, and tracer element sorption onto package components. Tooling is often needed to restrict flexible package expansion or nonfixed component movement upon differential pressure exposure. Tooling minimizes stress on flexible package seals and ensures consistent differential pressure conditions across the leak path.

Tracer detection requires the use of either analytical detection instrumentation [e.g., UV-Vis spectrophotometry (*20*), phase induction spectrophotometry, or other] or visual inspection aids. Analytical detection offers the advantage of minimizing the error that is inherent in visual discernment of low dye concentrations. Optimal visual inspection requires the use of controlled inspection conditions, such as background color, lighting, pacing, fatigue breaks, and negative controls for comparison.

3.4.4 TEST PARAMETERS

The following are test parameters for tracer liquid:

- Differential pressure conditions used (vacuum and/or pressure): greater pressure differentials encourages tracer liquid passage.
- Submersion times during and after differential pressure application: longer times allow for greater tracer liquid passage through leak paths.
- Holding time between tracer liquid challenge and final inspection: some tracer liquids visibly fade or are sorbed onto package surfaces over time.
- Tracer liquid surface tension: lower surface tension allows for smaller leak detection.
- Tracer detection parameters
    - Analytical detection
        - Test sample content extraction procedure
        - Method-specific performance parameters
    - Visual inspection
        - Inspection environment parameters: lighting intensity and wavelength, background color, viewing angle, and test sample visibility
        - Time allowed for inspection (pacing) and breaks to lessen operator fatigue
        - Comparison with negative controls

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