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Container Closure Integrity: Regulations, Test Methods, Application - Test Methods: Overview

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INTRODUCTION

Leak Test Methods Discussed in USP <1207>

- ❖ Described in relevant peer-reviewed publications
- ❖ ASTM test with supportive precision and bias data
- ❖ Significant variation w/in technologies is seen among vendors
- ❖ Other methods not included may be acceptable
- ❖ No method is appropriate for all product-packages
- ❖ All methods are valuable when used appropriately

INTRODUCTION

Probabilistic Leak Test Methods

- ❑ **Leakage event: Stochastic in nature**
  - ➢ Relies on a series of sequential and/or simultaneous events each associated with uncertainties
  - ➢ Results are associated with random outcomes (probability distributions)
  - ➢ Some uncertainty in findings

Examples:

- ➢ Microbial challenge tests
- ➢ Bubble emission tests
- ➢ Tracer liquid tests (either qualitative or quantitative measurement)
- ➢ Tracer gas tests by sniffer probe

- ❑ **When detecting leaks near the detection limit, or rarely occurring leaks**
  - ➢ Large sample sizes required
  - ➢ Rigorous test condition controls needed

- ❑ **More difficult to design, develop, validate, implement**

- ❑ **Test sample preparation required**

Deterministic Leak Test Methods

- **Leakage event: Follows a predictable sequence**
  - Gas movement through an open leak path (at specific delta pressure or partial pressure)
  - Liquid presence near or in a leak path

Examples:

- Tracer gas (vacuum mode)
- Laser-based gas headspace analysis
- Pressure / Vacuum decay
- Mass extraction
- Electrical Conductivity and Capacitance Test (High Voltage Leak Detection)
- Optical Emission Spectroscopy (OES)

- **Leak Detection**
  - Based on physicochemical technologies readily controlled and monitored
  - Objective, quantitative data

Introduction

Deterministic Leak Test Methods

- Little or no test sample preparation
- Reliable detection of leaks at the detection limit or rarely occurring leaks
- Less difficult to design, develop, validate, implement

INTRODUCTION

Deterministic or Probabilistic?

❑ Deterministic

- For determining inherent CCI via definitive results
- When a compatible method exists for a given product-package

❑ Probabilistic

- When deterministic methods are incompatible with product-package
- When a specific method outcome is required, e.g.,
  - Leak location detection
  - Microbial grow-through check

Probabilistic Leak Test Methods

- ❖ Bubble Emission
- ❖ Microbial Challenge by Immersion Exposure
- ❖ Tracer liquid detection (Dye Immersion)
- ❖ Tracer Gas Detection (Sniffer Mode)

Requirements:

Package

- ❖ Nonporous, rigid
- ❖ Porous material require masking to limit airflow through material
- ❖ Flexible or non-fixed components may employ optional restraint mechanism
- ❖ Must tolerate submersion or surfactant wetting
- ❖ All types of vials, bottles, syringes, blisters, pouches, bags, etc.
- ❖ Small volume generally (< few liters)
- ❖ Plastics (with limited helium permeability), glass, metal
- ❖ Rigid to flexible to non-fixed components (restraint mechanism may be desirable)

Probabilistic Leak Test Methods

Application:

- Packages must be able to tolerate submersion or wetting (immersion tests).
- Gas or liquid must be present and be able to migrate through leak path; product, debris, or air locks can block leak path.
- Test fixture or restraint mechanism at test sample site of contact may block leak path.
- Outgassing of sorbed gas on test sample surface (helium tracer test) or release of entrapped air pockets (bubble emission test) may falsely simulate leakage.
- Several minutes to weeks per test sample.

Probabilistic Leak Test Methods

Destructive (most probabilistic methods):

- Internal pressurization results in test sample barrier breach
- Submersion or surface wetting is destructive to test samples

Detection Limit: Varies with

- Leak size, type, length, blockage
- Package material of construction, flexibility
- Challenge conditions, including time, pressure, sample positioning, immersion fluid surface tension and gas saturation
- Inspection conditions and operator technique/skill

Detection Range: ≤ 0.01 µm (tracer gas); 6-20 µm (all others) may be possible

Probabilistic Leak Test Methods

Reported Usage:

- **Bubble Emission**
  - ➢ Gas must be present at leak site
  - ➢ Best used for testing empty packages to prevent clogging of leak site
- **Microbial Immersion**
  - ➢ Unless the product itself is growth-supportive, test samples are not filled with product
  - ➢ Substitute product with sterile growth media
- **Dye Immersion**
  - ➢ Ingress Test – test samples are filled with placebo unless the product itself is compatible with tracer liquid
  - ➢ Egress Test – test samples are filled with tracer liquid
  - ➢ Subject to visual inspection if applicable
- **Tracer Gas (Sniffer Mode)**
  - ➢ Tracer gas must be present at leak site
  - ➢ Best used for testing empty packages to prevent product contamination of gas analyzer and test probe

Bubble Emission Test

- **Detection:** Bubble emission at leak site

Technology

- **Qualitative measure by visual inspection of bubbles escaping test sample while sample is submerged and subject to differential pressure**
- **Alternatively, sample surface may be coated with surfactant; leakage evidenced by foaming**
- **Outcome judged by operators trained using no-leak and with-leak controls; indicates leak presence, location and relative size**

Bubble Emission Test

- **Test (Internal Pressurization): ASTM F2096**
  - Insert positive pressure source with monitor into test sample.
  - Submerge test sample in water; apply air to target pressure; hold for pre-determined time.
  - Observe for continuous stream of bubbles.
- **Test (External Vacuum): ASTM D3078**
  - Submerge test sample in water or other suitable fluid in vacuum chamber.
  - Evacuate chamber to target pressure; hold for pre-determined time.
  - Observe for continuous stream of bubbles.
- **Test (Alternative to Submersion):**
  - Apply surfactant to test sample surfaces. Observe for foaming.

Bubble Emission Test

- **Application:**
  - Submersion liquid boiling under vacuum may mask leakage
  - Used in all product life-cycle phases, often as a leak forensics test. Not recommended for inherent CCI verification
  - Off-line test; On-line testing used for aerosol products
  - Several minutes per test sample
- **Detection Range:** 2 µm to mm may be possible
- **Destructive**

Example of bubble leak test (Package restraint may be preferred)

Surfactant solution used at suspected leak site

Microbial Challenge by Immersion Test

- **Detection:** Microbial growth in test sample resulting from passive ingress or active growth

- **Technology:**
  - Qualitative measure by visual inspection of microbial growth inside incubated test samples filled with growth-supportive media or product, post immersion in heavily contaminated challenge media over a pre-determined challenge time.
  - Pressure and/or temperature cycling may be used to encourage ingress.
  - Outcome judged by visual inspection by trained operators; verified by other analytic means.
  - Outcome indicates presence of leak path(s) capable of allowing passive or active microbial entry.

- **Requirements:**
  - Product must be supportive of microbial growth; otherwise, test samples must contain sterile growth supportive media.
  - Test sample headspace must include gas appropriate for microbial growth (e.g., oxygen for aerobic microorganisms).

Microbial Challenge by Immersion Test

- **Test:**
  - Fill test samples with sterile media (growth support capability must be verified). Incubate and inspect to confirm test sample content sterility.
  - Immerse test samples in media concentrated with challenge organisms for pre-determined time.

Recommendation: Cycle pressure and/or temperature conditions and extend exposure time to encourage entry.

  - Remove and clean test samples. Incubate under growth-promoting conditions.
  - Examine test sample contents for evidence of growth by visual or other means. Compare to test sample blanks and no-leak/with-leak controls.

- **Detection Range:** **20 µm to mm may be possible**

- **Destructive**

Microbial Challenge by Immersion Test

- **Application:**
  - Liquid must fill leak path to allow microbes to be mechanically swept in (passive entry) or to allow microbial growth into package (active entry).
  - Debris or air-locks in leaks will prevent microbial ingress.
  - Off-line test, although often used as part of aseptic processing validation runs to verify processing conditions.
  - Generally used in R&D to check inherent CCI (only recommended if deterministic methods are not applicable).
  - Weeks per test sample.

Tracer Liquid Test (Dye Immersion)

- **Detection:** Tracer liquid migration into (or out of) test sample
- **Technology (Qualitative Measurement):**
  - Visual inspection of tracer liquid inside test samples post immersion in tracer liquid while exposed to differential pressure conditions over a pre-determined time
  - Alternatively, test samples may be filled with tracer liquid and submerged in tracer-free fluid
  - Outcome judged by trained operators using blank standards and no-leak/with-leak controls
- **Technology (Quantitative Measurement):**
  - Appropriate analytical means (e.g., UV/Vis spectrophotometry for dye tracer) of test sample contents post immersion, as above
  - Alternatively, tracer-free submersion fluid is analyzed for tracer
  - Outcome judged by appropriate analytical means, versus blank solution standard and no-leak/with-leak controls
- **Outcome:** Indicates presence of leak path(s) capable of allowing tracer liquid entry

Tracer Liquid Test (Dye Immersion)

❑ Requirements:

- **Liquids –** If used, product must be compatible with tracer liquid; otherwise, test samples are to contain placebo solution.
- **Powders –** Product must be compatible with tracer liquid. For smallest leak detection powders will required constitution with tracer-free liquid for analysis or inspection.

❑ Test (Tracer Ingress):

- Immerse test samples in tracer liquid for pre-determined time and temperature.
- Remove and clean test samples. Control and limit time to inspection.
- Examine test sample contents for evidence of tracer liquid by visual or analytical means. Compare findings to test sample blanks, no-leak and with-leak controls.

❑ Test (Tracer Egress):

- Fill test samples with tracer liquid.
- Immerse test samples in tracer-free liquid for pre-determined time and temperature.
- Examine immersion liquid for evidence of tracer liquid by visual or analytical means Compare findings to test sample blanks, no-leak and with-leak controls.

Tracer Liquid Test (Dye Immersion)

- **Recommended for Both Methods:**
  - Minimize volume of tracer-free liquid per test sample. Liquids should be clean and of low surface tension.
  - Cycle temperatures and/or pressure conditions and extend exposure time to encourage tracer migration.
  - Control/limit time and conditions of sample storage prior to examination.
- **Application:**
  - Off-line test.
  - Used in R&D or stability to check CCI (only recommended if deterministic methods are not applicable). May be used in package forensics analysis.
  - Minutes to hours per test sample.

Tracer Liquid Test (Dye Immersion)

- **Detection Limit:** Varies with:
  - Leak size, type, length, material of construction, blockage.
  - Tracer concentration, surface tension, cleanliness.
  - Tracer compatibility with product (ingress test) or immersion fluid (egress test).
  - Challenge conditions of time, temperature, pressure, sample positioning.
  - Inspection conditions and operator training/skill.
  - Analytical detection sensitivity and test sample preparation.
- **Detection Range:** 6-10 µm to mm may be possible
- **Destructive**

Tracer Gas Detection (Sniffer Mode)

- **Detection:** Tracer gas leakage rate in mass flow units
- **Technology:**
  - Quantitative measure of tracer gas leak rate from a gas-charged test sample into the atmosphere captured using a sniffer probe connected to a spectroscopic analyzer.
  - Output analyzed by spectroscopic means.
  - Leak rate above a baseline pass/fail limit indicative of leak presence and relative size.
  - Calibrated leak standards used to verify method accuracy and reliability.
- **Test:**
  - Flood tracer gas into test sample. Use tooling to restrain and/or compress flexible package or package with non-fixed components as required.
  - At test start, scan test sample surfaces with sniffer probe connected to spectroscopic analyzer specific for tracer gas (for Helium: Mass spec analysis).
  - Gas mass flow rate is continually reported. Reading above a pre-determined baseline is indicative of leak presence (pass/fail test). Reading magnitude may correlate to relative leak size.

Reference: ASTM F2391

Tracer Gas Detection (Sniffer Mode)

- **Application:**
  - Best performed on empty test sample – product drawn into analyzer or probe may damage instrument.
  - Used in all product life-cycle phases, but not recommended for inherent CCI verification.
  - Useful for leak forensics analysis.
  - Generally performed off-line.
  - Requires minutes per test sample.
- **Nondestructive:** If tracer gas introduction into test sample poses no threat to product sterility/quality
- **Detection Limit:**
  - Tracer gas permeation through package may be mistaken as leakage
  - Tracer gas background in testing environment can influence test results
  - Varies with operator technique and sniffer probe design
- **Detection Range:** ≤ 0.01 µm to mm may be possible

Tracer Gas Detection (Sniffer Mode)

MD-490S Helium/Hydrogen Leak Detector - VIC Leak Detection

Helium Sniff Test Application

APPENDIX I

Comparison of Tracer Liquid Test Methods

Probabilistic leak test methods

Closure Re-seal Method Parameters	USP 31 <381> Ph. Eur. 3.2.9	ISO 8362-5 Annex C	ISO test modified to maximize sensitivity
Dye	0.1% aq. Methylene Blue		
Vacuum	-27 KPa	-25 KPa	-37 KPa
Time at Vacuum	10 min	30 min	30 min
Time at Ambient	30 min	30 min	30 min
Detection method	Visual inspection		

H. Wolf, T. Stauffer, S-Chen Chen, et al, PDA J Pharm Sci & Technol., 63, 2009, p. 489 - 498

Probabilistic leak test methods

Test samples

BD Glass Syringes

- ✓ 1mL volume
- ✓ Staked Needle
- ✓ Water-filled

H. Wolf, T. Stauffer, S-Chen Chen, et al, PDA J Pharm Sci & Technol., 63, 2009, p. 489 - 498

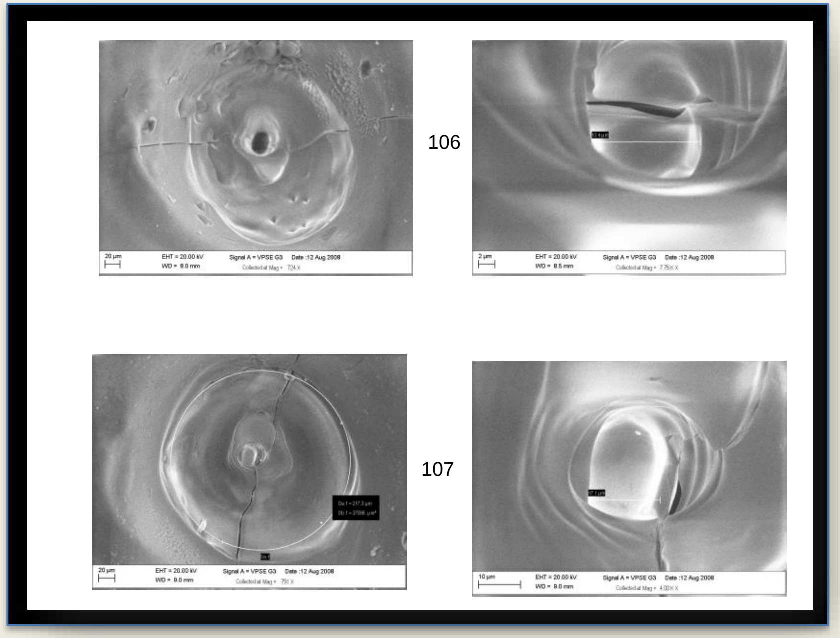
Probabilistic leak test methods

- Inspector Qualification Study
  - **Test Samples**
    - 1mL water-filled syringes WITH and WITHOUT methylene blue
    - Known (-) controls for comparison
  - **Logistics**
    - 3 Test sites, 3 Inspection stations, 10 Inspectors
    - 10 sec pacing, randomized, blinded
    - Inspection stations varied: lighting type, intensity, position, background angle and position
  - **Results**
    - **Detection limit varied from 0.2 to 0.5 ppm**

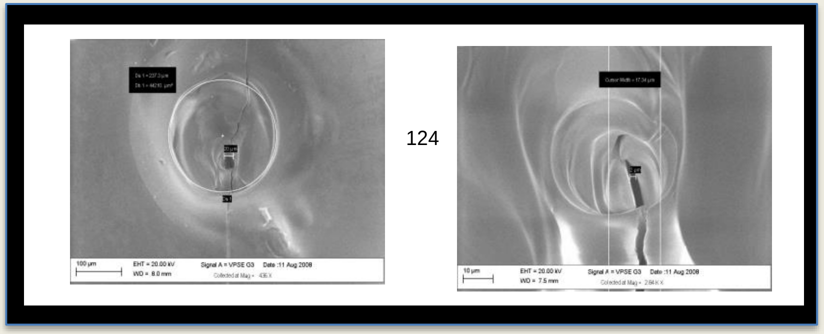
H. Wolf, T. Stauffer, S-Chen Chen, et al, PDA J Pharm Sci & Technol., 63, 2009, p. 489 - 498

Probabilistic leak test methods

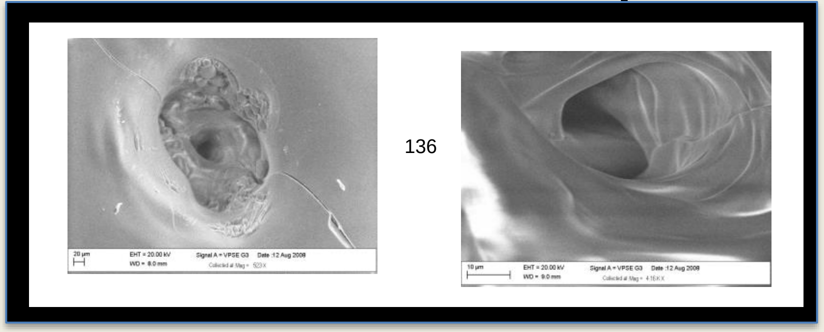
Glass Syringe Defects by Lenox Laser



Nominal hole size 5 µm



Nominal hole size 10 µm



Nominal hole size 15 µm

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Probabilistic leak test methods

Test Samples	USP/Ph.Eur. Dye Test (-27 kPa 10 min, amb 30 min) YES (Dye visible) or NO (Not visible)		
	Inspector 1	Inspector 2	Inspector 3
Negative Controls	No	No	No
	No	No	No
	No	No	No
	No	No	No
	No	No	No
5 µm	No	No	Yes
	No	Yes	Yes
	No	Yes	Yes
	No	No	No
	No	No	Yes
10 µm	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	No	No	Yes
	No	No	No
15 µm	No	No	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes

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Probabilistic leak test methods

USP/Ph. Eur. Dye Ingress Test Samples

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Probabilistic leak test methods

Test Samples	ISO Dye Test (-25 kPa 30 min, amb 30 min) YES (Dye visible) or NO (Not visible)		
	Inspector 1	Inspector 2	Inspector 3
Negative Controls	No	No	No
	No	No	No
	No	No	No
	No	No	No
	No	No	No
5 µm	No	No	No
	No	No	Yes
	No	Yes	Yes
	No	No	Yes
	No	No	No
10 µm	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	No	No	Yes
	No	No	No
15 µm	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes

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Probabilistic leak test methods

Test Samples	**MODIFIED ISO Dye Test** (-37 kPa 30 min, amb 30 min) YES (Dye visible) or NO (Not visible)		
	Inspector 7	Inspector 8	Inspector 10
Negative Controls	No	Yes	No
	No	Yes	No
	No	No	Yes
	No	Yes	Yes
	Yes	No	No
5 µm	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
10 µm	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
15 µm	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes
	Yes	Yes	Yes

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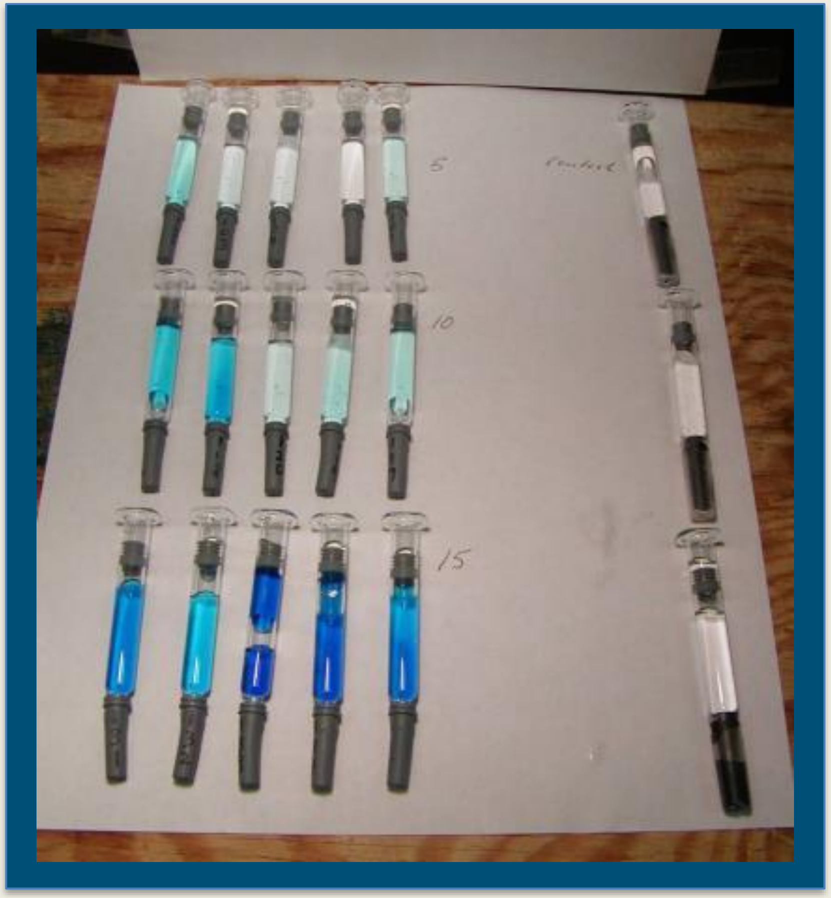
Probabilistic leak test methods

Modified ISO - Dye Ingress Test Samples

5 µm

10 µm

15 µm



Negative Controls

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Probabilistic leak test methods

Compendial and ISO dye ingress methods

Summary

- Inspector capabilities varied
- Visual inspection conditions not defined
- All methods lacked sensitivity, reliability for smallest leaks
- The 'optimized' ISO method resulted detected more leaking packages, but greater number of 'good' syringes were falsely reported as leaking
- Other disadvantages
  - **False negative risks**
    - Proteins, salts, etc. clog leak paths, inhibiting dye ingress
    - Dye dilution in larger volumes
    - Dye may fade over time
  - **False positive risks**
    - Inspector error
    - Sample contamination (if analytically analyzed)
  - **Destructive method**

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Probabilistic leak test methods

Dye test used for seal gap check