Container Closure Integrity: Regulations, Test Methods, Application

Test Methods: Overview

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Probabilistic leak test methods

Leakage event: Stochastic in nature

- Relies on a series of sequential and/or simultaneous events each associated with uncertainties
- Results:
  - 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

Probabilistic leak test methods

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)
  - Tracer gas (vacuum mode)
  - Laser-based gas headspace analysis
  - Pressure / Vacuum decay
  - Mass extraction

- Liquid presence near or in a leak path
  - electrical conductivity and capacitance test

Deterministic leak test methods

Leak detection

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

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

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

Leak test methods discussed

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

Probabilistic leak test methods

Bubble emission

Microbial challenge by immersion exposure

Tracer liquid detection

Tracer gas detection (sniffer mode)

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

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

Product: Headspace gas only must be present at leak site

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

- Packages must be able to tolerate submersion or wetting
- Gas must be at leak path; product or debris 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 or release of entrapped air pockets may falsely simulate leakage
- 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

Bubble emission test

Destructive

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: 20 µm to mm may be possible

Bubble emission test

Reported usage

Formulations

- Gas must be present at leak site
- Best used for testing empty packages to prevent clogging of leak site

Packages

- All types of vials, bottles, syringes, blisters, pouches, bags, etc.
- Small volume generally (< few liters)
- Plastics, glass, metal
- Rigid to flexible to non-fixed components (restraint mechanism may be desirable)

Bubble emission test

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

Microbial challenge by immersion test

Requirements

Package

- Nonporous, rigid
- Flexible or non-fixed components may employ optional restraint mechanism
- Must tolerate submersion

Product

- Must be demonstrated to 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.
  - **Recommend**: 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.

Microbial challenge by immersion test

Application

- Packages must be able to tolerate submersion
- 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
- Test fixture or restraint mechanism at test sample site of contact may block leak path
- 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

Microbial challenge by immersion test

Destructive

Detection limit

Varies with

- Leak size, type, length, material of construction, blockage
- Challenge organism type and concentration, media
- Challenge conditions, including temperature/pressure cycling, time, sample positioning

Detection range: 20 µm to mm may be possible

Microbial challenge by immersion test

Reported usage

Formulations

- Unless the product itself is growth-supportive, test samples are not filled with product

Packages

- All types of vials, bottles, syringes, blisters, pouches, bags, etc.
- Smaller volume generally (< 1 liter)
- Plastics, glass, metal
- Rigid to flexible to non-fixed components (restraint mechanism may be required)

Tracer liquid test

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

Requirements

Package

- Nonporous, rigid
- Flexible or non-fixed components may employ optional restraint mechanism
- Must tolerate submersion

Product

- 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

Tracer liquid test

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

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

Tracer liquid test

Application

- Packages must be able to tolerate submersion
- Liquid must be able to migrate through leak path
- Debris or air-locks in leaks will block liquid migration
- Test fixture or restraint mechanism at test sample site of contact may block leak path
- 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

Destructive

Tracer liquid test

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

Tracer liquid test

Reported usage

Formulations

- 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

Packages

- All types of vials, bottles, syringes, blisters, pouches, bags, etc.
- Smaller volume generally (< 1 liter)
- Plastics, glass, metal
- Subject to visual inspection if applicable
- Rigid to flexible to non-fixed components (restraint mechanism may be required)

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

Reference ASTM F2391

Tracer gas detection (sniffer mode)

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

Tracer gas detection (sniffer mode)

Application

- Best performed on empty test sample.
  - Product or debris can block leak path
  - Test sample fixture or compression tooling can block leak path
  - Product drawn into analyzer or probe may damage instrument
- Used in all product life-cycle phases
  - Not recommended for inherent CCI verification
  - Useful for leak forensics analysis
- Generally performed off-line
- Requires minutes per test sample

Tracer gas detection (sniffer mode)

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)

Reported usage

Formulations

- Tracer gas must be present at leak site
- Best used for testing empty packages to prevent product contamination of gas analyzer and test probe

Packages

- All types of vials, bottles, syringes, blisters, pouches, bags, etc.
- Small volume to large volume
- Plastics (with limited helium permeability), glass, metal
- Rigid to flexible to non-fixed components (tooling for package restraint and/or compression may be required)

Tracer gas detection (sniffer mode)

MD-490S helium/hydrogen leak detector
VIC Leak Detection

Helium sniff test application

Group Exercise: Methodology

Methodology Group Exercise

Objective:

- Compile classification and summary table detailing the key attributes of each CCI test methodology

Instructions:

- Each team will be assigned one CCI test method to evaluate
- Team will work as a group to classify the test method and identify the following:
  - Test method classification: deterministic v. probabilistic, destructive v. non-destructive, qualitative v. quantitative
  - Best case limit of detection achievable
  - Applicable product life cycle phase(s)
  - Major advantages and limitations
  - Any key considerations
- Each team will present findings in the morning of Day 2

Test Method	Technology Classification	Limit of Detection	Applications	Major Advantages	Major Limitations	Key Considerations
Tracer Gas (helium) in Vacuum Mode						
Vacuum/ Pressure Decay						
Mass Extraction						
Laser-based Headspace Analysis						
High Voltage Leak Detection						

Test Method: Dye Ingress/Microbial Immersion

Technology Classification	Limit of Detection	Applications	Major Advantages	Major Limitations	Key Considerations
• Probabilistic • Destructive • Qualitative	20µm - 50µm	• Routine QC testing	• Applied for decades • Familiarity • ”Last resort” when all else fails	• Reduced sensitivity • Destructive • Detection is probabilistic	• Potential identification of defect location

Appendix 1

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		

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Probabilistic leak test methods

Test samples

BD Glass Syringes

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

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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**

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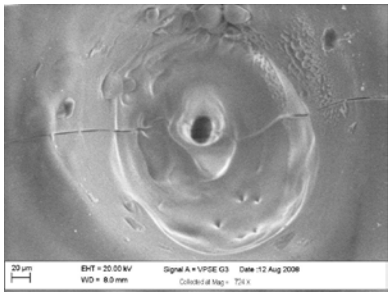
Probabilistic leak test methods

Glass Syringe Defects by Lenox Laser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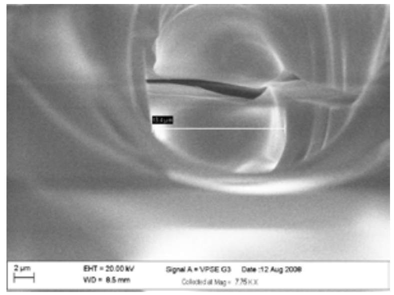

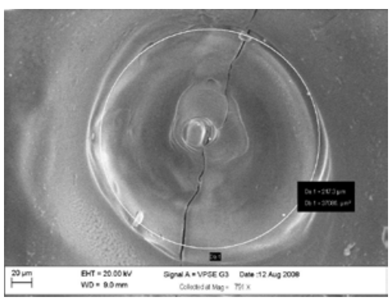
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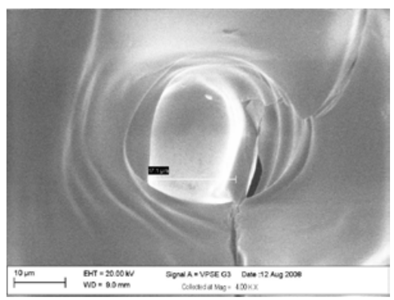
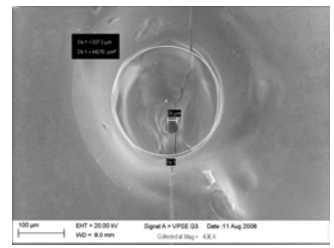


Nominal hole size 5 µm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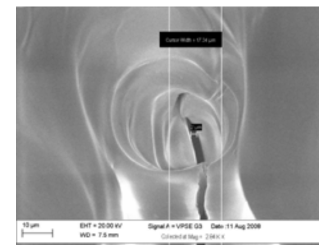
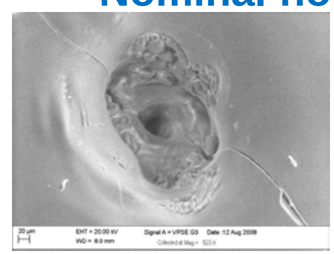


Nominal hole size 10 µm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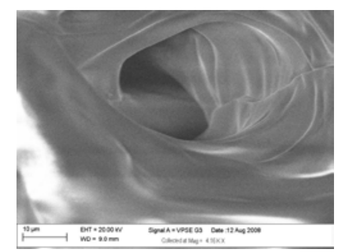

Nominal hole size 15 µm

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Probabilistic leak test methods

Test Samples	USP/Ph.Eur. Dye Test (-27kPa 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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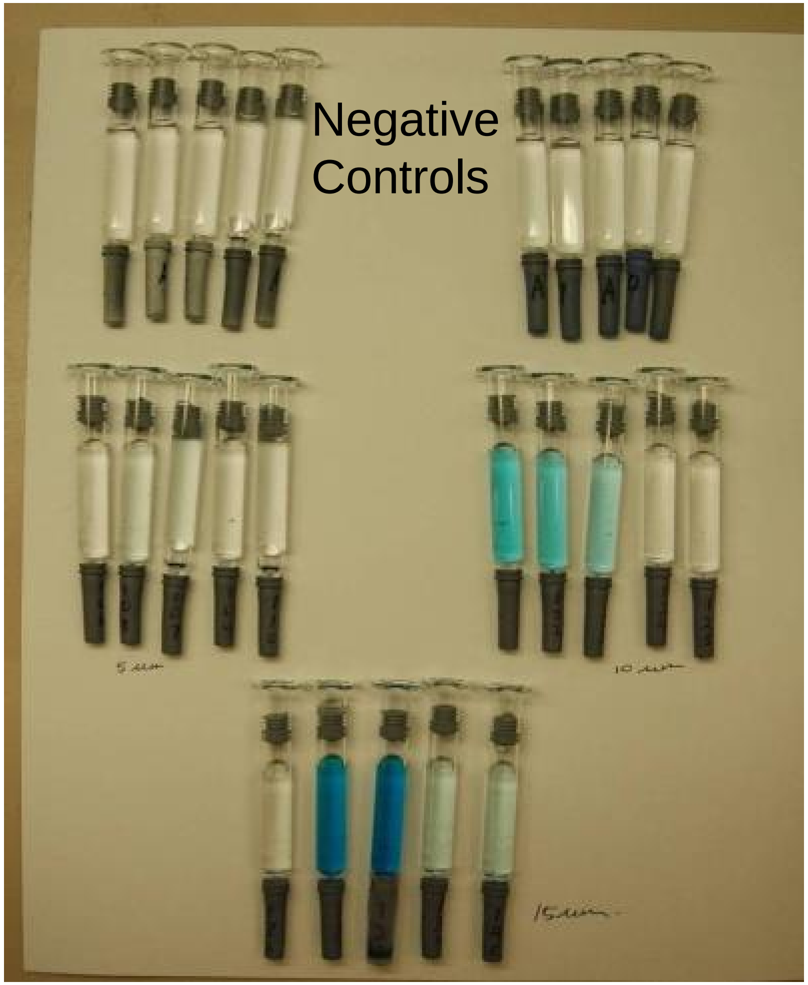
Probabilistic leak test methods

USP/PhEur Dye Ingress Test Samples



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Probabilistic leak test methods

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Test Samples	ISO Dye Test (-25kPa 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

Probabilistic leak test methods

Test Samples	MODIFIED ISO Dye Test (-37kPa 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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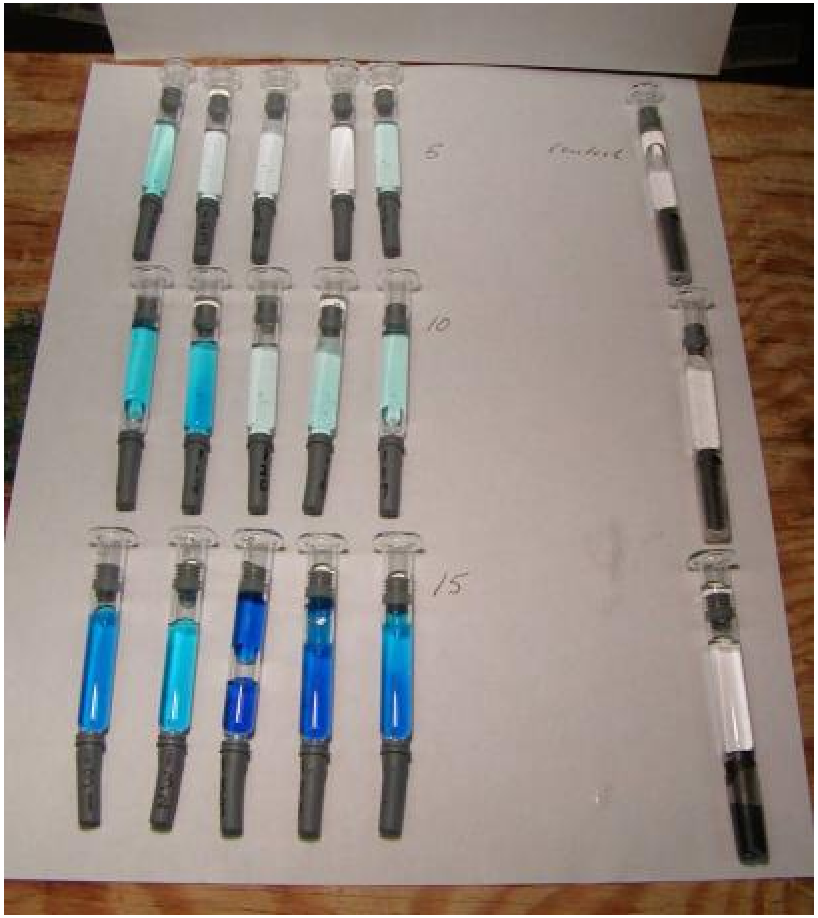
Probabilistic leak test methods

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

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Probabilistic leak test methods

• Compendial and ISO dye ingress methods

• Summary

- 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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Dye test used for seal gap check