Sterile Product Package Integrity Testing

Current Practice, Common Mistakes, New Developments

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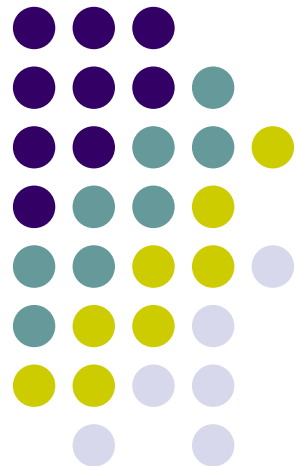
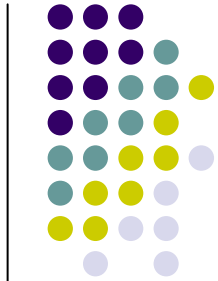


PDA Metro Chapter
May 17, 2010

Sterile Product Package Integrity Testing
Current Practice, Common Mistakes, New Developments



Part 1 Marketed Sterile Products
Package integrity related recalls

Part 2 Dye Ingress Leak Tests
"Best practices"?

Part 3 Best Practices Leak Test Methods
Validation Concepts

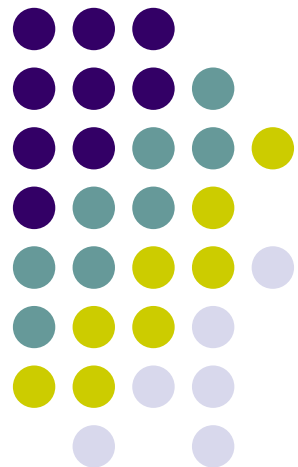
Part 4 Best Practices Leak Test Methods
Proven Nondestructive Methods

Summary

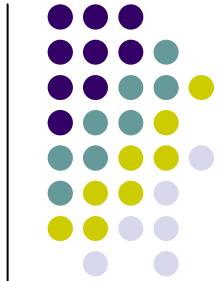
Part 1

Marketed Sterile Products

Package integrity related recalls



Recent Package Integrity Related Recalls



- **PRODUCT**
  AMO COMPLETE Multi-Purpose Solution

- **RECALLING FIRM/MANUFACTURER**
  Recalling Firm: Abbott Medical Optics Inc (AMO), Santa Ana, CA, by letter on July 28, 2010
  Manufacturer: Advanced Medical Optics Manufacturing Spain, S.L., Alcobendas (Madrid), Spain

- **REASON**
  A limited number of the flip top caps used during production of these solutions may leak and, although unlikely, the sterility of the product may be compromised. Products that are non-sterile have the potential to cause eye infections, which may be sight threatening

- **VOLUME OF PRODUCT IN COMMERCE**
  34,224 units

Recent Package Integrity Related Recalls

- **PRODUCT**
  Midazolam Injection, USP, 2 mg/2 mL (1 mg/mL), 10 x 2 mL Single-dose Sterile Cartridge Unit with Luer Lock per carton

- **RECALLING FIRM/MANUFACTURER**
  Recalling Firm: Hospira, Inc., Lake Forest, IL, by letter dated June 29, 2010
  Manufacturer: Hospira, Inc., McPherson, KS

- **REASON**
  Quality procedures were incomplete prior to the release of the product which could result in cracked vials which could compromise the sterility of the product

- **VOLUME OF PRODUCT IN COMMERCE**
  840 cartons

Recent Package Integrity Related Recalls

- **PRODUCT**

  Epinephrine injection, USP, auto-injector

- **RECALLING FIRM/MANUFACTURER**

  Recalling Firm: Shionogi Pharma, Inc., Atlanta, GA, by letter on/about October 28, 2010

  Manufacturers: Hospira, Inc., McPherson, KS; Covidien LP, Deland, FL;

  Phillips Plastics Corp, Phillips Medical, Menomonie, WI

- **REASON**

  Possibility exists a small number of sheaths covering the needle may have pinholes

- **VOLUME OF PRODUCT IN COMMERCE**

  34,629 units

Recent Package Integrity Related Recalls

- **PRODUCT**
  Cancidas (Caspofungin acetate) for Injection, for Intravenous Use, 50 mg

- **RECALLING FIRM/MANUFACTURER**
  Recalling Firm: Merck Sharp & Dohme, West Point, PA, by letter June 7, 2010.
  Manufacturer: Merck & Company, Inc., West Point, PA

- **REASON**
  Lack of Assurance of Sterility (cracked vials)

- **VOLUME OF PRODUCT IN COMMERCE**
  482 vials

Recent Package Integrity Related Recalls

- **PRODUCT**
  Invega syringes, 234mg

- **RECALLING FIRM/MANUFACTURER**
  Recalling Firm: Johnson & Johnson, Feb 15, 2011

- **REASON**
  May have cracks which possibly could affect the drug's sterility. The crack is completely covered by the label and is not detectable by the user

- **VOLUME OF PRODUCT IN COMMERCE**
  70,000 est

Recent Package Integrity Related Recalls

- **PRODUCT**
  Glucagon [rDNA Origin] for Injection, 1mg

- **RECALLING FIRM/MANUFACTURER**
  Recalling Firm: Novo Nordisk, Inc., Princeton, NJ, by letters on November 11, 2010
  Manufacturer: Novo Nordisk A/S, Gentofte, Denmark

- **REASON**
  There is a potential for cracked vials of Glucagon powder within the kit

- **VOLUME OF PRODUCT IN COMMERCE**
  13,698 vials

Recent Package Integrity Related Recalls

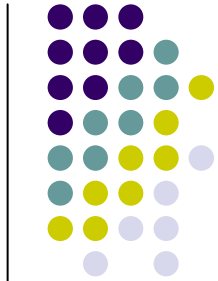
- **PRODUCT**
  Enbrel (etanercept) SureClick Autoinjector, 50 mg/mL, For Subcutaneous Use Only

- **RECALLING FIRM/MANUFACTURER**
  Amgen Manufacturing, Limited, Juncos, PR, by letter on September 14, 2009 and January 18, 2010

- **REASON**
  Syringe barrel flange that slightly deviated from the center line of the syringe barrel, resulted in broken or cracked syringes

- **VOLUME OF PRODUCT IN COMMERCE**
  2,948,741 syringes

Recent Package Integrity Related Recalls



- **PRODUCT**

  0.9% Sodium Chloride Injection, USP, latex free IV bags

- **RECALLING FIRM/MANUFACTURER**

  Recalling Firm: Hospira Inc., Lake Forest, IL, by letter on March 4, 2011 and March 23, 2011

  Manufacturer: Hospira, Inc., Austin, TX

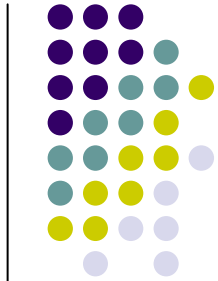
- **REASON**

  The product is being recalled due to defective containers. The bags containing the 0.9% Sodium Chloride Injection, USP solution has the potential to leak. Leaking bags have the potential to result in contamination

- **VOLUME OF PRODUCT IN COMMERCE**

  518,376 bags

Recent Package Integrity Related Recalls



- **PRODUCT**
  Exacta Mix TPN (total parenteral nutrition) Bag

- **RECALLING FIRM/MANUFACTURER**
  Baxa Corp., Englewood, CO, by letter on November 12, 2009 and November 17, 2009

- **REASON**
  TPN bags may leak fluid due to inadequate sealing

- **VOLUME OF PRODUCT IN COMMERCE**
  5,513 cases (US) 353 cases (International)

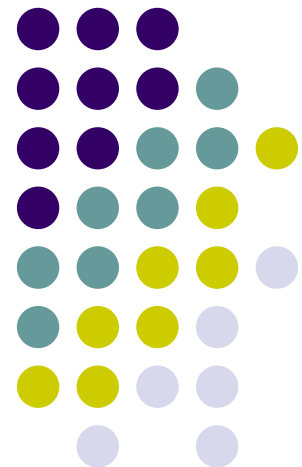
Recent Recalls Summary

- Package integrity related recalls continue to plague industry
- Multiple package types are impacted
  - Syringes, cartridges
  - Vials
  - IV bags
  - Ophthalmic solution bottles
- Current leak testing and package development practices are ineffective in preventing major recalls

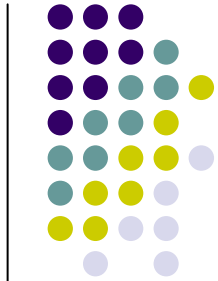
Part 2

Dye Ingress Leak Tests

“Best practices”?



Dye Ingress Tests



- Likely, most common pharma leak test method
- Reliance on dye ingress tests *does not represent "best practices"*
- Why?
  - Lack of validation
    - 'Standard' dye methods – USP/PharmEur, ISO
    - Company-specific methods
  - Validation studies have shown a lack of sensitivity and reliability

For example…

Dye Ingress Method Comparison

Closure Re-seal Method Parameters	USP 31 <381> Ph.Eur. 3.2.9	ISO 8362-5 Annex C	Modified ISO
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, et al, PDA J Pharm Sci & Technol., 63, 2009, p. 489 - 498

Test samples

BD Glass Syringes, 1mL, Staked Needle, Water-filled

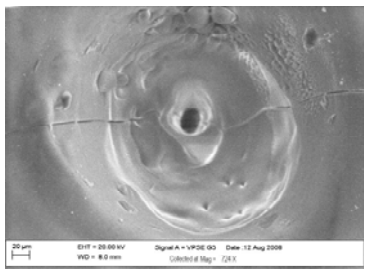
Dye Ingress Method Comparison

- 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
    - **LOD varied from 0.2 to 0.5 ppm**

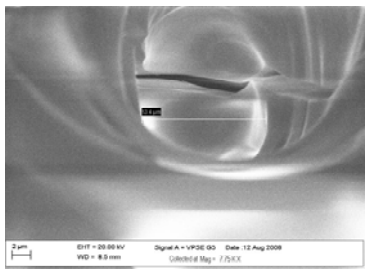
H. Wolf, et al, PDA J Pharm Sci & Technol., 63, 2009, p. 489 - 498

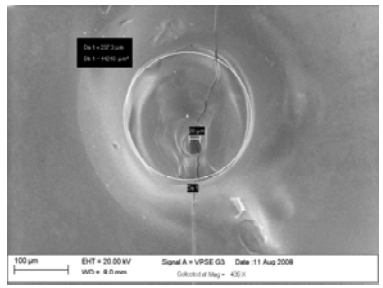
Dye Ingress Method Comparison

Glass Syringe Defects by Lenox Laser



106





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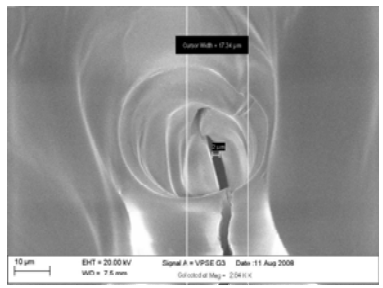
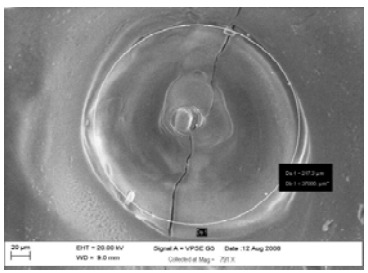


Nominal hole size 10 µm



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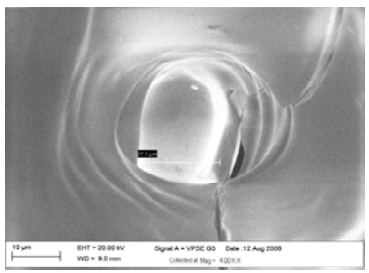

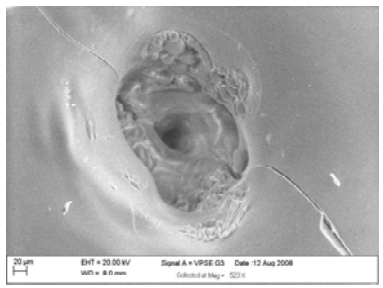

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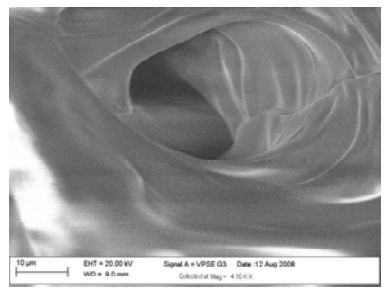


Nominal hole size 5 µm

Nominal hole size 15 µm

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

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

USP/PhEur Dye Ingress Test Samples

Negative Controls

5 µm

10 µm

15 µm

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

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

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

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

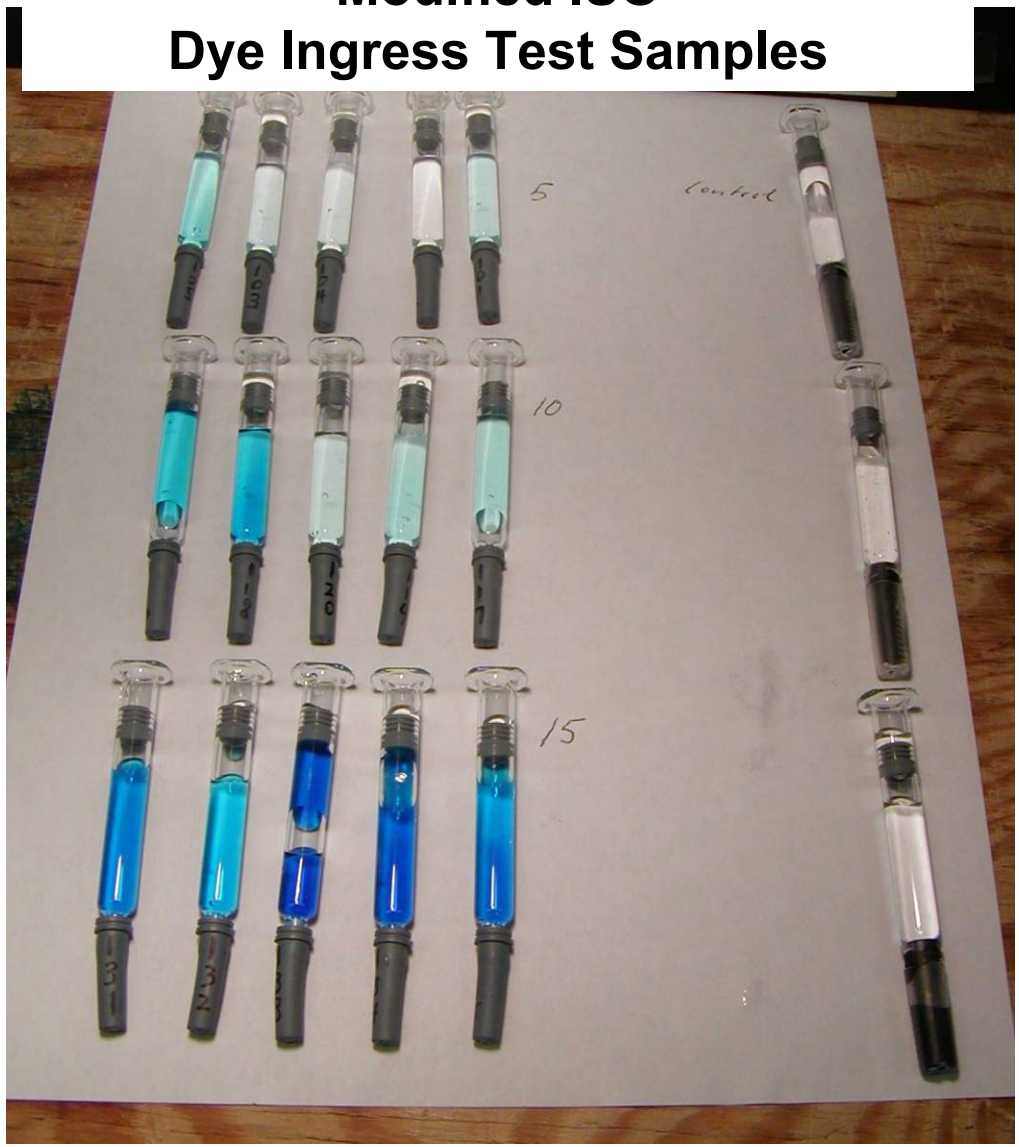
H. Wolf, et al, PDA J Pharm Sci & Technol., 63, 2009, p. 489 - 498

Modified ISO
Dye Ingress Test Samples

5 µm

10 µm

15 µm



Negative Controls

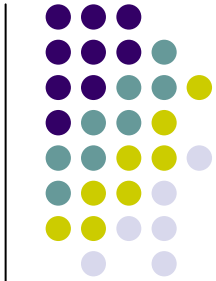
H. Wolf, et al, PDA J Pharm Sci & Technol., 63, 2009, p. 489 - 498

Dye Ingress Tests

- Comparison study observations
  - Inspector capabilities varied
  - ‘Standard’ inspection conditions not defined
  - ‘Standard’ methods lacked sensitivity, reliability
  - ‘Optimized’ method resulted in > false positives

No dye ingress advantages reported

Dye Ingress Tests



- Other disadvantages
  - False negative risks
    - Proteins 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

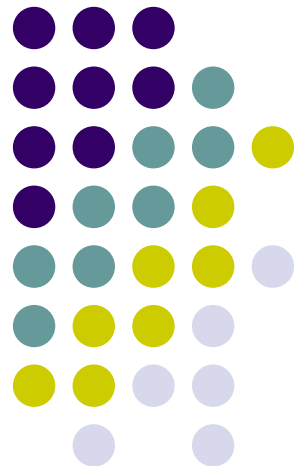
Dye Ingress Tests

- *Any* advantages?
  - Useful for gross leak detection
  - Useful as a lab tool for leak visualization, location

Part 3

Best Practices Leak Test Methods

Validation Concepts

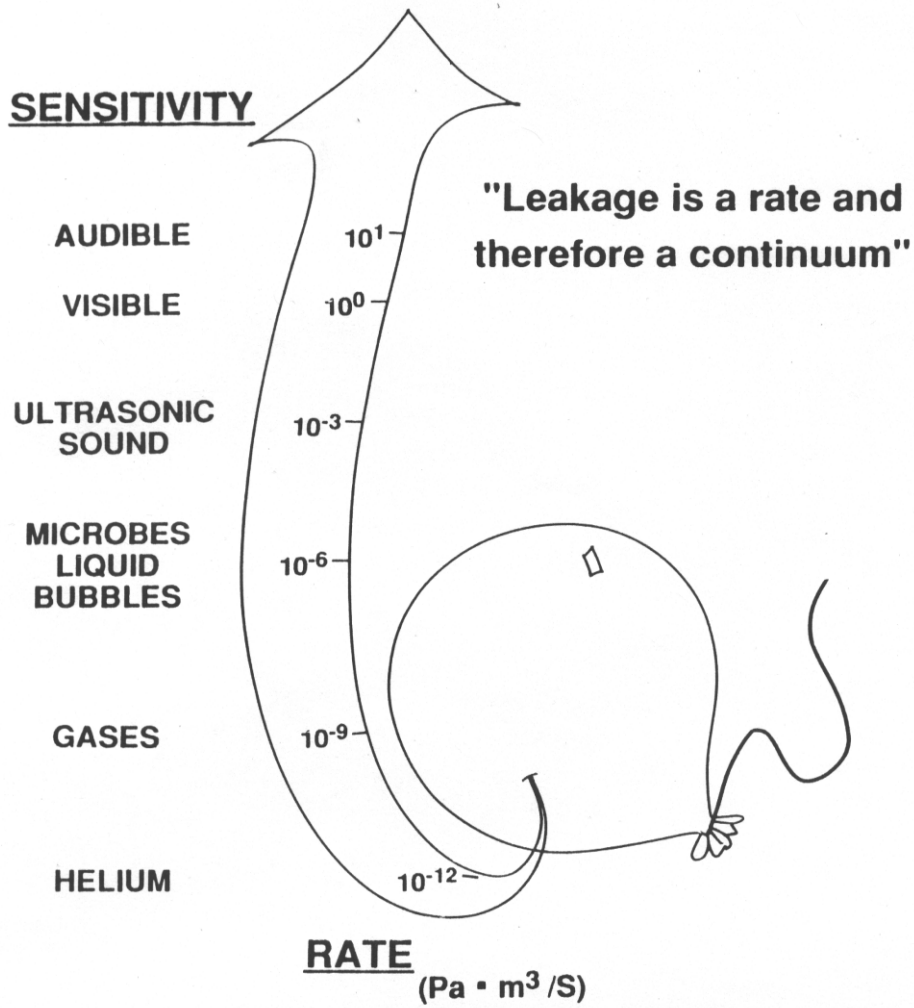


Best Practice Leak Test Methods

Meet validation criteria

- Sensitive
  - Proven using various *defect types* and *sizes*
- Reliable
  - Proven using a random mix of *positive (with-leak) and negative (no-leak) controls*

Therefore, positive control test samples with leaks of appropriate size and type are required



D. Guazzo, "Package Integrity Testing" Chapter 4, *Parenteral Quality Control*, 2nd Ed.,Marcel Dekker, NYC, 1994

Critical Leak Spec

- Sterile product “critical leak” rate or defect size
  - Risks microbial ingress

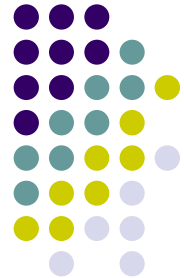
    → ***sterility loss***
  - Loss of critical headspace gases

    → ***instability***
  - Loss of headspace vacuum

    → ***instability***

    → ***product access difficulty***

Sterility Assurance Critical Leak Spec



- *Published Study* **Lee Kirsch,** et al 1997- 99
  - Glass micro-pipettes through wall of stoppered glass vial
    - Sized via helium mass spec
    - 0.1 to 10µm diameter
  - Microbial challenge by immersion + liquid tracer element
    - 10^8 to 10^{10} *P. diminuta* and *E. coli* cfu/mL
    - Tween 80 additive
    - Mg ion tracer for liquid path verification
      - Detection by atomic absorption
  - Challenge conditions
    - Airlock elimination procedure
      - Water bath immersion 60ºC 2hr, then 25ºC 1hr
    - 24 hr immersion, ambient pressure

Kirsch vial test unit

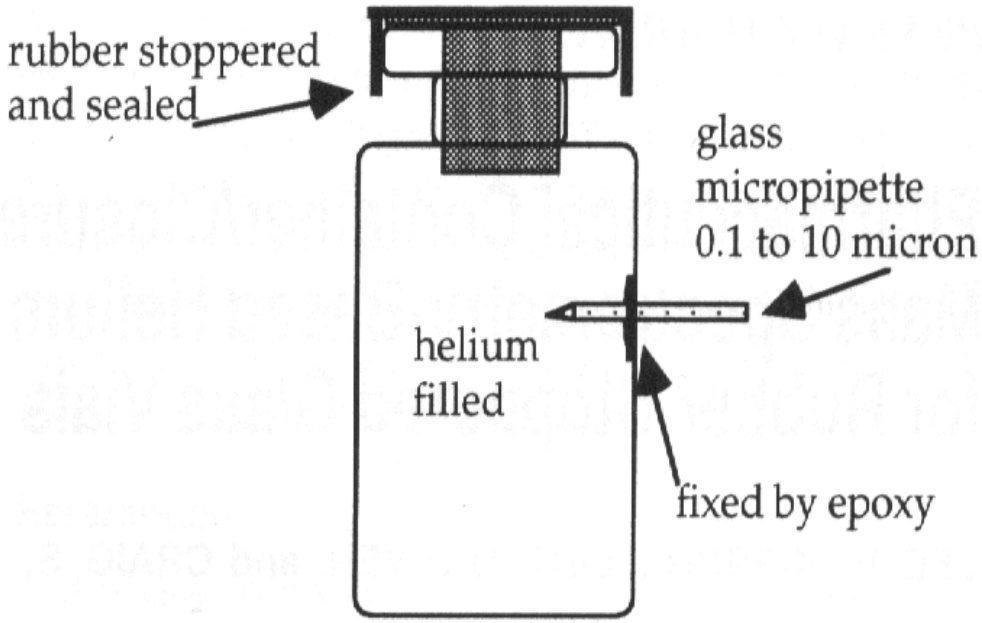


Figure 1—Schematic description of the modified pharmaceutical vials used as test units for the evaluation of mass spectrometry-based helium leak rate measurements.

Kirsch, et al, *PDA J Pharm Sci & Technol* 51, 5, 1997 p. 188

Microbial ingress vs. Micro-pipette diameter vs. Helium leak rate

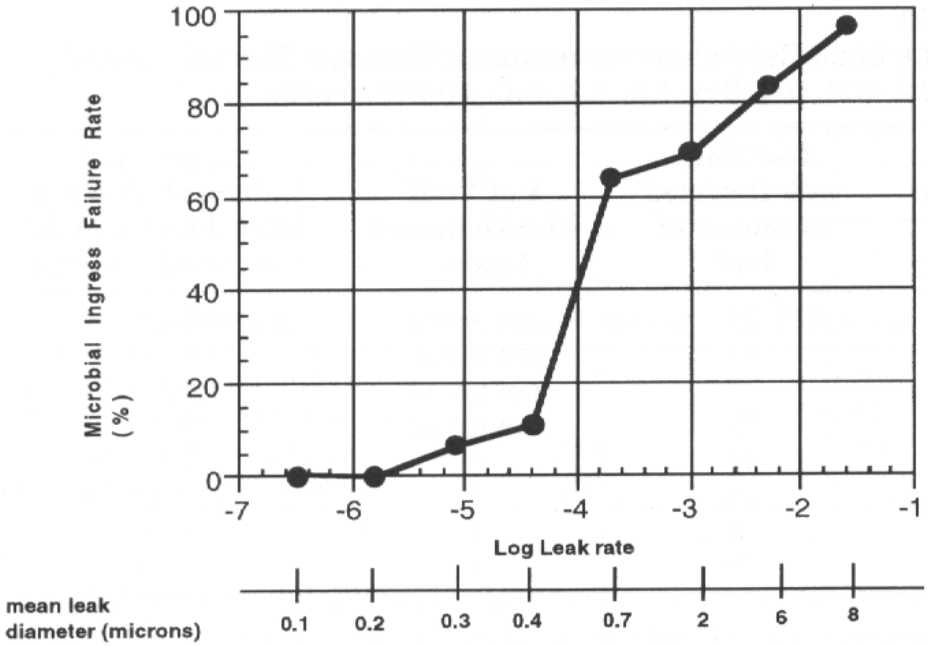


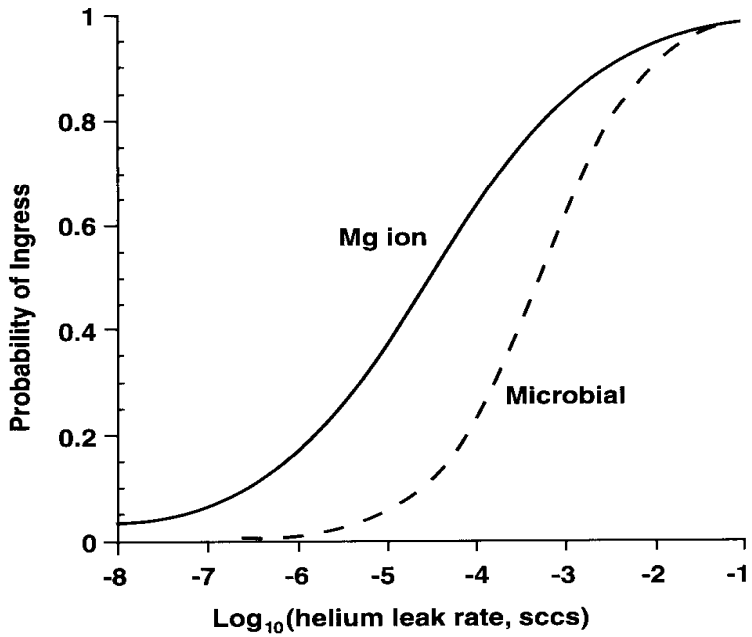
Figure 2—The correlation of microbial failure rate (%) and the mean logarithm of the absolute leak rate and nominal leak diameter for modified SVPs. The absolute leak rate (standard cubic centimeters per second) was determined by mass spectrometry-based helium leak rate detection. Microbial failure was measured by microbial ingress after 24 hour immersion in a bath (37°C) containing 10^8 to 10^{10} *P. diminuta* and *E. coli* organisms/mL and a 13 day, 35°C incubation.

- Ingress risk dropped dramatically
  - Log -3.8 sccs
  - < ~1μm
- No ingress
  - Log -5 to -5.8 sccs
  - ~0.3 to 0.2μm

Kirsch, et al, *PDA J Pharm Sci & Technol* 51, 5, 1997 p. 200

Liquid vs. Microbial ingress vs. Helium leak rate

Figure 1: Logistical regression models describing the probability of microbial or liquid tracer (Mg ion) as a function of the logarithm of the helium leak rates. Curves were generated using Equation 1 and parameters estimated with the logistical regression platform in the software JMP (10).



- Microbial ingress *required* liquid flow
  - > Liquid flow =
    > microbial ingress *risk*
- Liquid flow ≠ microbial ingress

Kirsch, *PDA J Pharm Sci & Technol* 54, 2000 p. 309

Sterility Assurance Critical Leak Spec

Study Author	Challenge medium	Challenge microbe	Challenge path	Challenge conditions	Threshold path size
Kirsch JPDA '97-'99	Liquid	*P. diminuta* *E. coli*	Glass micro-pipette	Airlock elimination step + 24 hr ambient	0.3 μm
Burrell JPDA 2000	Liquid	*E. Coli*	Poly-coated glass micro-tube	ISO closure reseal: 30 min 22"Hg + 30 min ambient	10 μm
Keller *J Applied Pkgg Res 2006*	Aerosol	*P. Fragi*	Nickel micro-tube	Varied: -20 kPa to +20 kPa 4 to 37ºC	5 μm

- "Critical leak" threshold ranged from *0.3 to 10μm*
- Leak path *liquid presence* is required for microbial ingress
  - > Liquid flow = > microbial ingress *potential*
    - Liquid presence *does not guarantee* microbial ingress
  - Liquid presence may be more important than *challenge medium*

Sterility Assurance Critical Leak Spec

- Critical leak spec remains undefined for "real leaks"
  - Real leak paths are *not holes, tubes, pipettes*
    - Natural defects are long, complex, irregular channels
    - Defects consist of actual package materials
  - Air pockets, debris, even product may *block flow*

Positive Control Leakage Behavior

- *Published Study* **Bradley Morrical, et al** 2007
  - Leakage of two leak types compared
    - Glass vial packages
      - **Micro-hole** in metal plate on stopper 0.5 to 15 µm
      - **Copper wire** between stopper and vial 10 to 120 µm
    - Leak methods
      - Helium trace test — Mass spectrometry
      - Microbial challenge
        - *Serratia marcenscens* — ≥ 10^8 cfu/mL
        - Vacuum — - 0.4 bar 1 hr
        - Pressure — + 0.4 bar 1 hr

Morrical vial test unit with micro-hole

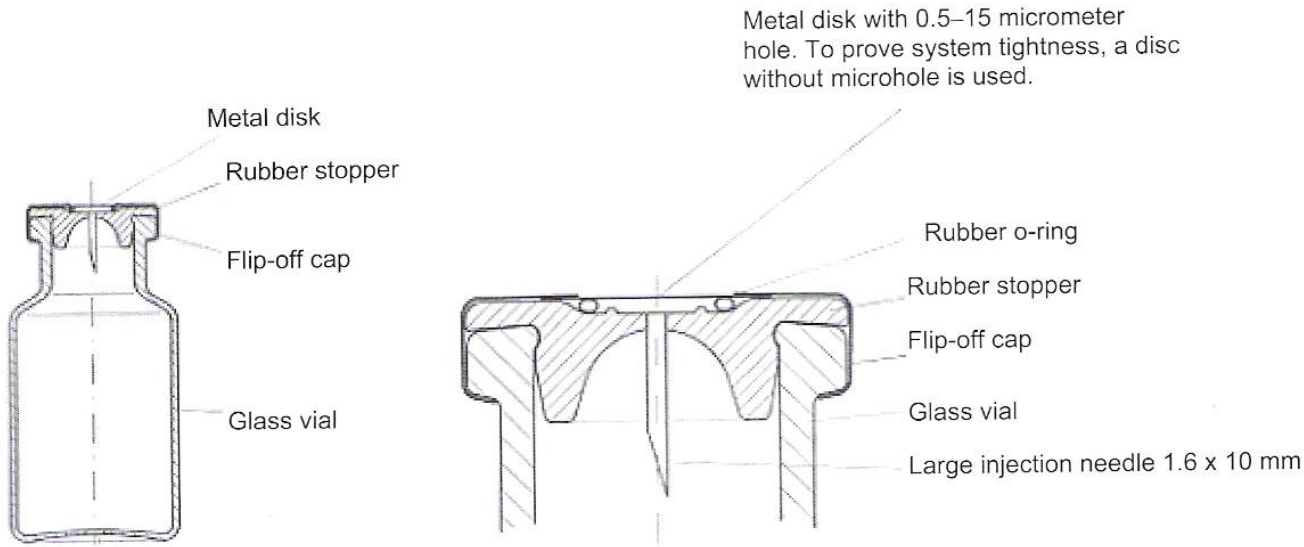


Figure 3

Schematic of vial with microhole. The use of an injection needle to penetrate the rubber stopper ensured the leak was only due to the microdrilled hole. A small o-ring provides a proper seal on the backside of the microhole.

Morrical, et al, *PDA J Pharm Sci & Technol* 61, 2007 p. 226 – 236

Morrical vial test unit with wire leak

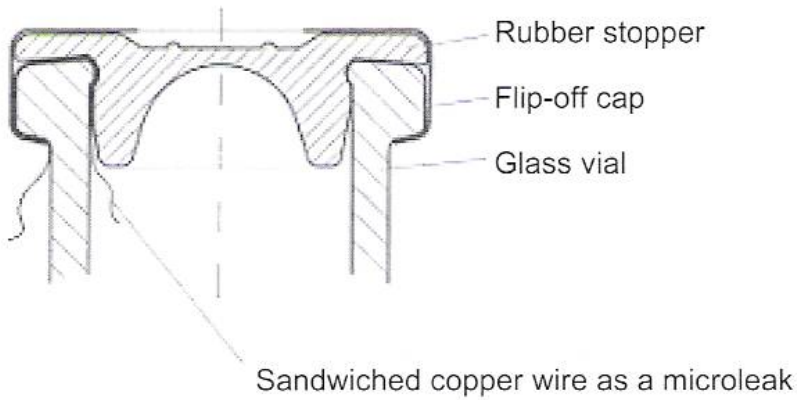


Figure 4

Schematic of vial with copper wire as a microleak. Wire was laid carefully over the rim of the glass vial and visually inspected to ensure the wire remained intact after sealing.

Morrical, et al, *PDA J Pharm Sci & Technol* 61, 2007 p. 226 – 236

Morrical He+ mass spec test fixture

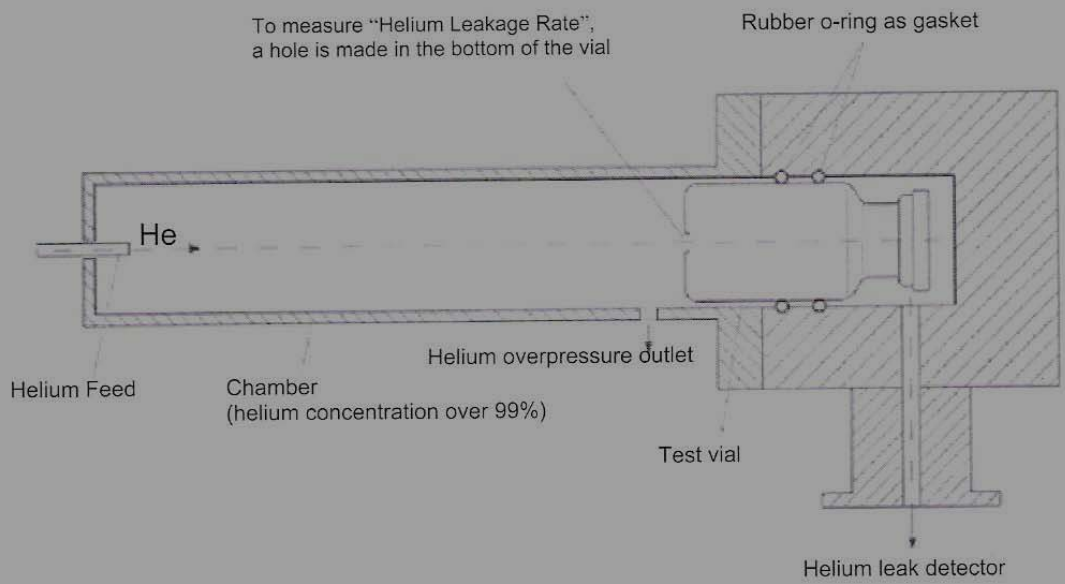


Figure 1

Helium leak rate test apparatus for glass vials. Helium is flowed in through a tube and an outlet maintains ambient pressure. The rubber o-ring seals isolate the test leak from the helium inlet. Measurement is made with a mass spectrometric helium leak detector.

Morrical, et al, *PDA J Pharm Sci & Technol* 61, 2007 p. 226 – 236

Positive Control Leakage Behavior

Defect type	Defect size (µm)	He+ leak rate (mbarL/s)	Microbial ingress observed (%)
Hole	1	4.8 log -4	0
	2	1.4 log -3	0
	4	6.1 log -3	20
	8	2.8 log -2	30
	15	9.3 log -2	90
Wire*	15	1.3 log -5	0
	20	2.2 log -5	35
	28	1.5 log -4	85
	40	1.6 log -3	95
	60	5.3 log -3	100

* Data represent 'machine-sealed' units. See reference for 'hand-sealed' data

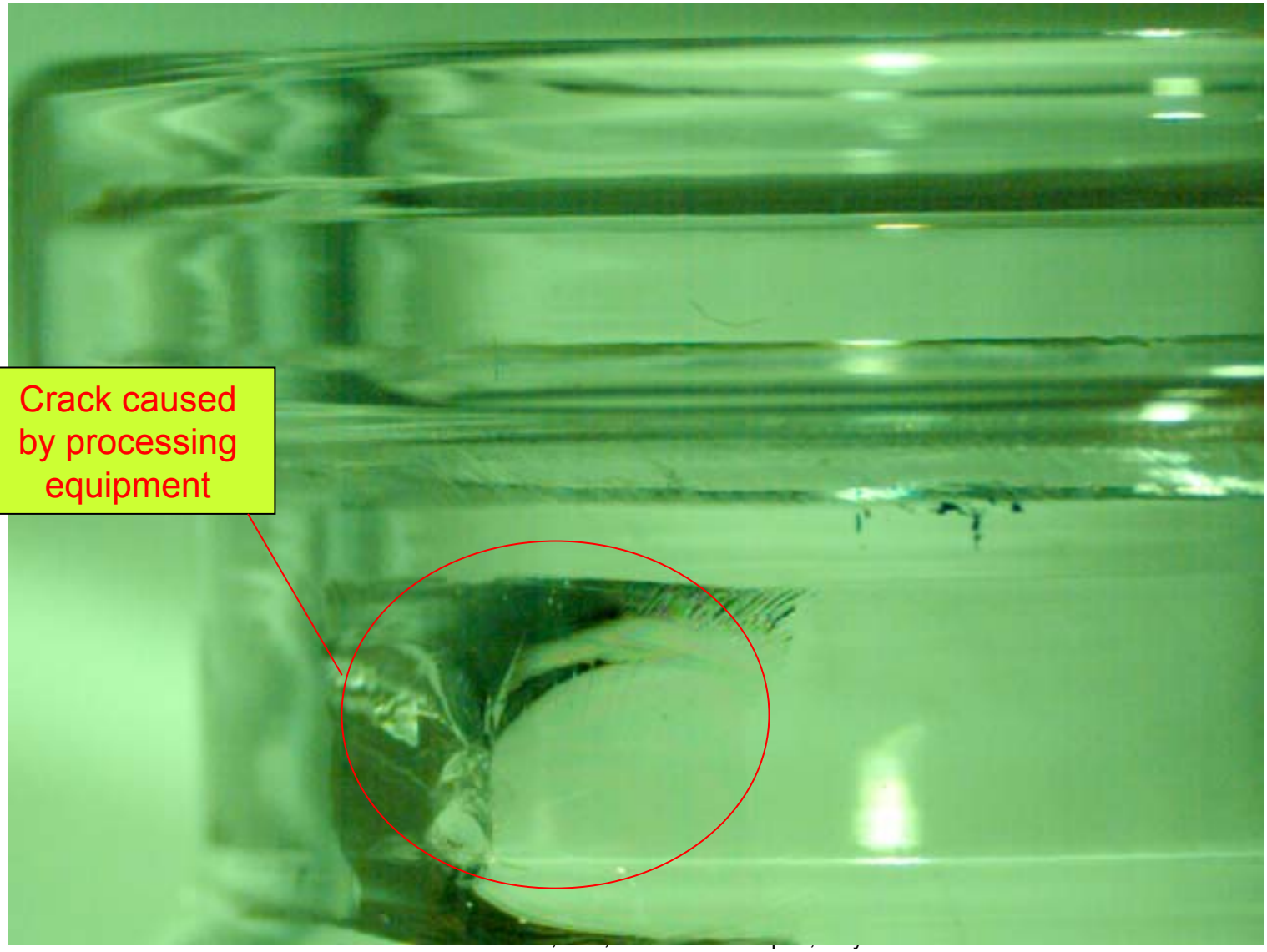
- **Holed vial** helium flow matched theoretical predictions for orifice
- **Wired vial** helium flow followed less predictable, more complex dynamics

Morrical, et al, *PDA J Pharm Sci & Technol* 61, 2007 p. 226 – 236

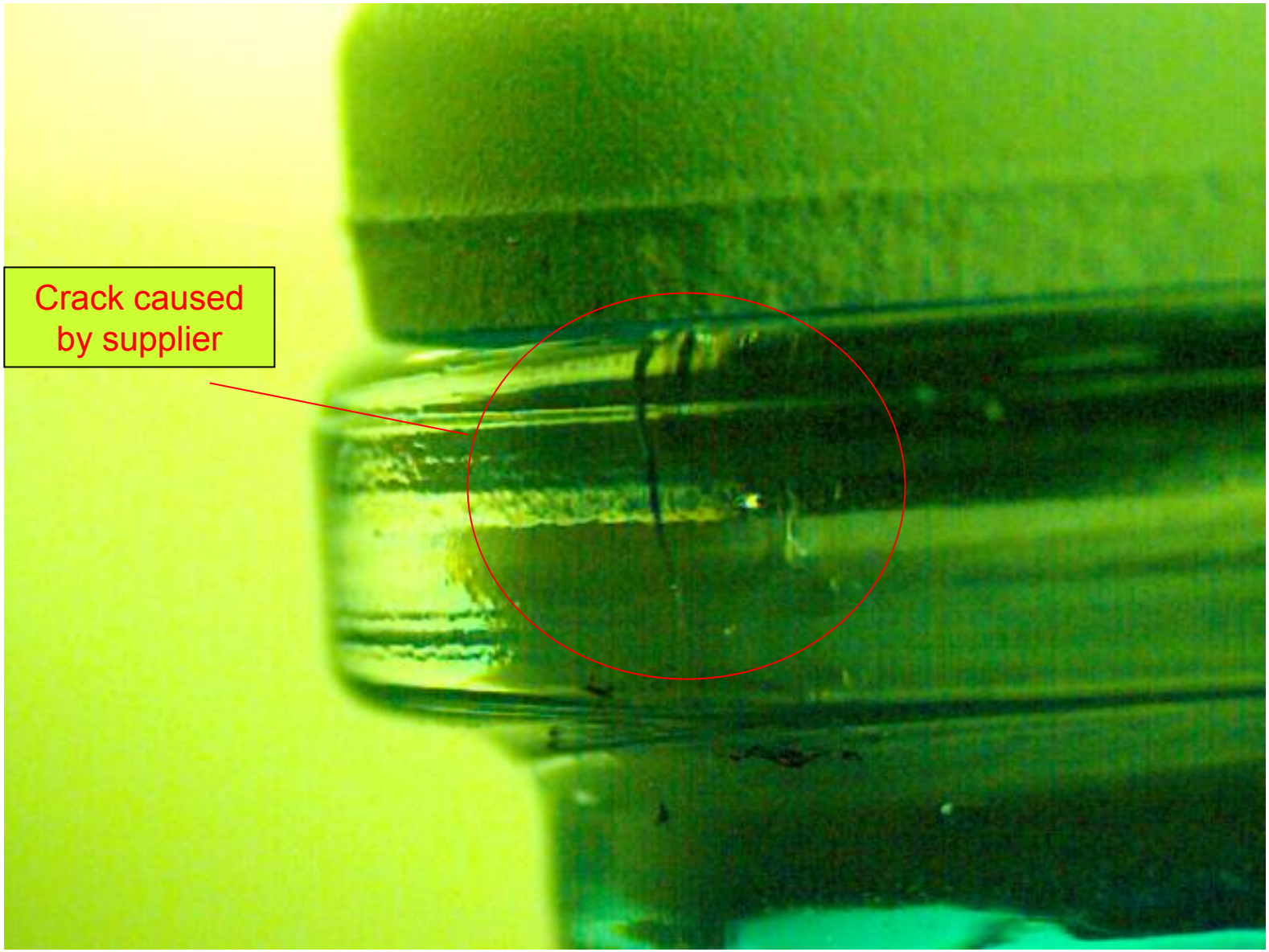
Positive Control Recommendations

- **Laser-drilled holes**
  - Benefit
    - Closely simulates package wall crack, pinhole
    - Product and package impact on leak detection checked
  - Size
    - ≥ 5 μm for most materials (plastic, glass, films)
      - May vary according to material and wall thickness
      - Smaller sizes difficult to create, certify and readily clog
  - Location
    - Above and below product-fill level
    - As close to critical seal area as possible

Naturally Occurring Defects



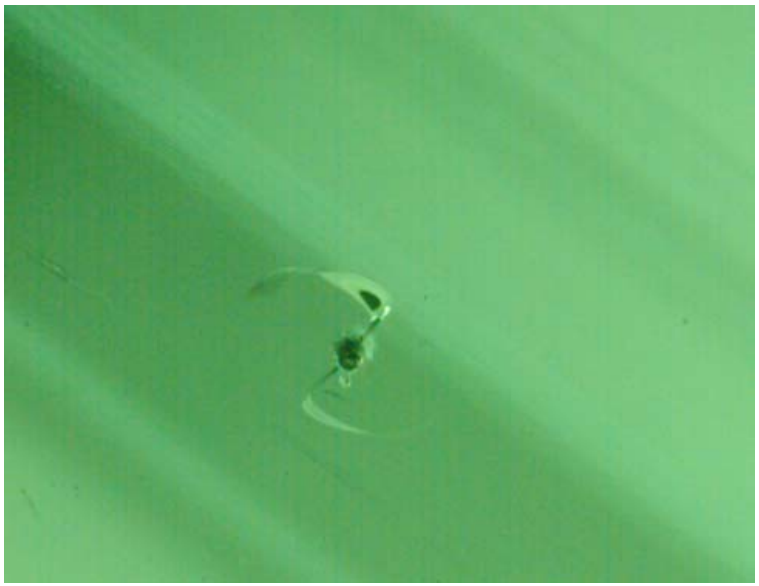
Naturally Occurring Defects



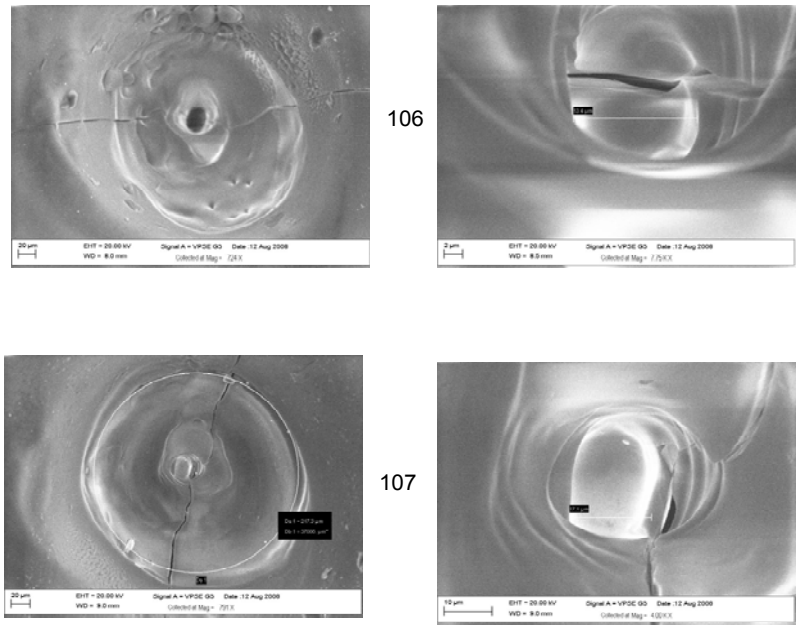
Positive Control Example

Glass Syringe Defects by Lenox Laser

Nominal hole size 5 µm



Microscope photo by BMS



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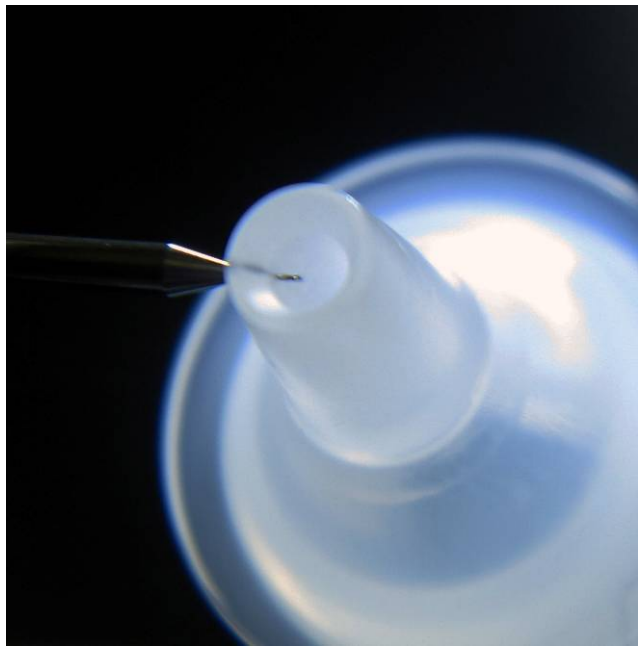
Electron-microscope photo by Amgen

Positive Control Recommendations

- **"Type defects"**
  - Examples
    - Loose cap, damaged stopper
    - Scored land sealing surface
    - Gap or channel in heat seal
    - Needle protruding through needle-shield
  - Benefit
    - Verifies ability of CCI method to find defects likely to occur
    - Greatest benefit during method development studies
  - Size
    - Exact sizing may not be feasible
    - 'Type' defects are often 'large' leaks

Ironically, larger defects are the cause for product recalls

Positive Control Type Defect Example

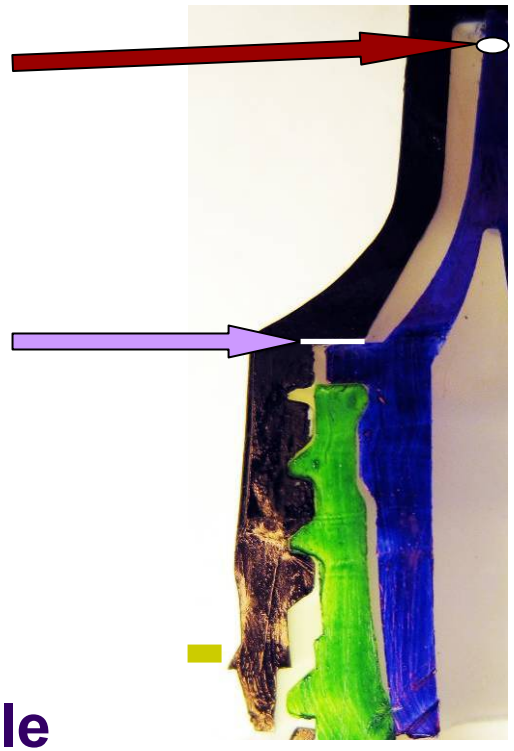


Hole creation
0.10 – 0.16 mm

Positive Control Type Defect Example

Hole defect

Channel defect



Screw capped bottle with application insert

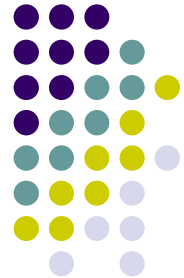
Positive Controls are *NOT* LOD Standards

- Positive controls
  - Product-filled with-defect packages
  - Used to verify actual leaking package detection capability
- Limit of detection standards
  - A known, fixed standard
  - Evaluates instrument detection capability under *ideal* conditions

Positive Controls are *NOT* LOD Standards

Test	Method LOD Standard
Microbial ingress	Growth promotion test
Dye ingress	Minimum detectable dye concentration
Vacuum decay	• Minimum detectable NIST airflow rate • Smallest detectable in-line fixed orifice
High voltage leak detection (HVLD)	Minimum detectable voltage
Helium mass spectrometry	Standard Helium flowmeter detection limit
Frequency modulation spectroscopy (FMS)	Minimum detectable oxygen concentration or partial pressure

Negative Control Recommendations

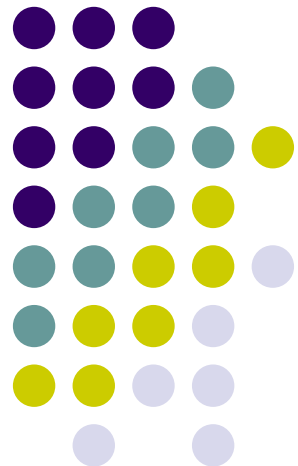


- No-leak packages
  - Ideally, normal distribution is represented
    - Assembly operations
    - Component fit
    - Multiple sources or lots
  - Product- or placebo-filled

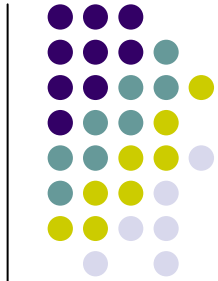
Part 4

Best Practices Leak Test Methods

Proven Nondestructive Methods



Proven Nondestructive Methods



- "Proven"

  Validation and suitability supported by data in peer-reviewed publications

- Test methods

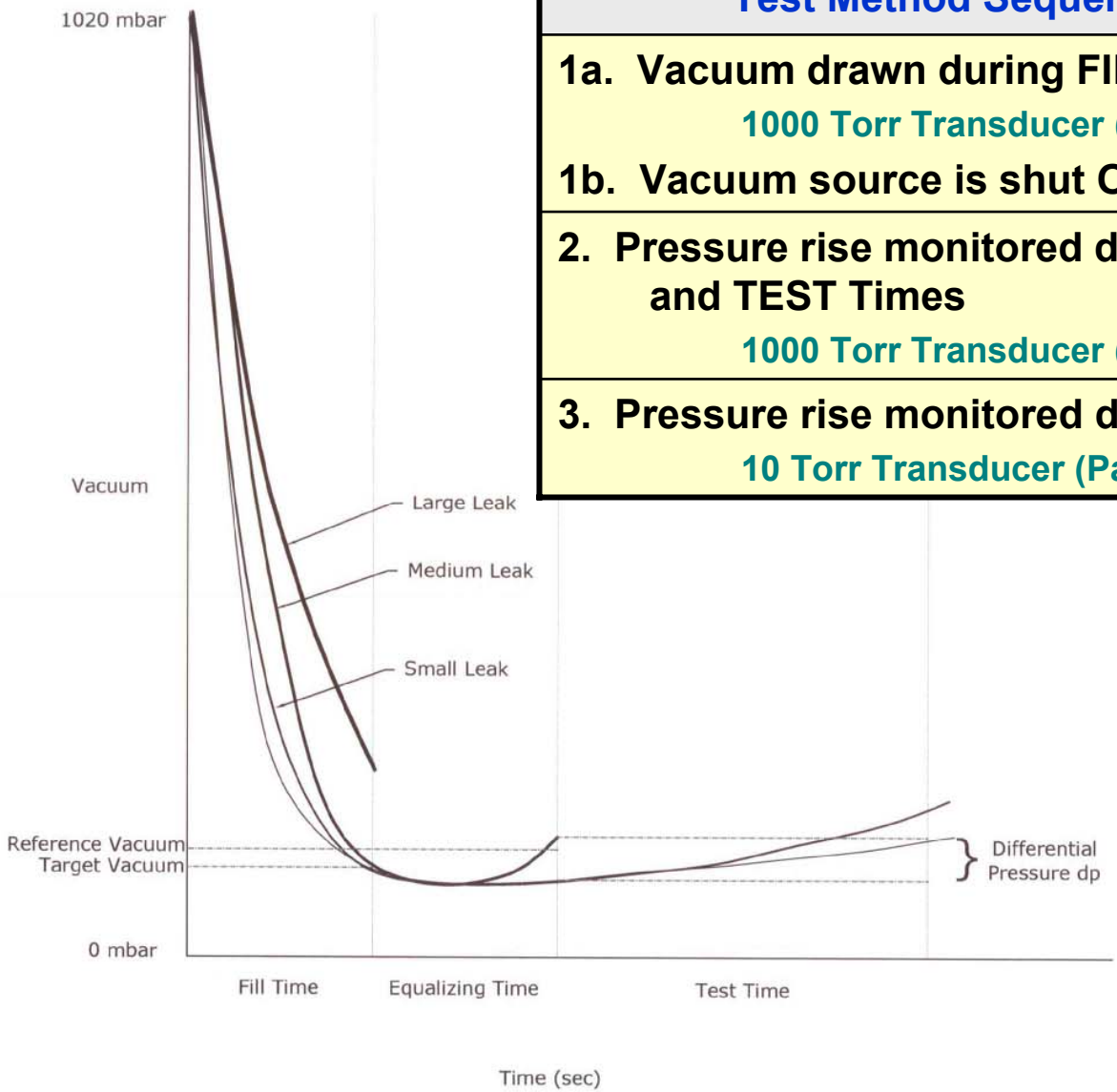
  1. Vacuum decay
  2. High voltage leak detection (HVLD)
  3. Laser-based headspace detection (FMS)

1. Vacuum Decay

- For dry or liquid products, most package systems
- Detects pressure rise from gas or vapor egress
- Limitations
  - Protein clogging often prevents leak detection
  - Liquid leaks may contaminate test chamber
- Considerations
  - Faster tests limit sensitivity
  - Instrument design/make can influence test results
    - Transducers and internal system design
    - No-leak baseline stability

ASTM F2338-09

Test Method Sequence of Events
1a. Vacuum drawn during FILL Time 1000 Torr Transducer (mbar) 1b. Vacuum source is shut OFF
2. Pressure rise monitored during EQUALIZATION and TEST Times 1000 Torr Transducer (mbar)
3. Pressure rise monitored during TEST Time 10 Torr Transducer (Pa)



ASTM F2338-09 Round Robin Study

- Packages — 1mL glass syringes by BD
- Positive controls — Laser-drilled holes 5, 10, 15 µm
- Vacuum decay tests
  - **Study 3** — NIST calibrated airflow meter
  - **Study 4** — Air-filled syringes
  - **Study 5** — Water-filled syringes
- Logistics
  - 3 Test sites — Amgen, BMS, PTI
  - 3 Instruments — PTI VeriPac 325-LV
  - 3 Replicates of ea. study at ea site, 2 days per site
  - Samples randomized within ea. study

H. Wolf, et al, PDA J Pharm Sci & Technol., 63, 2009, p. 477 - 488

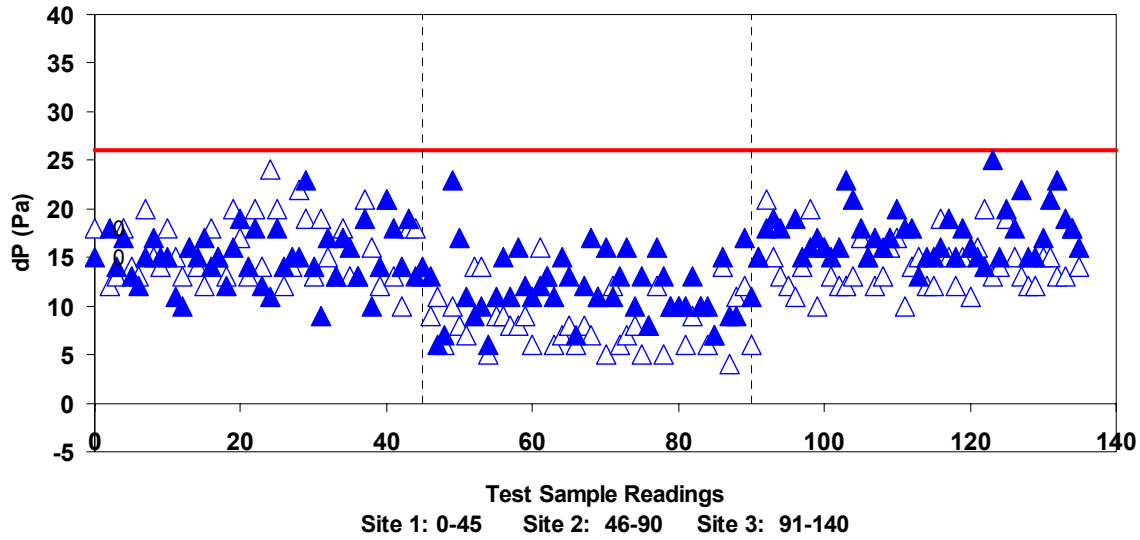
ASTM F2338-09
Vacuum decay test parameters

Leak test parameters	Parameter limits
Evacuation (Fill) time	6 s
Equalization time	0.2 s
Test time	8 s
Pressure rise reference limit 1000 Torr transducer	2 mbar (abs)
Pressure rise reference limit 10 Torr transducer	25 Pa (differential)

Test instrument by Packaging Technologies & Inspection, LLC
Model PTI VeriPac 325/LV

H. Wolf, et al, PDA J Pharm Sci & Technol., 63, 2009, p. 477 - 488

Vacuum decay
Negative control syringes

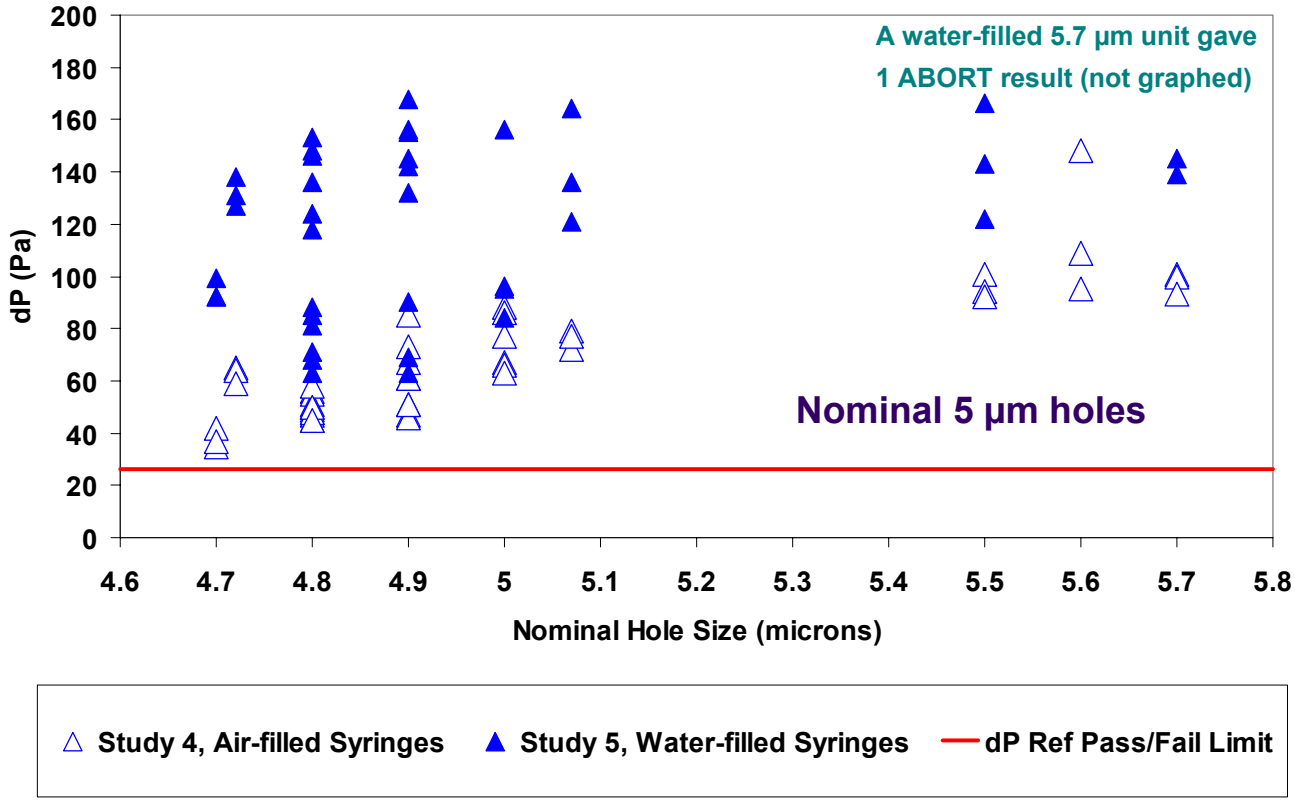


H. Wolf, et al, PDA J Pharm Sci & Technol., 63, 2009, p. 477 - 488

Study	No. Packages Tested	No. Tests	No. FAILED	No. PASSED	% Accurate
Study 4: Water	**15**	**135**	**0**	**135**	**100**
Study 5: Water	**15**	**134**	**0**	**134**	**100**

Vacuum decay

Positive control syringes
Air- vs. water-filled

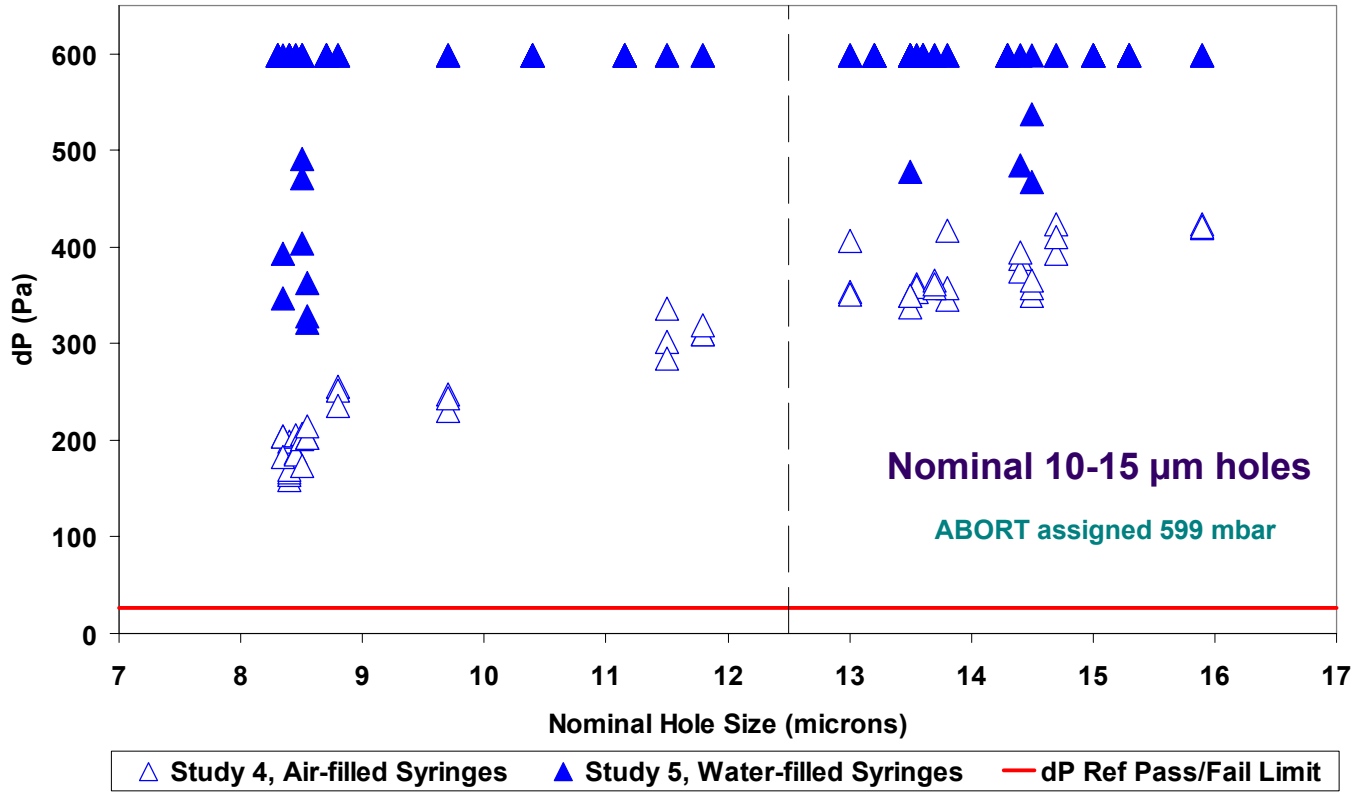


H. Wolf, et al, PDA J Pharm Sci & Technol., 63, 2009, p. 477 - 488

Syringe Contents	No. Packages Tested	No. Tests	No. FAILED	No. PASSED	% Accurate
Study 4: Air	15	45	45	0	100
Study 5: Water	15	45	45	0	100

Vacuum decay

Positive control syringes
Air- vs. water-filled



Syringe Contents	No. Packages Tested	No. Tests	No. FAILED	No. PASSED	% Accurate
Study 4: Air	30	90	90	0	100
Study 5: Water	30	90	90	0	100

Test Samples	Air-filled Syringe Vac decay dP (Pa) Pass or Fail	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	11	No	No	No
	10	No	No	No
	12	No	No	No
	9	No	No	No
	9	No	No	No
5 µm	25 (4.7 µm)	No	No	Yes
	71	No	Yes	Yes
	80	No	Yes	Yes
	43	No	No	No
	42	No	No	Yes
10 µm	217	Yes	Yes	Yes
	177	Yes	Yes	Yes
	264	Yes	Yes	Yes
	231	No	No	Yes
	161	No	No	No
15 µm	ABORT	No	No	Yes
	344	Yes	Yes	Yes
	342	Yes	Yes	Yes
	350	Yes	Yes	Yes
	281	Yes	Yes	Yes

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

Test Samples	Air-filled Syringe Vac decay dP (Pa) Pass or Fail	ISO Dye Test (-25kPa 30 min, amb 30 min) YES (Dye visible) or NO (Not visible)		
		Inspector 1	Inspector 2	Inspector 3
Negative Controls	7	No	No	No
	6	No	No	No
	7	No	No	No
	6	No	No	No
	7	No	No	No
5 µm	22 (4.7 µm)	No	No	No
	66	No	No	Yes
	79	No	Yes	Yes
	44	No	No	Yes
	42	No	No	No
10 µm	205	Yes	Yes	Yes
	175	Yes	Yes	Yes
	260	Yes	Yes	Yes
	221	No	No	Yes
	154	No	No	No
15 µm	388	Yes	Yes	Yes
	346	Yes	Yes	Yes
	335	Yes	Yes	Yes
	337	Yes	Yes	Yes
	301	Yes	Yes	Yes

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

Test Samples	Air-filled Syringe Vac decay dP (Pa) Pass or Fail	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	9	No	Yes	No
	9	No	Yes	No
	10	No	No	Yes
	9	No	Yes	Yes
	17	Yes	No	No
5 µm	57	Yes	Yes	Yes
	96	Yes	Yes	Yes
	43	Yes	Yes	Yes
	41	Yes	Yes	Yes
	51	Yes	Yes	Yes
10 µm	ABORT	Yes	Yes	Yes
	191	Yes	Yes	Yes
	ABORT	Yes	Yes	Yes
	ABORT	Yes	Yes	Yes
	188	Yes	Yes	Yes
15 µm	ABORT	Yes	Yes	Yes
	ABORT	Yes	Yes	Yes
	ABORT	Yes	Yes	Yes
	ABORT	Yes	Yes	Yes
	ABORT	Yes	Yes	Yes

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

2. High Voltage Leak Detection

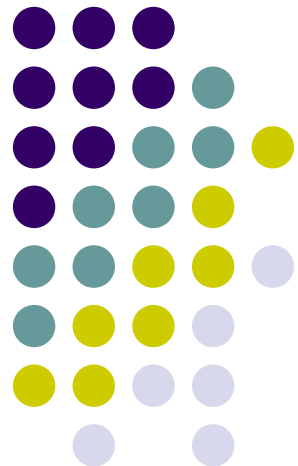
- For nonflammable conductive liquid product in electrically insulating package
  - small molecule or proteinaceous active
- Detects liquid present near leak path
- Fast, clean test method
- Considerations
  - Method-product compatibility to be checked
  - Whole package vs. spot location checks
  - Package rotation to capture leaks in headspace region
  - Instrument make/design can influence test results

Glass Vial Finish Defects

Leak detection and product risk assessment

Stephen T. Orosz, Jr. PhD
ImClone Systems
a wholly-owned subsidiary of Eli Lilly & Co.
Branchburg, NJ

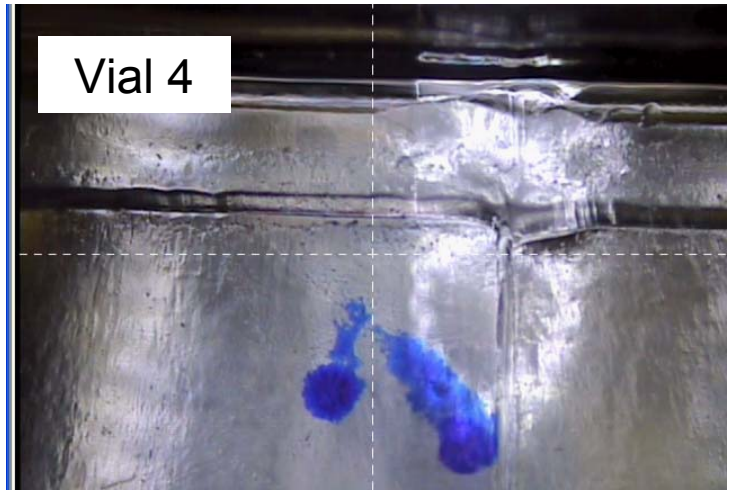
Dana Morton Guazzo, PhD
RxPax, L.L.C. Bridgewater, NJ
WHITEHOUSE ANALYTICAL LABORATORIES, LLC Whitehouse, NJ

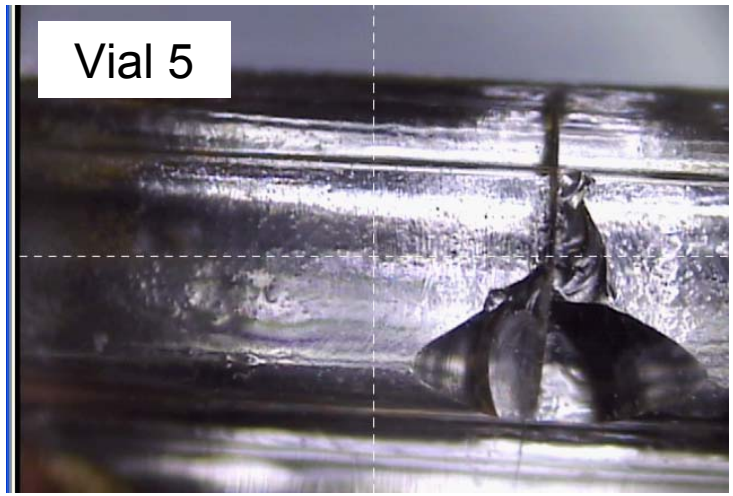


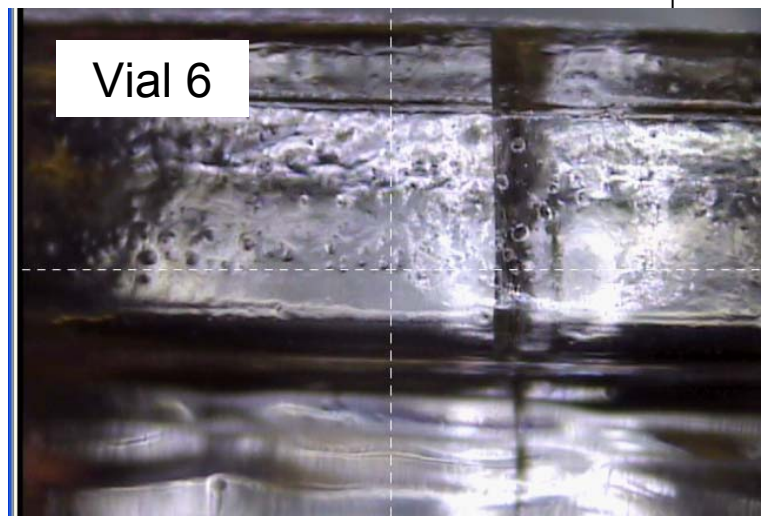
PDA Annual Meeting, Packaging Science Interest Group
March 16, 2010 Orlando, FL

Glass Vial Finish Defects Study

- Challenge
  - 50-mL 20-mm molded glass vials with finish defects
- Project scope
  - ID defects sources, risk of propagation and leakage
  - ID a nondestructive leak test able to find such defects in finished product packages
    - Aqueous solution formulations
    - 20mm elastomeric serum stopper
    - 20mm aluminum flip seal

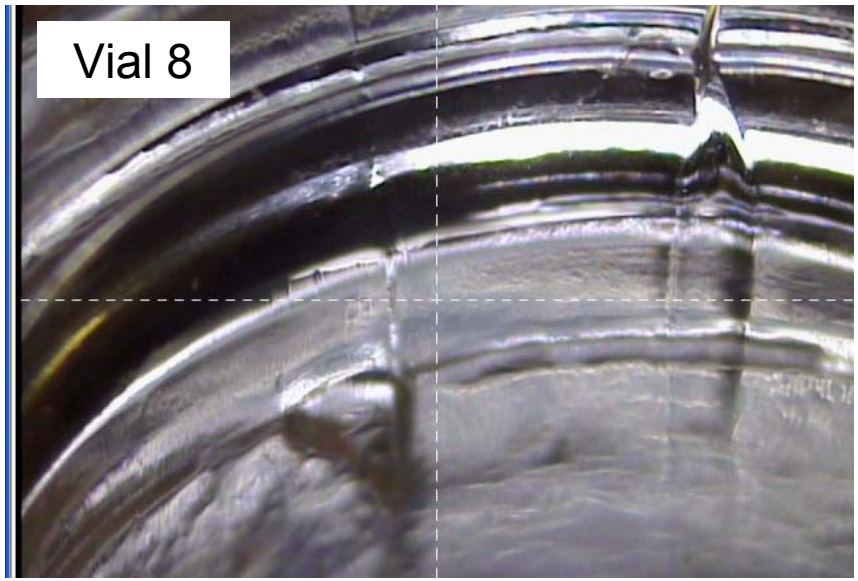


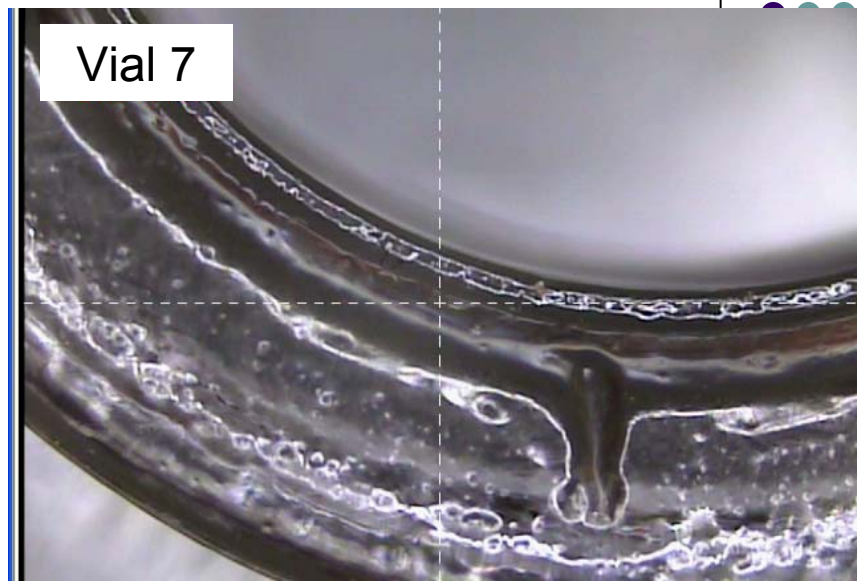




135X, Magellan V20 Video Microscope

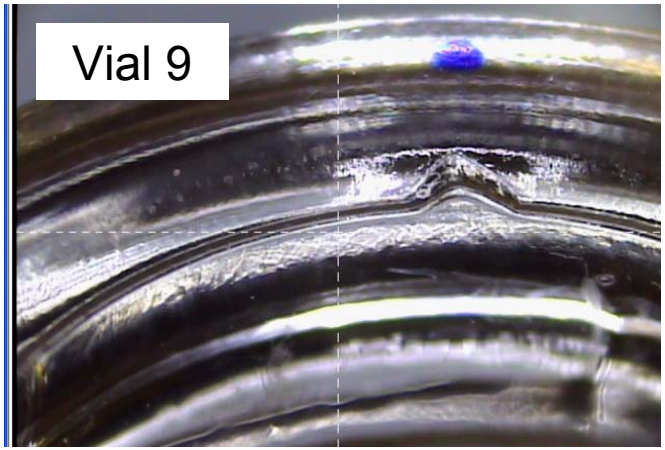
Vial ID code	Analyzed by	Description	Propagation risk	Leakage risk
4, 5, 6	**AGR, GPT**	**Large split**	**Moderate to high under certain handling conditions**	**Very likely**

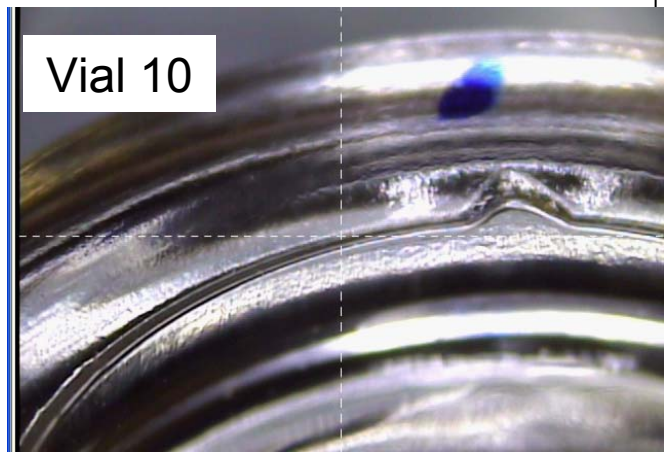


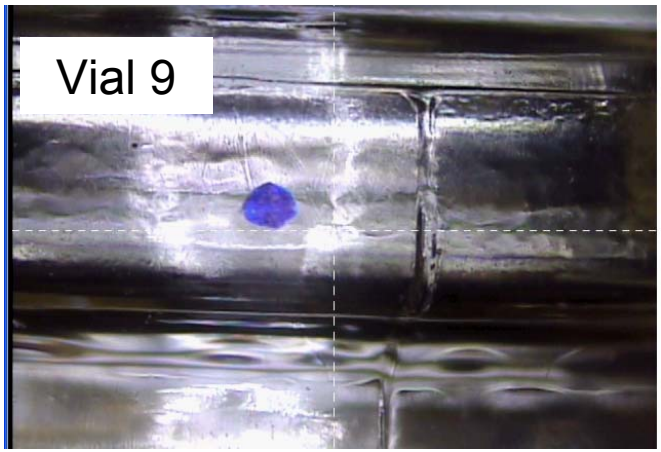


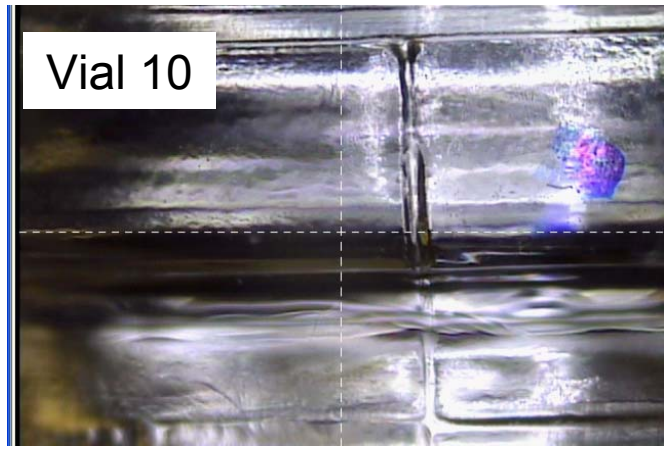
135X, Magellan V20 Video Microscope

Vial ID code	Analyzed by	Description	Propagation risk	Leakage risk
8	AGR	Smaller split	Not likely	Possible if not capped properly
	GPT	Open check or chip	Possible, may lead to split finish	Possible if finish splits
7	AGR	Rough surface Unfilled finish flaw	Not likely	Possible if not capped properly
	GPT	Rough surface Plunger mark	Not likely	Possible if not capped properly



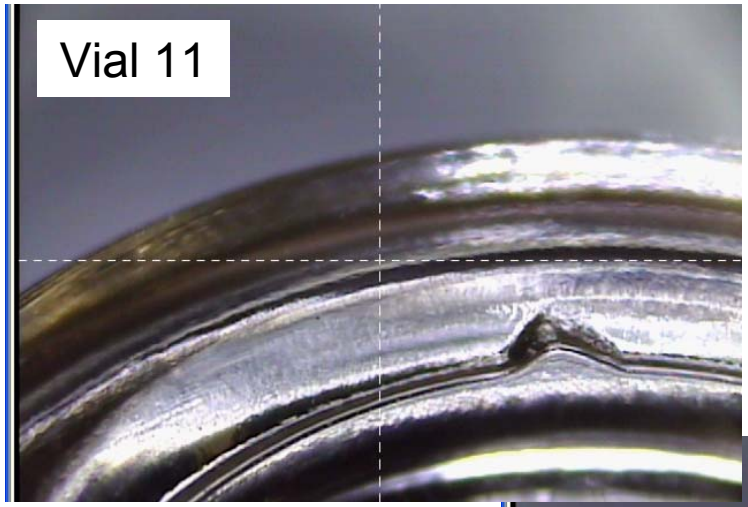


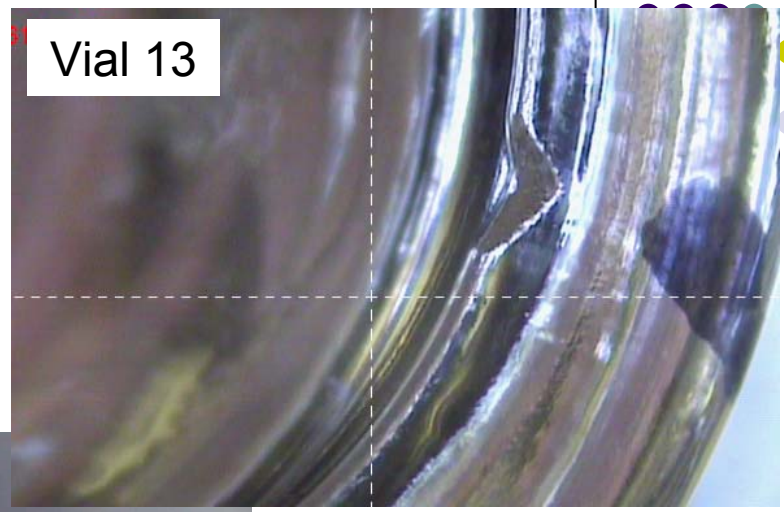


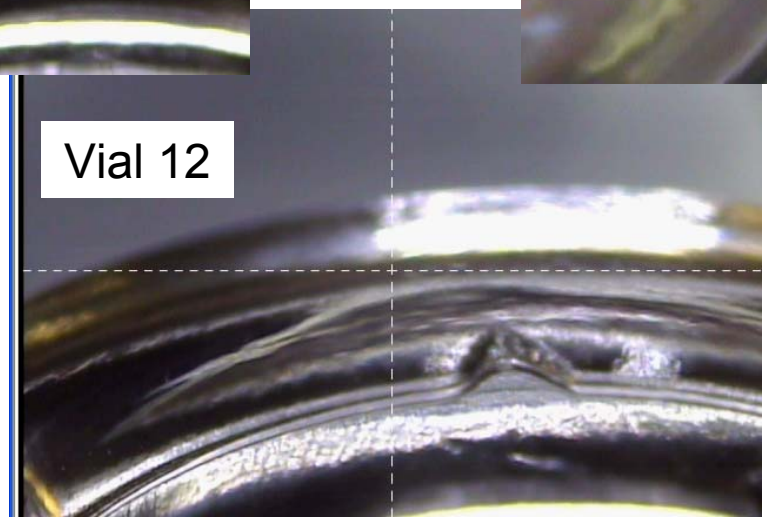


135X, Magellan V20 Video Microscope

Vial ID code	Analyzed by	Description	Propagation risk	Leakage risk
9, 10, 11, 12, 13	AGR	Neck ring seams Knockout on inside lip	Not likely	Not likely
	GPT	Mismatched neck ring seam, Plunger mark Somewhat healed split finish	Healed split finish might extend	Possible if finish split opens

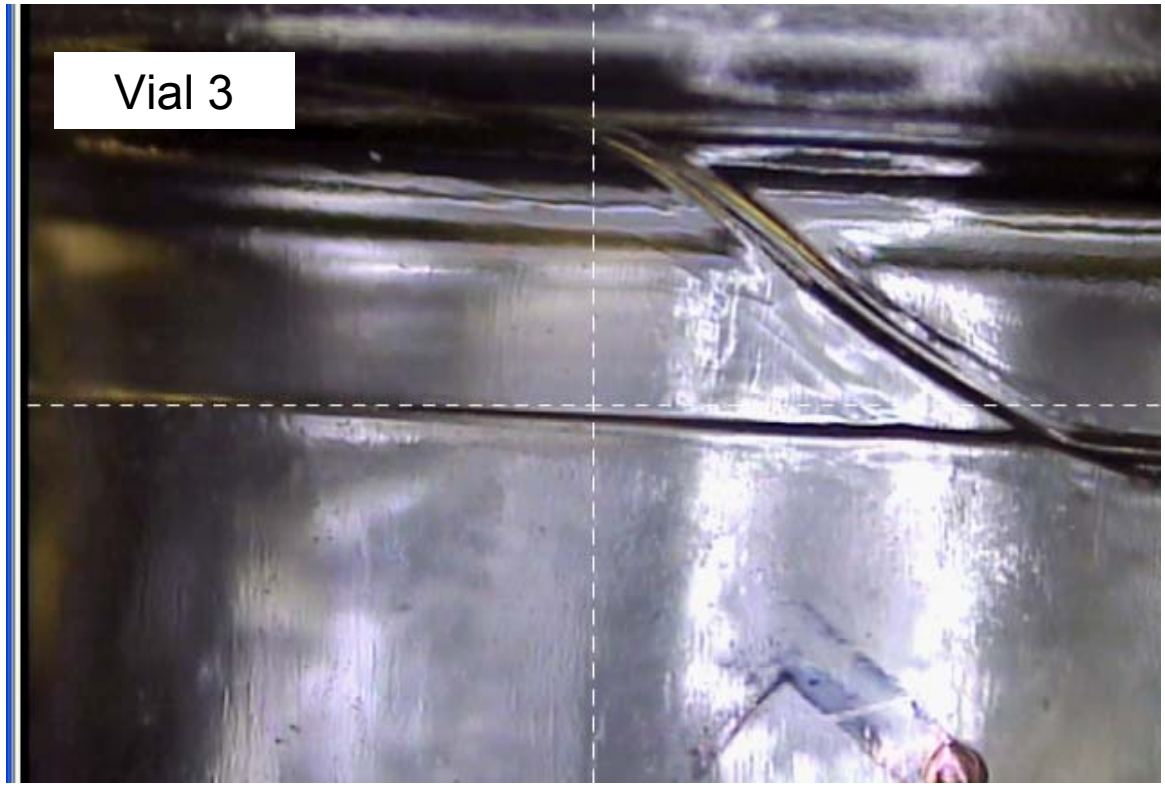






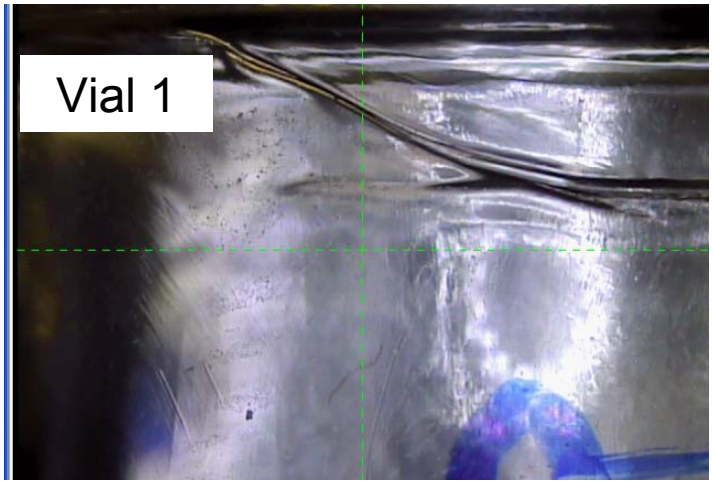
135X, Magellan V20 Video Microscope

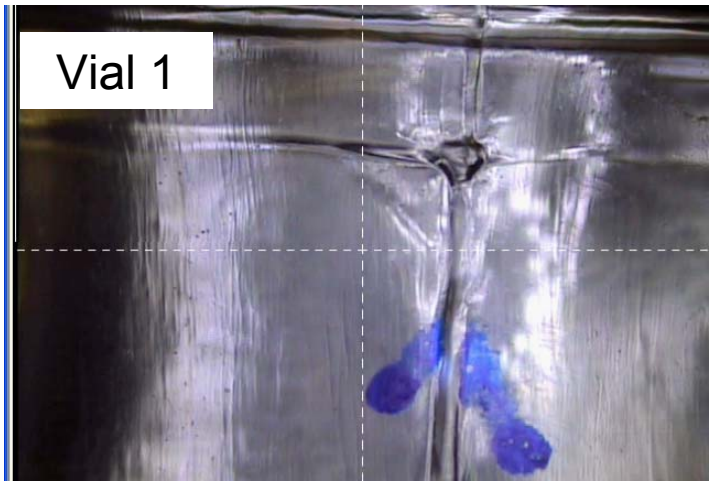
Vial ID code	Analyzed by	Description	Propagation risk	Leakage risk
9, 10, 11, 12, 13	AGR	Neck ring seams Knockout on inside lip	Not likely	Not likely
	GPT	Mismatched neck ring seam, Plunger mark Somewhat healed split finish	Healed split finish might extend	Possible if finish split opens

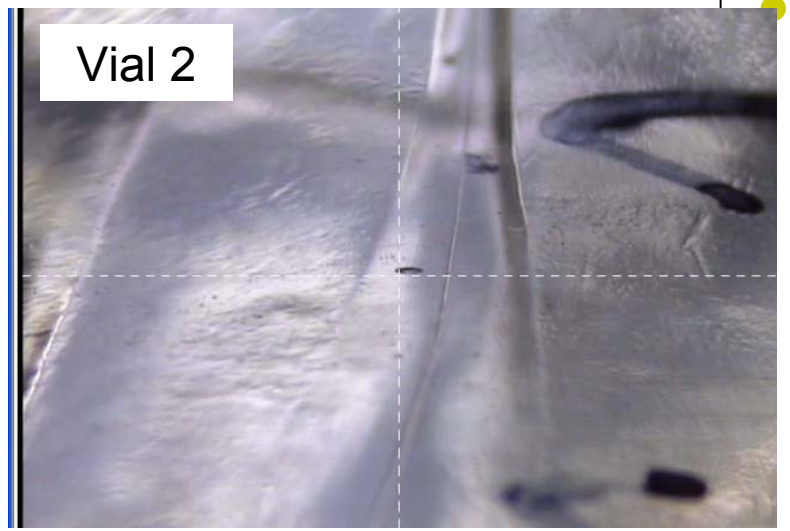


135X, Magellan V20 Video Microscope

Vial ID code	Analyzed by	Description	Propagation risk	Leakage risk
3	AGR	Fold defect Loading mark defect or knockout defects	Not likely	Not likely
	GPT	Heavy lap in neck	Small risk	Not likely



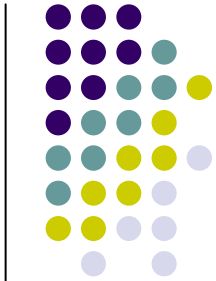




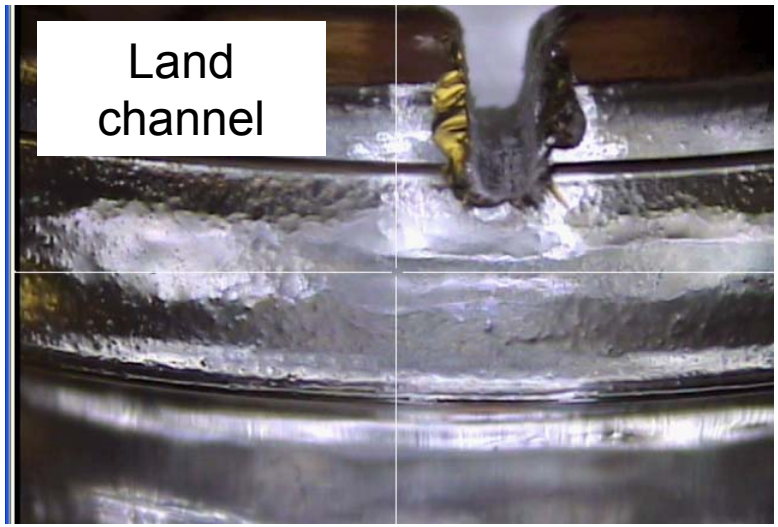
135X, Magellan V20 Video Microscope

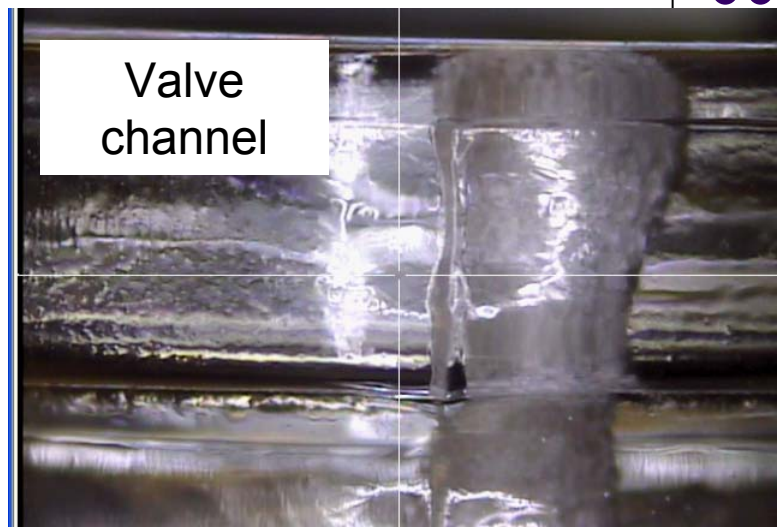
Vial ID code	Analyzed by	Description	Propagation risk	Leakage risk
1, 2	AGR	Fold defect Loading mark defect or knockout defects	Not likely	Not likely
	GPT	Laps Mismatched and/or heavy neck ring seams Cords, Loading marks	Not likely	Not likely

Glass Vial Finish Defects Study

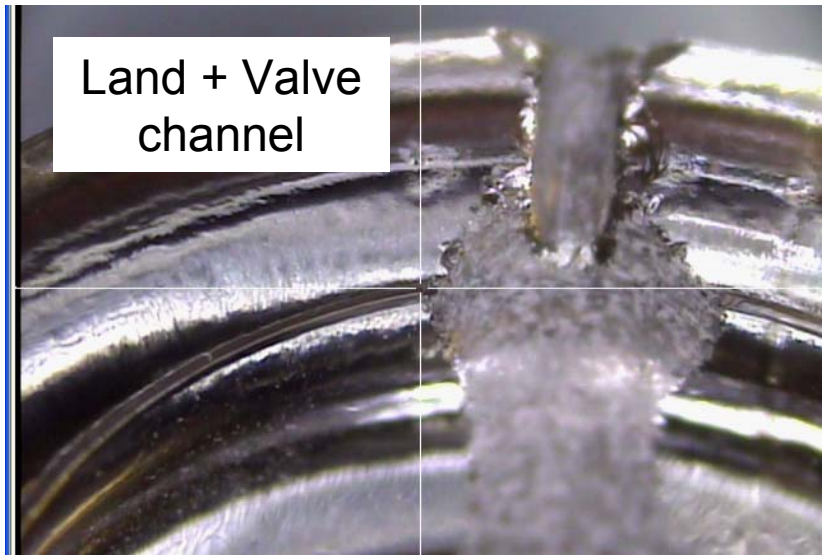


- Artificial defects created for leak testing
  - Holes through vial neck - Laser drilled
    - Lenox Laser, Glen Arm, MD
    - Sizes 15, 25, 50 µm nominal diameter
  - Channel defect – Dremel® saw
    - Land surface (horizontal, top)
    - Valve surface (vertical, neck)
    - Land + valve surfaces
    - Sizes 0.7-3.1 mm (W) x 0.6-1.5 mm (H)
- No defect – Negative controls





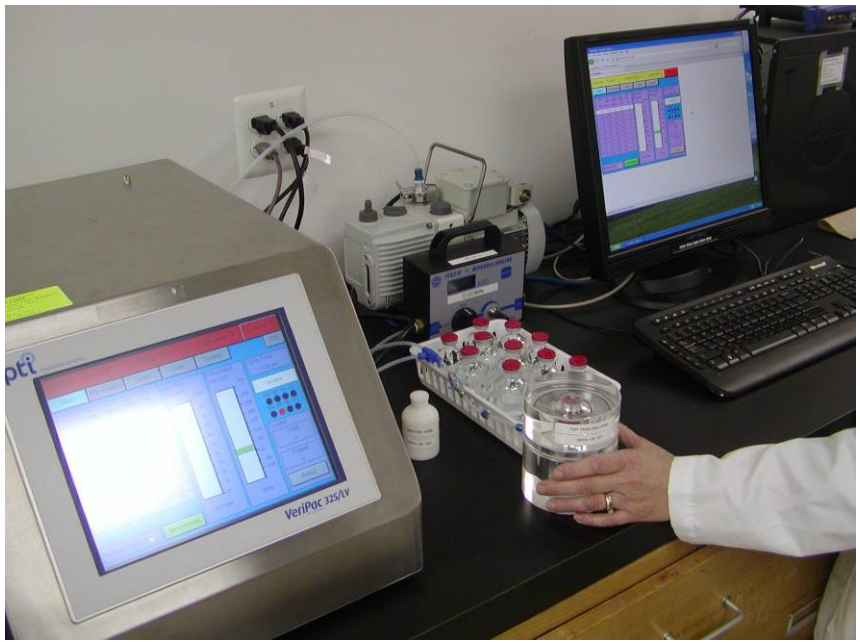
135X, Magellan V20 Video Microscope



Glass Vial Finish Defects Study

- Vacuum decay leak test
  - Packaging Technologies & Inspection
- High voltage leak test
  - Nikka Densok U.S.A.

Vacuum Decay Leak Test
ASTM F2338-09



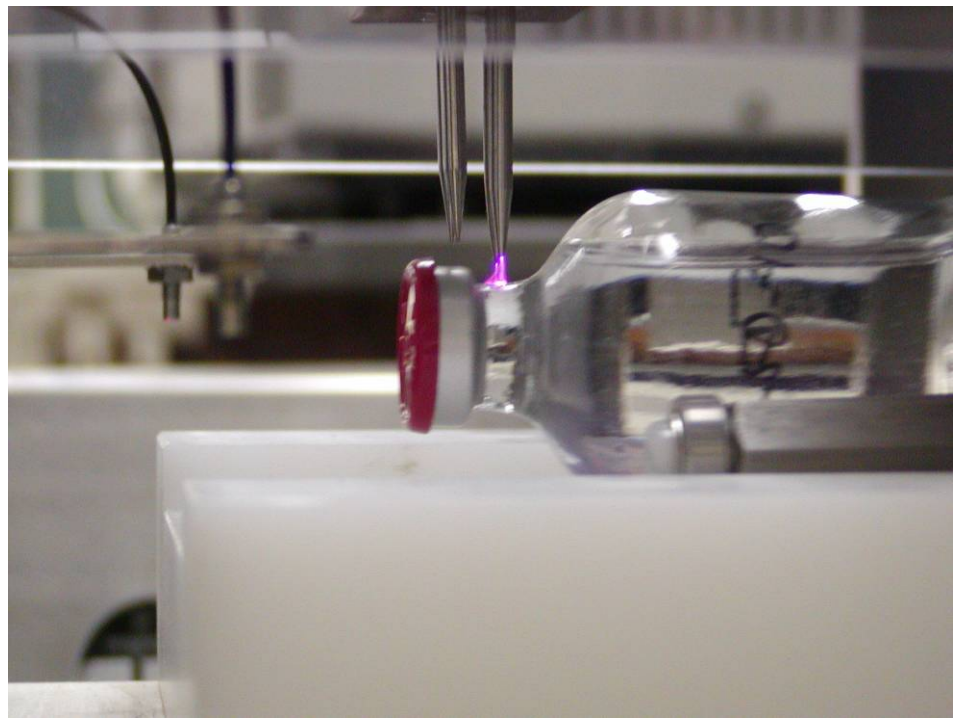
PTI VeriPac 325/LV

Test chamber

High Voltage Leak Test

Nikka Densok HVLD Model HDT1

Positive leak detection



Leak Detection vs. Defect Size & Type

- Test samples
  - Negative controls, no defect packages
  - Positive controls
    - Natural defect vials
    - Laser-drilled holes through glass vial neck
    - Channels cut along seal surfaces
  - Package contents
    - Artificial defects: 1/2 = active product 1/2 = placebo
    - Natural defects all contained active product

Leak Detection vs. Defect Size & Type

Hole size (µ)	Package contents	# Packages tested	# Packages ID'd as LEAKING	
			Vacuum decay	HVLD
15	Placebo	10	10	10
	Active	10	8	10
25	Placebo	10	10	10
	Active	10	9	10
50	Placebo	10	10	10
	Active	10	10	10

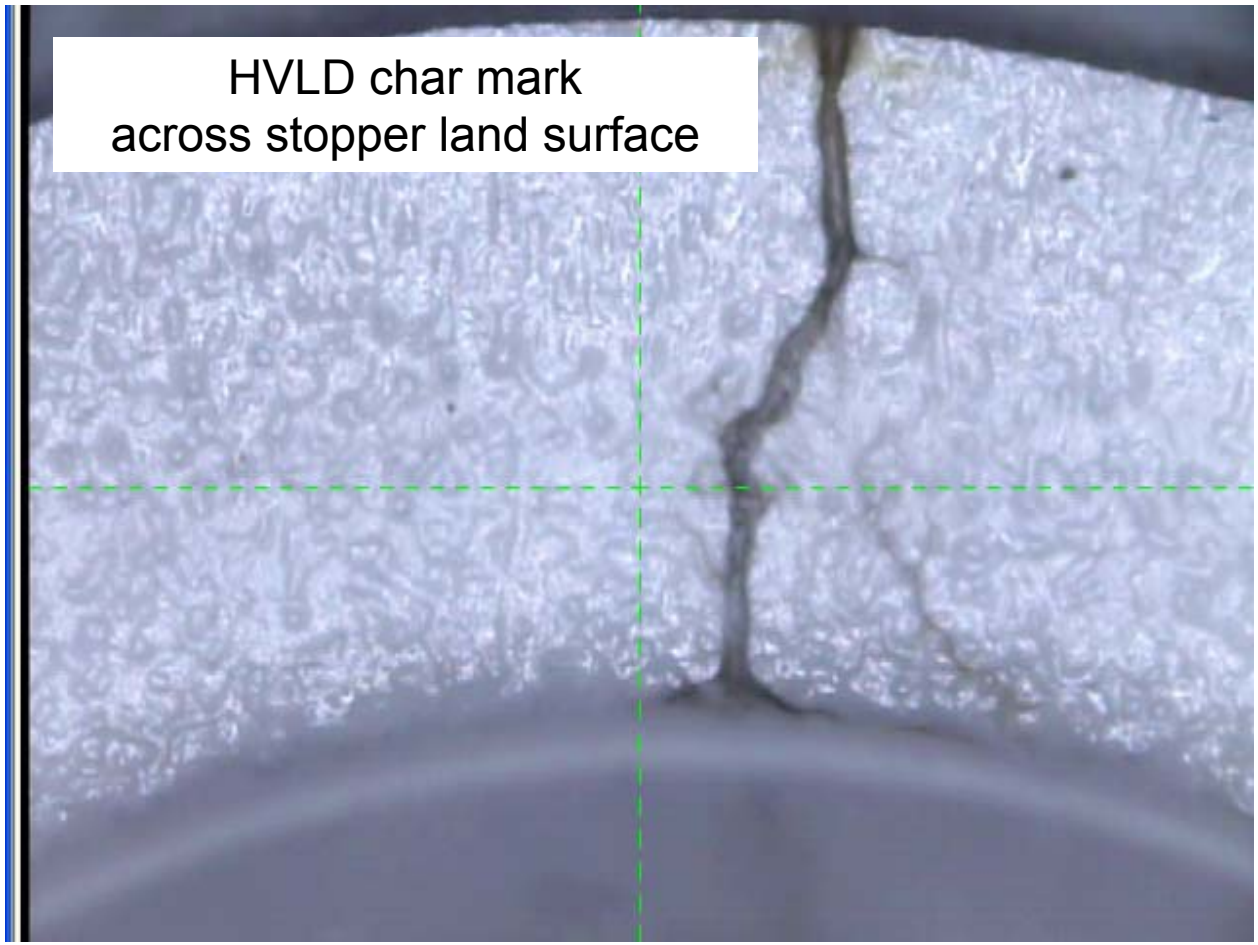
Leak Detection vs. Defect Size & Type

Channel location	Package contents	# Packages tested	# Packages ID'd as LEAKING	
			Vacuum decay	HVLD
None	Placebo	50	0	0
	Active	51	0	2*
Valve	Placebo	10	0	0
	Active	10	0	0
Land + Valve	Placebo	10	10	10
	Active	10	10	10

** Second HVLD failure was confirmed for a total of 5 HVLD tests. Both packages demonstrated **HVLD char marks** across vial and stopper land surfaces.*

continued

Leak Detection vs. Defect Size & Type



Leak Detection vs. Defect Size & Type

Natural defects

Vial ID code	Defect description	Leakage risk	ACTIVE PRODUCT-FILLED LEAKING Vial Packages	
			Vacuum Decay	HVLD
5, 6	Large split	Very likely	5	5, 6
8	Smaller split	Possible if not capped properly	---	8
	Open check or chip	Possible if finish splits		
7	Rough surface Unfilled finish flaw	Possible if not capped properly	---	---
	Rough surface Plunger mark	Possible if not capped properly	---	---

Leak Detection vs. Defect Size & Type

Natural defects

Vial ID code	Defect description	Leakage risk	ACTIVE PRODUCT-FILLED LEAKING Vial Packages	
			Vacuum Decay	HVLD
9, 10, 11, 12, 13	Neck ring seams Knockout on inside lip	Not likely	---	---
	Mismatched neck ring seam, Plunger mark Somewhat healed split finish	Possible if finish split opens	---	---
3	Fold defect Loading mark defect or knockout defects	Not likely	---	---
	Heavy lap in neck	Not likely	---	---
1, 2	Fold defect Loading mark defect or knockout defects	Not likely	---	---
	Laps Mismatched and/or heavy neck ring seams Cords, Loading marks	Not likely	---	---

Leak Detection vs. Defect Size & Type

SUMMARY

- HVLD and Vacuum decay are effective leak detection methods
  - Channel defects
    - land seal surface
    - land + valve seal surfaces
  - Hole defects in vial wall
  - Split or cracked finish defects

- However,
  - HVLD detected a larger % of potential leaking packages

Leak Detection vs. Product Formulation, Storage time

- Purpose
  - To determine effects of product formulation, product storage time on HVLD and vacuum decay results
- Test samples
  - Vials - laser drilled holes (15, 25, 50 µ)
  - Packages contained either
    - **Proteinaceous active product solution**
    - **Placebo solution**
- Experiment
  - Samples leak tested in random order on days 1 and 29
  - Vacuum decay first, then HVLD on each test day

Leak Detection vs. Product Formulation, Storage time

Vial hole size (µ)	Packages tested (#)	# Packages ID'd as LEAKING DAY 1		# Packages ID'd as LEAKING DAY 29	
		Vacuum decay	HVLD	Vacuum decay	HVLD
PRODUCT-FILLED					
15	10	8	10	2	10
25	10	9	10	2	10
50	10	10	10	3	10
PLACEBO-FILLED					
15	10	10	10	10	10
25	10	10	10	10	10
50	10	10	10	10	10

Leak Detection vs. Product Formulation, Storage time

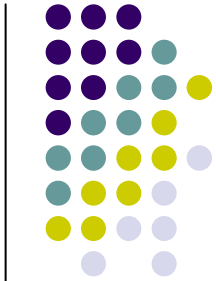
SUMMARY

- Vacuum decay FAILED to find package defects
  - Protein blockage of defect leak path suspected
- HVLD DETECTED all leaks
  - HVLD not influenced by protein presence
- Protein blockage risk increases over time

HVLD Exposure Effects on Product P-C Properties

- Purpose
  - Determine HVLD exposure effects on proteinaceous product
- Test samples
  - Three different **proteinaceous active products**
- Experiment
  - Product exposed to HVLD at 25kV 0x, 1x, 10x
  - Assays: Monomeric peak, High and Low MW species

HVLD Exposure Effects on Product P-C Properties



ImClone Systems Products

HVLD Exposure	Product A				Product B				Product C			
	Monomeric Peak		High MW Species	Low MW Species	Monomeric Peak		High MW Species	Low MW Species	Monomeric Peak		High MW Species	Low MW Species
	Rel. MW	% Purity	% Purity	% Purity	Rel. MW	% Purity	% Purity	% Purity	Rel. MW	% Purity	% Purity	% Purity
None	142	97.6	1.5	1.0	138	98.0	0.5	1.1	170	99.1	0	0.9
1 x 25kV	142	97.5	1.5	1.0	138	98.0	0.5	1.1	170	99.1	0	0.9
10 x 25kV	142	97.5	1.5	1.0	138	98.0	0.5	1.1	170	99.1	0	0.9

SUMMARY: HVLD exposure demonstrated no impact

Advances in HVLD Technology

E-Scan Laboratory HVLD Instrument

Nikka/PTI collaboration

3. Laser-based Headspace Detection

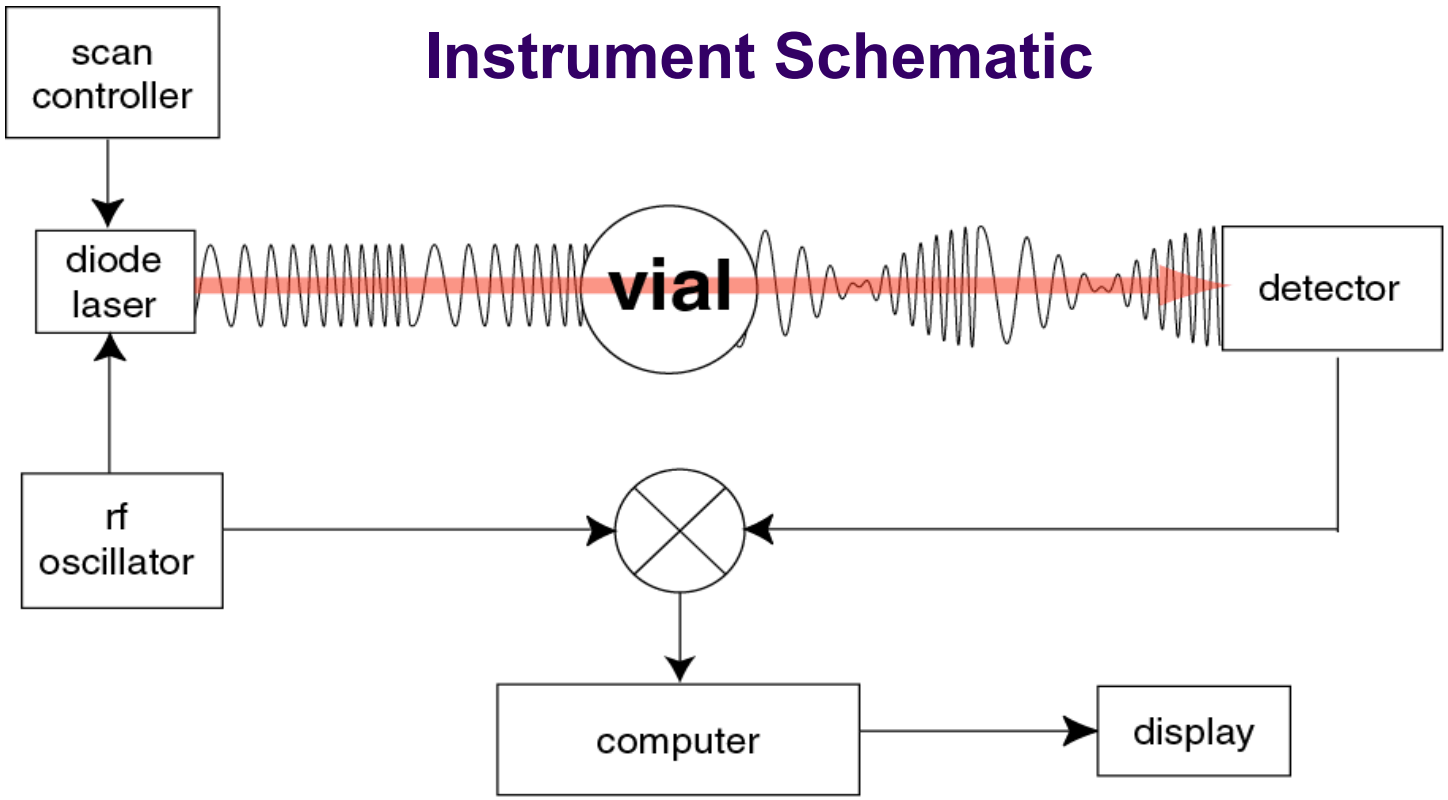
- Frequency Modulated Spectroscopy (FMS)
  - For dry or liquid product in transparent package
  - Detects headspace content
    - Oxygen, CO2, H20
    - Partial pressure
  - Instrument make can influence results
    - Sensitivity, reliability, testing speed

Laser-based Headspace Detection

- Method
  - Laser passed through container headspace
  - Laser frequency tuned to match internal absorption frequency of target molecule
    - Absorption is proportional to pressure
    - Amplitude is proportional to concentration
  - Differential absorption and phase sensitive detection techniques to enhance sensitivity

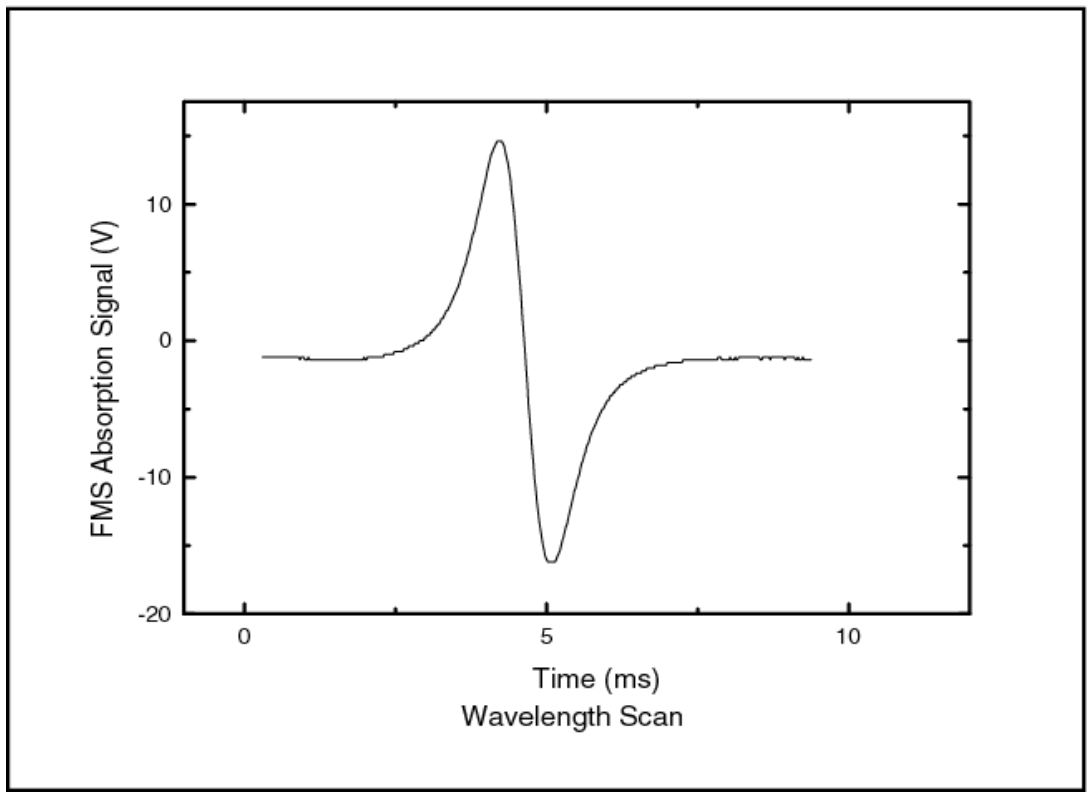
Laser-based Headspace Detection

Instrument Schematic



Lighthouse Instruments, Inc.

Laser-based Headspace Detection

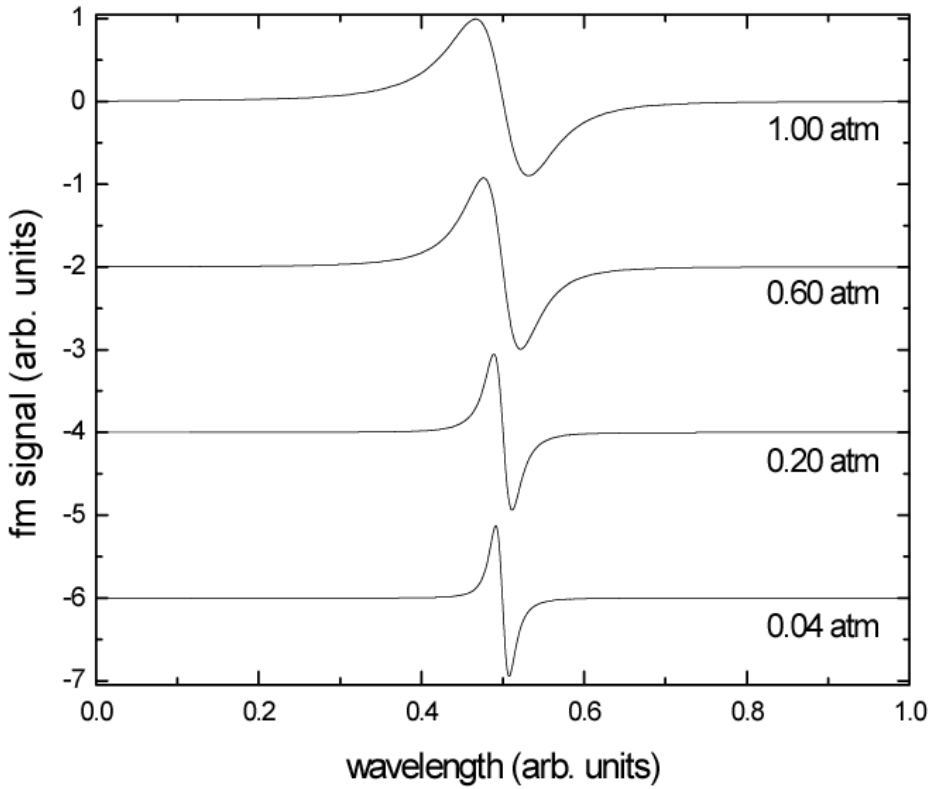


Absorption Signal Example

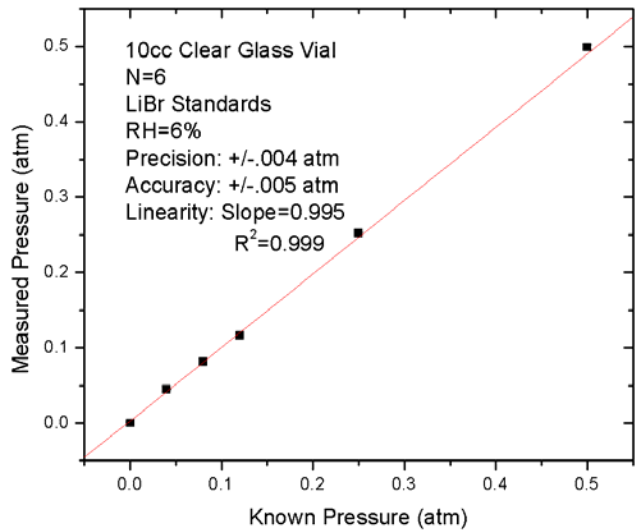
Lighthouse Instruments, Inc.

Laser-based Headspace Detection

Pressure vs. Peak Width



Linearity validation



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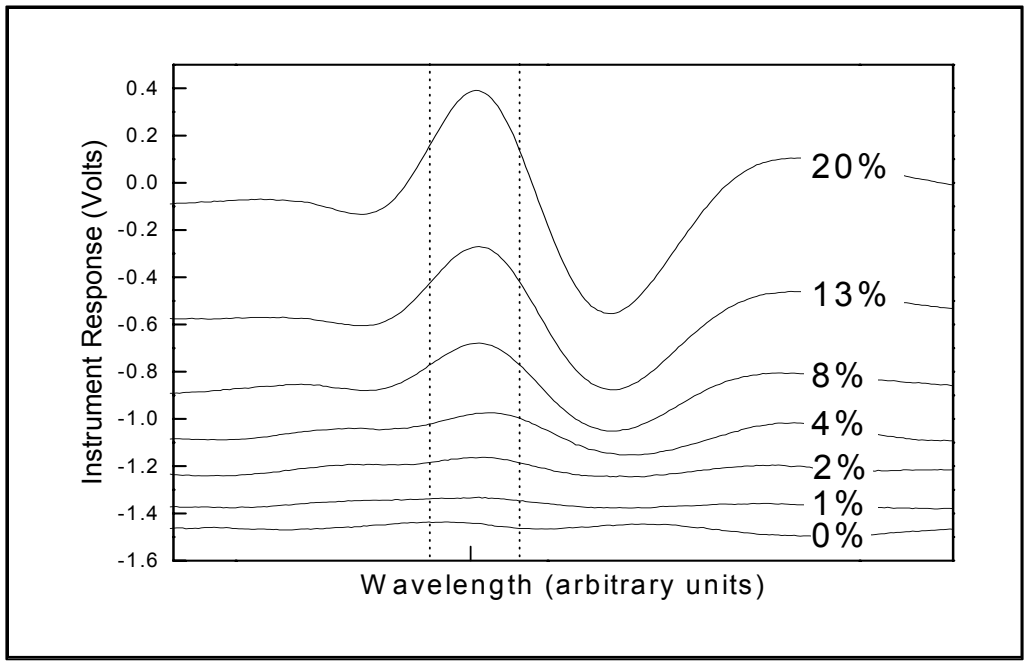
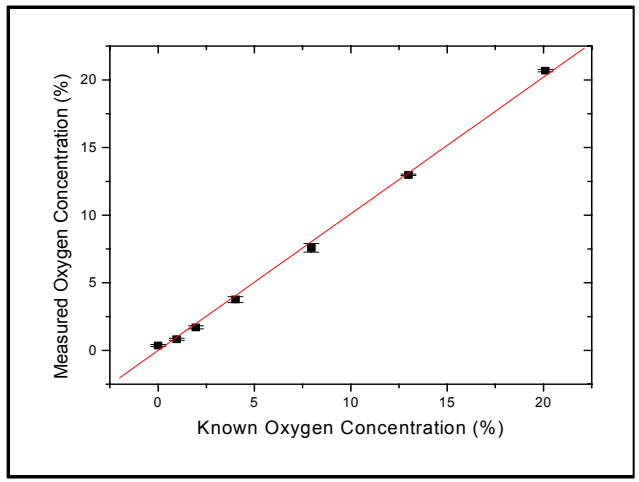
Laser-based Headspace Detection

O_2 Concentration vs. Signal Amplitude



Linearity validation



Lighthouse Instruments, Inc.

Laser-based Headspace Detection

- Specifications
  - Headspace analysis
    - O_2 inert gas environment
    - H_2O dry product
    - Vacuum < ~500 mbar absolute
  - Non-destructive, rapid (<1 s)
- Applications
  - Glass or transparent plastic packages
    - Vials, ampoules, syringes
  - On-line or off-line systems

Laser-based Headspace Detection

Inert Gas Loss over Time
10 mL vial container

Predicted rise in package oxygen content		Time to reach predicted oxygen levels (Days)	
Partial pressure (atm)	Oxygen concentration (% atm)	5 µm Hole	2 µm Hole
0	0	0	0
0.005	0.5	<1	4
0.01	1	1	8
0.02	2	3	17
0.04	4	6	36
0.08	8	13	81

Initial oxygen partial pressure = 0 Torr
Hole path length assumed to be 0.1 mm

(Courtesy of Lighthouse Instruments, Inc., Charlottesville, VA)

Laser-based Headspace Detection

Vacuum Loss over Time
10 mL vial container

Time post package closing	Package headspace pressure (Torr)	
	5 µm Hole	2 µm Hole
0 minutes	0	0
1 minute	13	2.4
5 minutes	63	12
10 minutes	126	24
60 minutes	756	144
5 hours	760	720
8 hours	760	760

Initial headspace pressure = 0 Torr
Viscous flow kinetics assumed
- hole path length 1.5 mm
- air viscosity 1.8×10^{-7} Pa·s

(Courtesy of Lighthouse Instruments, Inc., Charlottesville, VA)

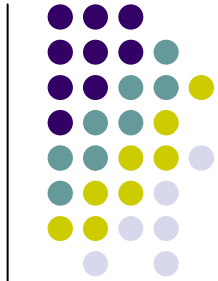
SUMMARY

- Package integrity related recalls continue to plague industry
- Current leak testing and package development practices are ineffective in preventing major recalls
- Commonly used dye ingress tests for CCIT are not considered ‘best practices’

SUMMARY

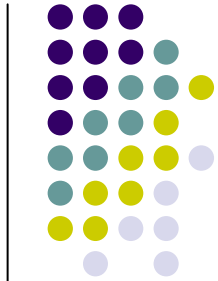
- 'Best practice' leak detection methods meet validation criteria of sensitivity and reliability
- Validation studies require appropriate positive and negative control test samples
- CCIT validation studies must reflect specific instruments, methods, packages, and products

SUMMARY



- 'Best practice' leak test methods are supported by data in peer-reviewed publications

- Best practice methods examples include
  - Vacuum decay
  - High voltage leak detection
  - Laser-based Headspace Detection



Thank you