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**
  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**
  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**
  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

<table>
<thead>
<tr>
<th>Closure Re-seal Method Parameters</th>
<th>USP 31 &lt;381&gt; Ph.Eur. 3.2.9</th>
<th>ISO 8362-5 Annex C</th>
<th>Modified ISO</th>
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<tbody>
<tr>
<td>Dye</td>
<td>0.1% aq. Methylene Blue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vacuum</td>
<td>-27 KPa</td>
<td>-25 KPa</td>
<td>-37 KPa</td>
</tr>
<tr>
<td>Time at Vacuum</td>
<td>10 min</td>
<td>30 min</td>
<td>30 min</td>
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<tr>
<td>Time at Ambient</td>
<td>30 min</td>
<td>30 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Detection method</td>
<td>Visual inspection</td>
<td></td>
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</tbody>
</table>

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

Dye Ingress Method Comparison

Glass Syringe Defects by Lenox Laser

Nominal hole size 10 µm

Nominal hole size 5 µm

Nominal hole size 15 µm
### USP/Ph.Eur. Dye Test
(-27kPa 10 min, amb 30 min)

**YES (Dye visible) or NO (Not visible)**

<table>
<thead>
<tr>
<th>Test Samples</th>
<th>Inspector 1</th>
<th>Inspector 2</th>
<th>Inspector 3</th>
</tr>
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<tbody>
<tr>
<td>Negative Controls</td>
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</table>

USP/PhEur Dye Ingress Test Samples

Negative Controls

5 µm

10 µm

15 µm

<table>
<thead>
<tr>
<th>Test Samples</th>
<th>ISO Dye Test (-25kPa 30 min, amb 30 min)</th>
<th>YES (Dye visible) or NO (Not visible)</th>
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<td>Inspector 1</td>
<td>Inspector 2</td>
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<td>5 µm</td>
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<td>10 µm</td>
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<td>15 µm</td>
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<td>Yes</td>
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</table>

**MODIFIED ISO Dye Test**

(-37kPa 30 min, amb 30 min)

**YES (Dye visible) or NO (Not visible)**

<table>
<thead>
<tr>
<th>Test Samples</th>
<th>Inspector 7</th>
<th>Inspector 8</th>
<th>Inspector 10</th>
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<td><strong>5 µm</strong></td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td><strong>10 µm</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Yes</td>
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<td>Yes</td>
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<td><strong>15 µm</strong></td>
<td>Yes</td>
<td>Yes</td>
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<td>Yes</td>
<td>Yes</td>
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<tr>
<td></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Modified ISO Dye Ingress Test Samples

5 µm

10 µm

15 µm

Negative Controls

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
"Leakage is a rate and therefore a continuum"

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  
  - 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 60°C 2hr, then 25°C 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.

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

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

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.

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

## Sterility Assurance Critical Leak Spec

<table>
<thead>
<tr>
<th>Study Author</th>
<th>Challenge medium</th>
<th>Challenge microbe</th>
<th>Challenge path</th>
<th>Challenge conditions</th>
<th>Threshold path size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirsch</td>
<td>Liquid</td>
<td><em>P. diminuta</em></td>
<td>Glass micro-pipette</td>
<td>Airlock elimination step + 24 hr ambient</td>
<td>0.3 µm</td>
</tr>
<tr>
<td>JPDA ’97-'99</td>
<td></td>
<td><em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burrell</td>
<td>Liquid</td>
<td><em>E. Coli</em></td>
<td>Poly-coated glass micro-tube</td>
<td>ISO closure reseal: 30 min 22”Hg + 30 min ambient</td>
<td>10 µm</td>
</tr>
<tr>
<td>JPDA 2000</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Keller</td>
<td>Aerosol</td>
<td><em>P. Fragi</em></td>
<td>Nickel micro-tube</td>
<td>Varied: -20 kPa to +20 kPa 4 to 37°C</td>
<td>5 µm</td>
</tr>
</tbody>
</table>

- “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
    - Microbial challenge
      - *Serratia marcenscens*  \( \geq 10^8 \text{ cfu/mL} \)
      - 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 vial test unit with wire leak**

![Diagram](image)

**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 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.

Positive Control Leakage Behavior

<table>
<thead>
<tr>
<th>Defect type</th>
<th>Defect size (µm)</th>
<th>He+ leak rate (mbarL/s)</th>
<th>Microbial ingress observed (%)</th>
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<tbody>
<tr>
<td>Hole</td>
<td></td>
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<tr>
<td></td>
<td>1</td>
<td>4.8 log -4</td>
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<td>2</td>
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<td>4</td>
<td>6.1 log -3</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2.8 log -2</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>9.3 log -2</td>
<td>90</td>
</tr>
<tr>
<td>Wire*</td>
<td>15</td>
<td>1.3 log -5</td>
<td>0</td>
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<td></td>
<td>20</td>
<td>2.2 log -5</td>
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<tr>
<td></td>
<td>60</td>
<td>5.3 log -3</td>
<td>100</td>
</tr>
</tbody>
</table>

* 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

Positive Control Recommendations

• Laser-drilled holes
  • Benefit
    • Closely simulates package wall crack, pinhole
    • Product and package impact on leak detection checked
  • Size
    • $\geq 5 \ \mu m$ 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

Crack caused by processing equipment
Naturally Occurring Defects

Crack caused by supplier
Positive Control Example

Glass Syringe Defects by Lenox Laser
Nominal hole size 5 µm

Microscope photo by BMS

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

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

## ASTM F2338-09

Vacuum decay test parameters

<table>
<thead>
<tr>
<th>Leak test parameters</th>
<th>Parameter limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evacuation (Fill) time</td>
<td>6 s</td>
</tr>
<tr>
<td>Equalization time</td>
<td>0.2 s</td>
</tr>
<tr>
<td>Test time</td>
<td>8 s</td>
</tr>
<tr>
<td>Pressure rise reference limit</td>
<td></td>
</tr>
<tr>
<td>1000 Torr transducer</td>
<td>2 mbar (abs)</td>
</tr>
<tr>
<td>Pressure rise reference limit</td>
<td></td>
</tr>
<tr>
<td>10 Torr transducer</td>
<td>25 Pa (differential)</td>
</tr>
</tbody>
</table>

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

Vacuum decay
Negative control syringes

\[
\begin{array}{cccc}
\text{dP (Pa)} & 0 & 5 & 10 \\
\triangle & 15 & 30 & 45 \\
\end{array}
\]

Test Sample Readings
Site 1: 0-45 Site 2: 46-90 Site 3: 91-140

\[\text{△ Study 4, Water-filled Syringes for Gas Leak Tests} \]
\[\text{△ Study 5, Water-filled Syringes for Liquid Leak Tests} \]
\[\text{dP Ref Pass/Fail Limit} \]

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

<table>
<thead>
<tr>
<th>Study</th>
<th>No. Packages Tested</th>
<th>No. Tests</th>
<th>No. FAILED</th>
<th>No. PASSED</th>
<th>% Accurate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 4: Water</td>
<td>15</td>
<td>135</td>
<td>0</td>
<td>135</td>
<td>100</td>
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<tr>
<td>Study 5: Water</td>
<td>15</td>
<td>134</td>
<td>0</td>
<td>134</td>
<td>100</td>
</tr>
</tbody>
</table>
Vacuum decay
Positive control syringes
Air- vs. water-filled

![Graph showing dP (Pa) vs. nominal hole size (microns) for air-filled and water-filled syringes.]

- Study 4, Air-filled Syringes
- Study 5, Water-filled Syringes

A water-filled 5.7 µm unit gave 1 ABORT result (not graphed)

Nominal 5 µm holes

<table>
<thead>
<tr>
<th>Syringe Contents</th>
<th>No. Packages Tested</th>
<th>No. Tests</th>
<th>No. FAILED</th>
<th>No. PASSED</th>
<th>% Accurate</th>
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</thead>
<tbody>
<tr>
<td>Study 4: Air</td>
<td>15</td>
<td>45</td>
<td>45</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Study 5: Water</td>
<td>15</td>
<td>45</td>
<td>45</td>
<td>0</td>
<td>100</td>
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Vacuum decay
Positive control syringes
Air- vs. water-filled

Nominal 10-15 µm holes
ABORT assigned 599 mbar

<table>
<thead>
<tr>
<th>Syringe Contents</th>
<th>No. Packages Tested</th>
<th>No. Tests</th>
<th>No. FAILED</th>
<th>No. PASSED</th>
<th>% Accurate</th>
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<tbody>
<tr>
<td>Study 4: Air</td>
<td>30</td>
<td>90</td>
<td>90</td>
<td>0</td>
<td>100</td>
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<td>Study 5: Water</td>
<td>30</td>
<td>90</td>
<td>90</td>
<td>0</td>
<td>100</td>
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<tr>
<td>Test Samples</td>
<td>Air-filled Syringe Vac decay dP (Pa)</td>
<td>USP/Ph.Eur. Dye Test (-27kPa 10 min, amb 30 min)</td>
<td></td>
<td></td>
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<tr>
<td>----------------------</td>
<td>--------------------------------------</td>
<td>-----------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pass or Fail</td>
<td>YES (Dye visible) or NO (Not visible)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inspector 1</td>
<td>Inspector 2</td>
<td>Inspector 3</td>
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<td>5 µm</td>
<td>25 (4.7 µm)</td>
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<td>71</td>
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<td>10 µm</td>
<td>217</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>161</td>
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<td>15 µm</td>
<td>ABORT</td>
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<td></td>
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<td>281</td>
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<table>
<thead>
<tr>
<th>Test Samples</th>
<th>Air-filled Syringe Vac decay $dP$ (Pa)</th>
<th>ISO Dye Test (-25kPa 30 min, amb 30 min)</th>
<th>YES (Dye visible) or NO (Not visible)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pass or Fail</td>
<td>Inspector 1</td>
<td>Inspector 2</td>
</tr>
<tr>
<td>Negative Controls</td>
<td>7</td>
<td>No</td>
<td>No</td>
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<td></td>
<td>6</td>
<td>No</td>
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<tr>
<td></td>
<td>7</td>
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<tr>
<td></td>
<td>7</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>5 µm</td>
<td>22 (4.7 µm)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>No</td>
<td>No</td>
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<td>79</td>
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<tr>
<td></td>
<td>44</td>
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<td>No</td>
</tr>
<tr>
<td></td>
<td>42</td>
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<td>10 µm</td>
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<td>Yes</td>
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<td>260</td>
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<td>Yes</td>
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<td>154</td>
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<td>15 µm</td>
<td>388</td>
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<td>Yes</td>
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<td>346</td>
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<td>Yes</td>
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<td>335</td>
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<td>Yes</td>
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<td>337</td>
<td>Yes</td>
<td>Yes</td>
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<td>301</td>
<td>Yes</td>
<td>Yes</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Test Samples</th>
<th>Air-filled Syringe Vac decay dP (Pa) Pass or Fail</th>
<th>MODIFIED ISO Dye Test (-37kPa 30 min, amb 30 min) YES (Dye visible) or NO (Not visible)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Controls</td>
<td>9 No 10 No 9 No 17 Yes</td>
<td>Inspector 7 Inspector 8 Inspector 10</td>
</tr>
<tr>
<td>5 µm</td>
<td>57 Yes 96 Yes 43 Yes 41 Yes 51 Yes</td>
<td>Yes Yes Yes Yes Yes</td>
</tr>
<tr>
<td>10 µm</td>
<td>ABORT 191 Yes ABORT 188 Yes</td>
<td>Yes Yes Yes Yes Yes</td>
</tr>
<tr>
<td>15 µm</td>
<td>ABORT Yes ABORT Yes ABORT Yes</td>
<td>Yes Yes Yes Yes Yes</td>
</tr>
</tbody>
</table>

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
<table>
<thead>
<tr>
<th>Vial ID code</th>
<th>Analyzed by</th>
<th>Description</th>
<th>Propagation risk</th>
<th>Leakage risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>4, 5, 6</td>
<td>AGR, GPT</td>
<td>Large split</td>
<td>Moderate to high under certain handling conditions</td>
<td>Very likely</td>
</tr>
</tbody>
</table>

135X, Magellan V20 Video Microscope
### Table: Analysis of Vials 7 and 8

<table>
<thead>
<tr>
<th>Vial ID code</th>
<th>Analyzed by</th>
<th>Description</th>
<th>Propagation risk</th>
<th>Leakage risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>AGR</td>
<td>Smaller split</td>
<td>Not likely</td>
<td>Possible if not capped properly</td>
</tr>
<tr>
<td></td>
<td>GPT</td>
<td>Open check or chip</td>
<td>Possible, may lead to split finish</td>
<td>Possible if finish splits</td>
</tr>
</tbody>
</table>
| 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 |
<table>
<thead>
<tr>
<th>Vial ID code</th>
<th>Analyzed by</th>
<th>Description</th>
<th>Propagation risk</th>
<th>Leakage risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>9, 10, 11, 12, 13</td>
<td>AGR</td>
<td>Neck ring seams, Knockout on inside lip</td>
<td>Not likely</td>
<td>Not likely</td>
</tr>
<tr>
<td></td>
<td>GPT</td>
<td>Mismatched neck ring seam, Plunger mark, Somewhat healed split finish</td>
<td>Healed split finish might extend</td>
<td>Possible if finish split opens</td>
</tr>
</tbody>
</table>

135X, Magellan V20 Video Microscope
<table>
<thead>
<tr>
<th>Vial ID code</th>
<th>Analyzed by</th>
<th>Description</th>
<th>Propagation risk</th>
<th>Leakage risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>9, 10, 11, 12, 13</td>
<td>AGR</td>
<td>Neck ring seams, Knockout on inside lip</td>
<td>Not likely</td>
<td>Not likely</td>
</tr>
<tr>
<td></td>
<td>GPT</td>
<td>Mismatched neck ring seam, Plunger mark, Somewhat healed split finish</td>
<td>Healed split finish might extend</td>
<td>Possible if finish split opens</td>
</tr>
</tbody>
</table>

135X, Magellan V20 Video Microscope
### Vial 3

#### 135X, Magellan V20 Video Microscope

<table>
<thead>
<tr>
<th>Vial ID code</th>
<th>Analyzed by</th>
<th>Description</th>
<th>Propagation risk</th>
<th>Leakage risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>AGR</td>
<td>Fold defect&lt;br&gt;Loading mark defect or knockout defects</td>
<td>Not likely</td>
<td>Not likely</td>
</tr>
<tr>
<td></td>
<td>GPT</td>
<td>Heavy lap in neck</td>
<td>Small risk</td>
<td>Not likely</td>
</tr>
<tr>
<td>Vial ID code</td>
<td>Analyzed by</td>
<td>Description</td>
<td>Propagation risk</td>
<td>Leakage risk</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>-----------------</td>
<td>--------------</td>
</tr>
<tr>
<td>1, 2</td>
<td>AGR</td>
<td>Fold defect</td>
<td>Not likely</td>
<td>Not likely</td>
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<tr>
<td></td>
<td></td>
<td>Loading mark defect or knockout defects</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GPT</td>
<td>Laps</td>
<td>Not likely</td>
<td>Not likely</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mismatched and/or heavy neck ring seams</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cords, Loading marks</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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
Land channel

Valve channel

Land + Valve channel

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

Positive leak detection

Nikka Densok HVLD Model HDT1
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

<table>
<thead>
<tr>
<th>Hole size (µ)</th>
<th>Package contents</th>
<th># Packages tested</th>
<th># Packages ID’d as LEAKING</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vacuum decay</td>
</tr>
<tr>
<td>15</td>
<td>Placebo</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Active</td>
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<td>8</td>
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<tr>
<td>25</td>
<td>Placebo</td>
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<td>10</td>
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<tr>
<td></td>
<td>Active</td>
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<td>9</td>
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<tr>
<td>50</td>
<td>Placebo</td>
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<td>Active</td>
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</table>
# Leak Detection vs. Defect Size & Type

<table>
<thead>
<tr>
<th>Channel location</th>
<th>Package contents</th>
<th># Packages tested</th>
<th># Packages ID’d as LEAKING</th>
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<td>Vacuum decay</td>
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<tr>
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<td>Placebo</td>
<td>50</td>
<td>0</td>
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<tr>
<td></td>
<td>Active</td>
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<td>0</td>
</tr>
<tr>
<td>Valve</td>
<td>Placebo</td>
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<td>0</td>
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<tr>
<td></td>
<td>Active</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Land + Valve</td>
<td>Placebo</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Active</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

* 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

HVLD char mark across stopper land surface
## Leak Detection vs. Defect Size & Type

### Natural defects

<table>
<thead>
<tr>
<th>Vial ID code</th>
<th>Defect description</th>
<th>Leakage risk</th>
<th>ACTIVE PRODUCT-FILLED LEAKING Vial Packages</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vacuum Decay</td>
<td>HVLD</td>
</tr>
<tr>
<td>5, 6</td>
<td>Large split</td>
<td>Very likely</td>
<td>5</td>
<td>5, 6</td>
</tr>
<tr>
<td>8</td>
<td>Smaller split</td>
<td>Possible if not capped properly</td>
<td>---</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Open check or chip</td>
<td>Possible if finish splits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Rough surface</td>
<td>Possible if not capped properly</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Unfilled finish flaw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rough surface Plunger mark</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Leak Detection vs. Defect Size & Type

### Natural defects

<table>
<thead>
<tr>
<th>Vial ID code</th>
<th>Defect description</th>
<th>Leakage risk</th>
<th>ACTIVE PRODUCT-FILLED LEAKING Vial Packages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vacuum Decay</td>
</tr>
</tbody>
</table>
| 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

<table>
<thead>
<tr>
<th>Vial hole size (µ)</th>
<th>Packages tested (#)</th>
<th># Packages ID’d as LEAKING DAY 1</th>
<th># Packages ID’d as LEAKING DAY 29</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vacuum decay</td>
<td>HVLD</td>
</tr>
<tr>
<td>PRODUCT-FILLED</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>50</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>PLACEBO-FILLED</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>50</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
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 | | | |
|--------------|-----------|---|---|---|-----------|---|---|---|---|-----------|---|---|---|---|---|
| Monomorphic Peak | % Purity | % Purity | % Purity | Rel. MW | % Purity | % Purity | % Purity | Rel. MW | % Purity | % Purity | % Purity | Rel. MW | % Purity | % 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

scan controller

diode laser

rf oscillator

vial

detector

computer

display

Lighthouse Instruments, Inc.
Laser-based Headspace Detection

Absorption Signal Example

Lighthouse Instruments, Inc.
Laser-based Headspace Detection

Pressure vs. Peak Width

Lighthouse Instruments, Inc.
Laser-based Headspace Detection

O₂ 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 $< \sim 500$ 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

<table>
<thead>
<tr>
<th>Partial pressure (atm)</th>
<th>Oxygen concentration (% atm)</th>
<th>5 µm Hole</th>
<th>2 µm Hole</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>0.5</td>
<td>&lt;1</td>
<td>4</td>
</tr>
<tr>
<td>0.01</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>0.02</td>
<td>2</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>0.04</td>
<td>4</td>
<td>6</td>
<td>36</td>
</tr>
<tr>
<td>0.08</td>
<td>8</td>
<td>13</td>
<td>81</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Time post package closing</th>
<th>Package headspace pressure (Torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 µm Hole</td>
</tr>
<tr>
<td>0 minutes</td>
<td>0</td>
</tr>
<tr>
<td>1 minute</td>
<td>13</td>
</tr>
<tr>
<td>5 minutes</td>
<td>63</td>
</tr>
<tr>
<td>10 minutes</td>
<td>126</td>
</tr>
<tr>
<td>60 minutes</td>
<td>756</td>
</tr>
<tr>
<td>5 hours</td>
<td>760</td>
</tr>
<tr>
<td>8 hours</td>
<td>760</td>
</tr>
</tbody>
</table>

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

(Courtesy of Lighthouse Instruments, Inc., Charlottesville, VA)
Sterile Product Package Integrity Testing

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’
Sterile Product Package Integrity Testing

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