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CCI:
Regulations, Test Methods, Application
Test Methods: Fundamentals

PDA® Parenteral Drug Association

PDA TRAINING

OUTLINE - Test Method Fundamentals

1. CCI Testing Principles
2. Leak & Positive Controls
3. Gas flow: Flow Rate & Leak Size
4. "Sizing" : Correlation between leak size & flow rate

RECAP

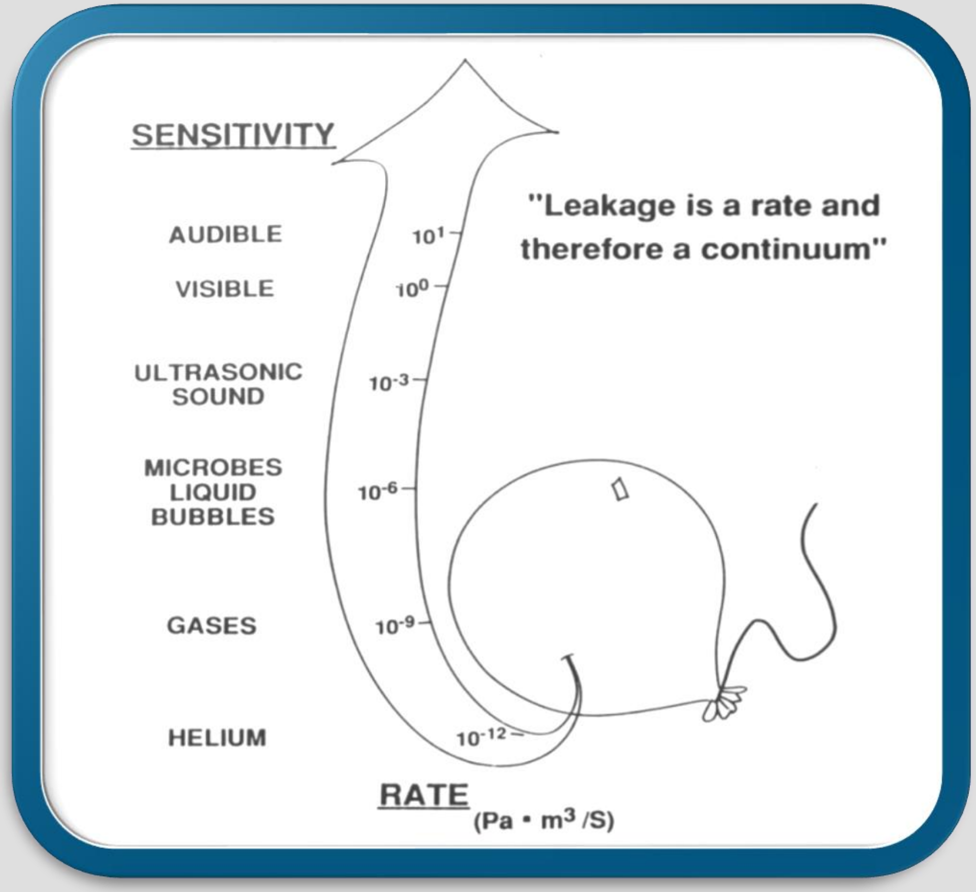
Most advanced CCIT technologies rely on gas flow:

- ❑ More reliable (for micron-size leaks)
- ❑ More predictable, some are quantitative

Smallest leaks **only** allow

Larger leaks **may also** allow

Largest leaks **may also** allow



CCI Testing Technology Overview

Most CCIT technologies do not detect leak (defects) directly.

Instead, they detect presence of leak by monitoring the **biological and/or physiochemical responses** caused by a medium passing through the leaking path, typically driven by certain challenge conditions.

Technology detection performance depends on:

1. Test medium: Liquid (with microbe/dye tracer) vs. gas
2. Challenge conditions: Pressure differential, high voltage
3. Response detection methods

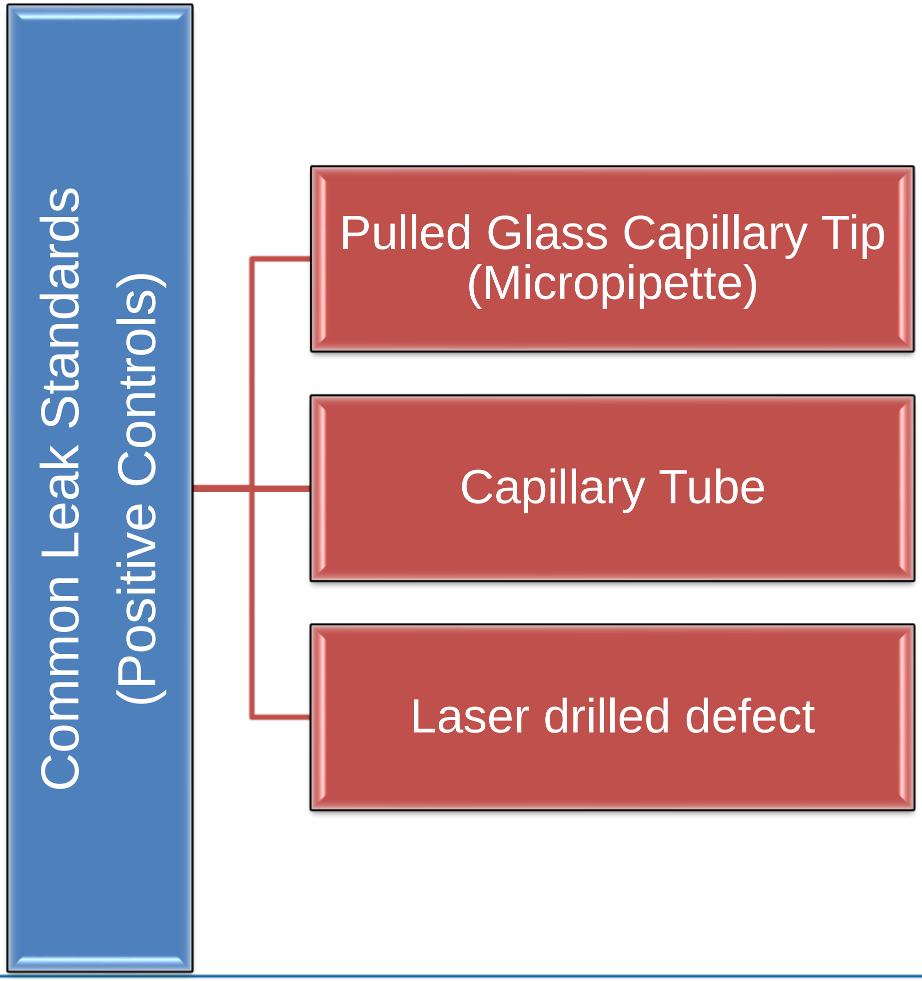
CCI Testing Technology Overview

Technology	Test Medium	Typical Challenge Condition	Response	Detection
Microbial Challenge	Liquid flow (Microbial species)	Pressure differential	Turbidity due to microbe growth	Visual
Dye ingress	Liquid flow (Dye solution)	Pressure differential	Dye presence	Visual or spectrophotometer
Vacuum Decay	Gas flow	Pressure differential	Pressure change	Pressure transducer
Mass Extraction	Gas flow	Pressure differential	Mass flow	Mass flow sensor
Headspace (e.g., oxygen) Analyzer	Gas flow (e.g., O_2)	Partial pressure differential (e.g., O2)	Oxygen	Laser absorption spectroscopy
Helium Leak Detection	Gas flow (Helium)	Pressure differential	Helium	Mass spectrometer
Optical Emission Spectrometry	Gas Flow (e.g., N_2, CO_2, Ar, H_2O)	Pressure differential	Air Leakage	OES
High Voltage Leak detection	Electron flow (Current)	High voltage	Current (electron flow)	Current to voltage converter

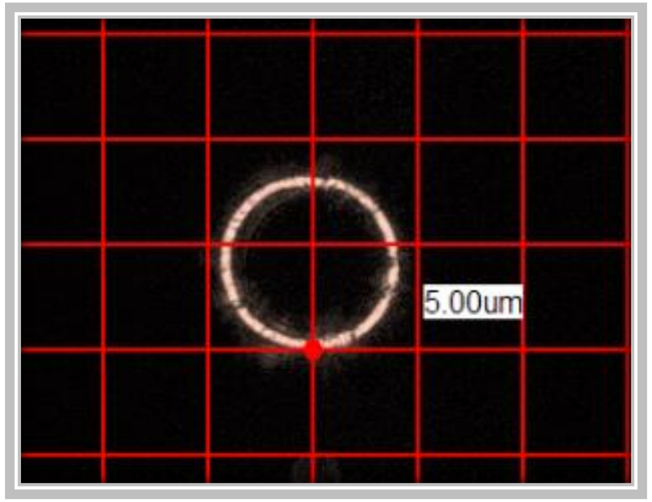
Artificial Standards (Positive Controls)

Leak

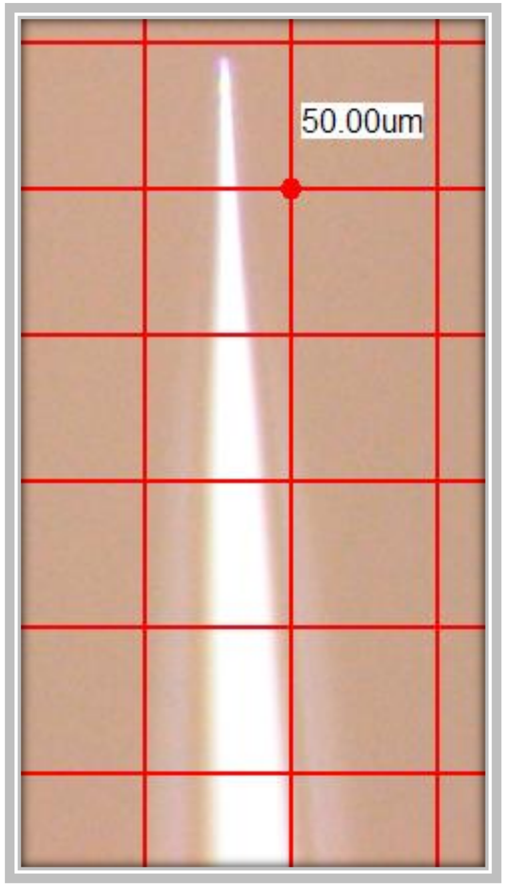
- A gap or breach in the container capable of permitting the passage of liquid or gas (Syn. “Leak path”).
- Real world leaks are usually complex, featuring various shapes, length, forms; some are transient and can change over time.



Pulled Glass Capillary Tip (Micropipette)



Top view showing the tip orifice



Side view showing approximate length

Glass tube with the tip pulled into micron size capillary

Capillary Tubes / Wires

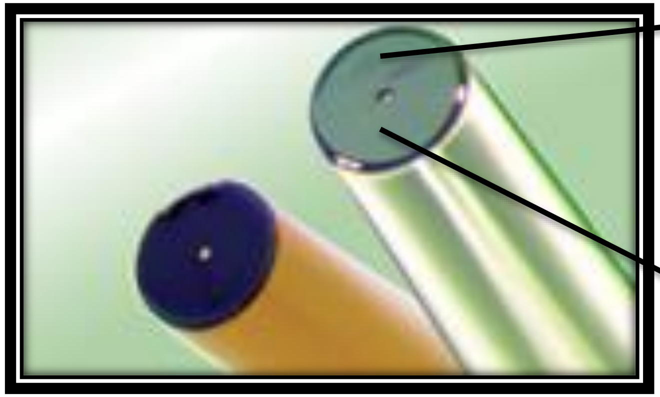
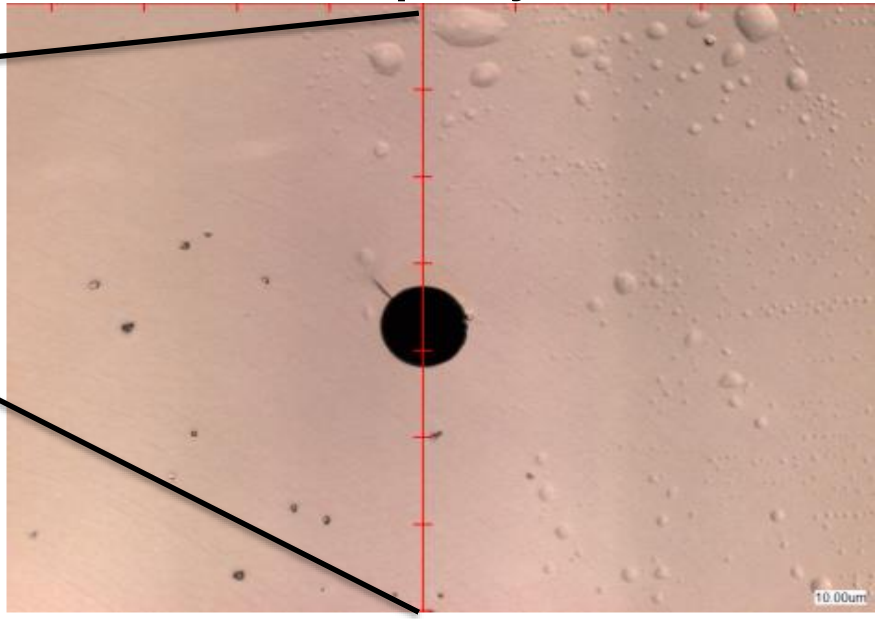
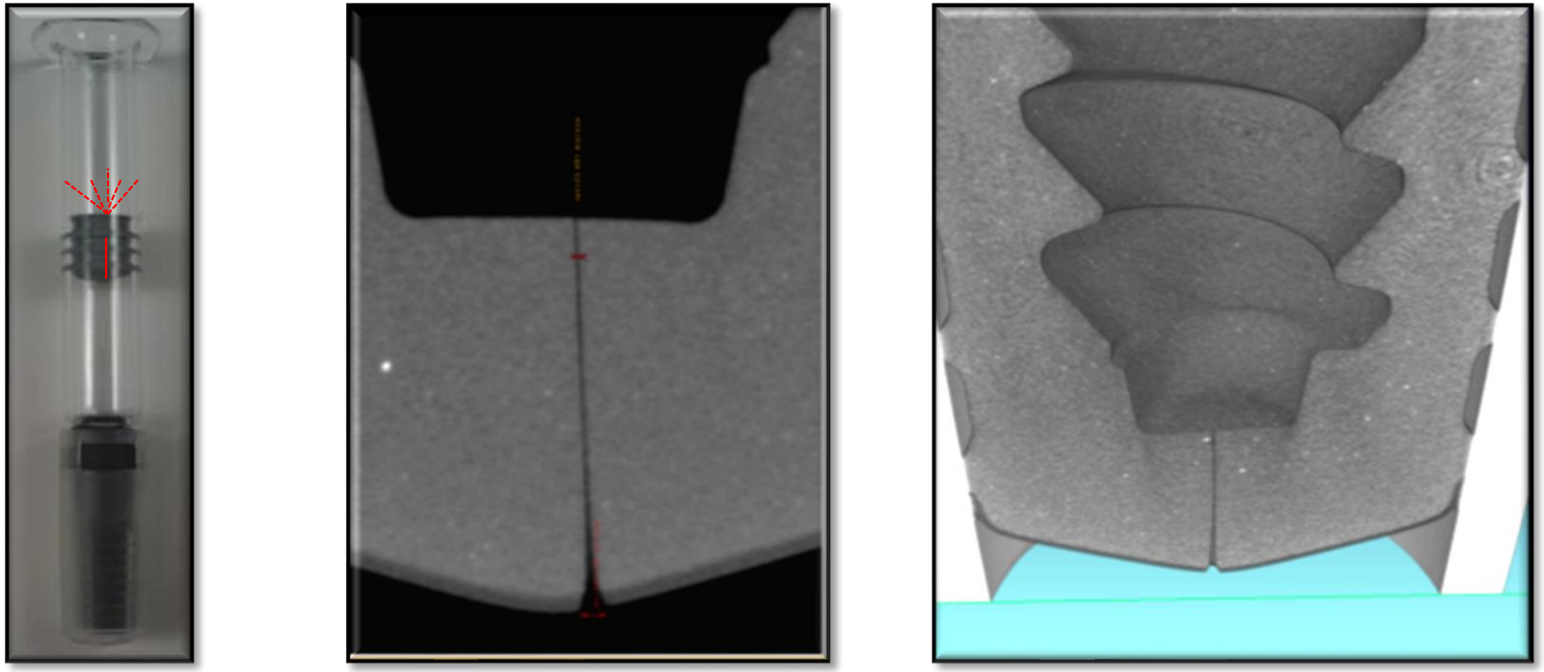


Image courtesy: Polymicro Technologies by Molex®

Uniform size through the length of the capillary tube



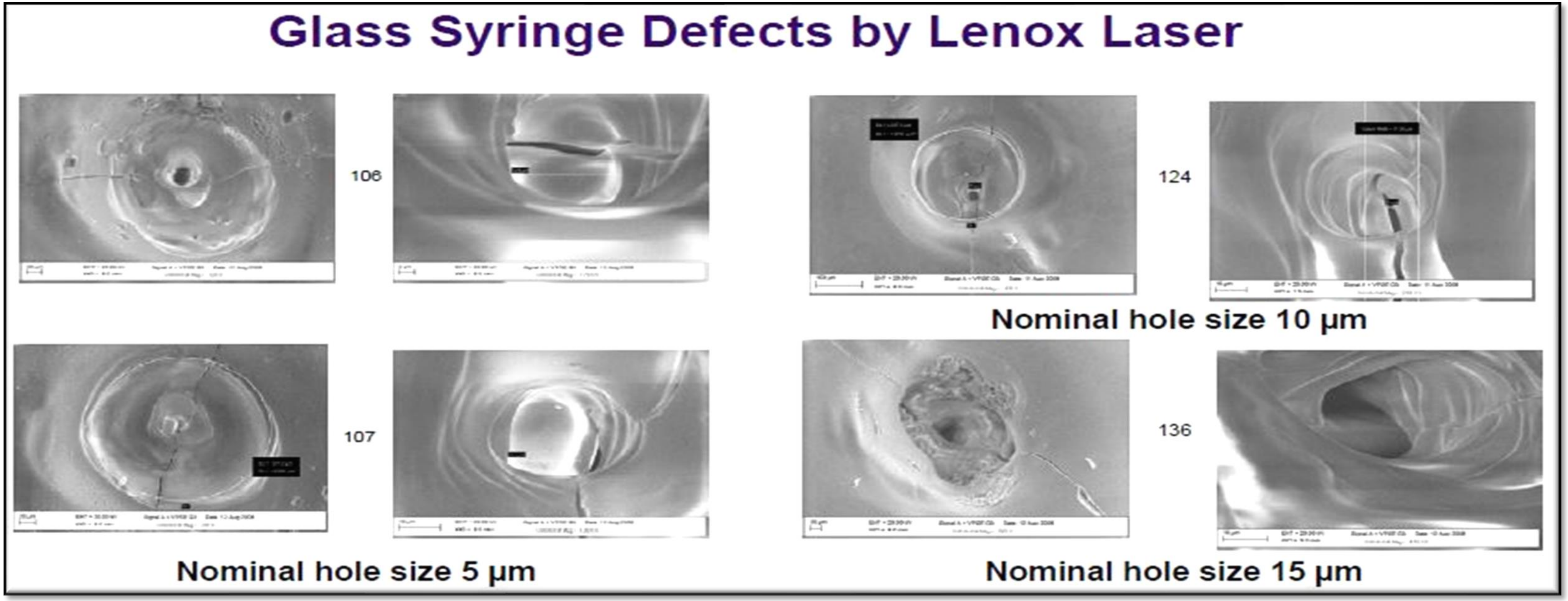
Laser Drilled Defect (Elastomer/Plastics)



- Laser drilled defect can be readily created in plastics or elastomer materials
- Defect size may change upon applying stress on elastomer materials (e.g., insertion in syringe barrel)

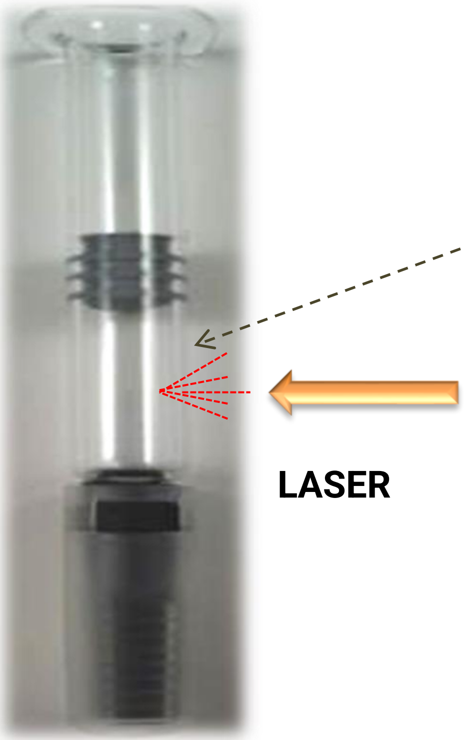
Laser Drilled Defects in Glass

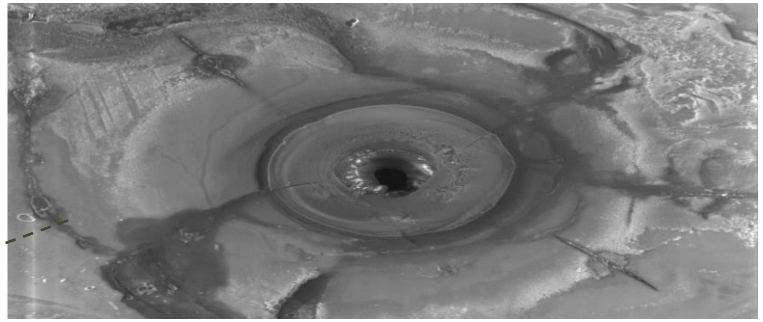
The laser-drilled defects in glass are not ‘ideal’ defects but realistic ‘tortuous path’ defects



Laser Drilled Defects in Glass

Example image of a laser drilled defect on the laser exit (i.e., glass interior surface)





Example image of a laser drilled defect on the laser entrance side (i.e., glass exterior surface)

- Laser drilled defects in glass are not well-defined holes of a uniform size.
- Some may be made to approximate “holes” while others may resemble a network of cracks – both are valuable as they represent specific types of “real-world” defects.
- Know the laser drill processes and the defect characteristics.

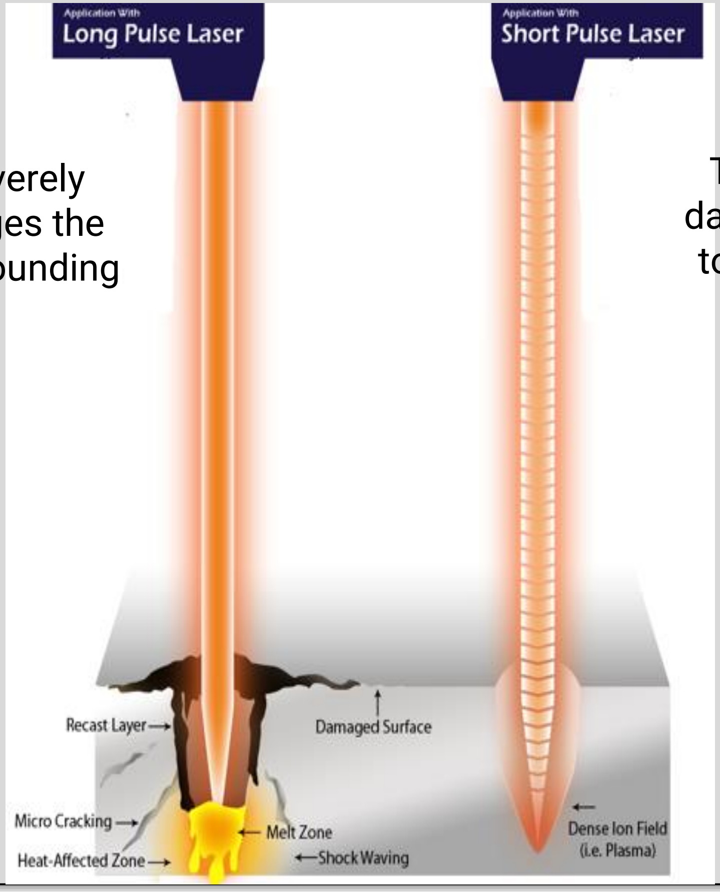
What is a "Calibrated Leak"?

Definition for CCIT case: Calibrated leak is the opening in wall of a container in a shape of orifice or crack that gives a specific flow rate for a given gas or liquid at given pressures applied to both sides of the material wall where the leak is made.

Slide courtesy of Greg Soylar, Lenox Laser

What is a “Calibrated Leak”?

This style of laser drilling severely damages the piece and changes the properties of the material surrounding the drilled hole.



This style of laser drilling with minimal damage to the piece. This is possible due to the creation of plasma in a technique called cold ablation.

Slide courtesy of Greg Soylar, Lenox Laser

Cross-Section view

Top view

Bottom view

Slide courtesy of Greg Soylar, Lenox Laser

Interwoven Plastic Bag

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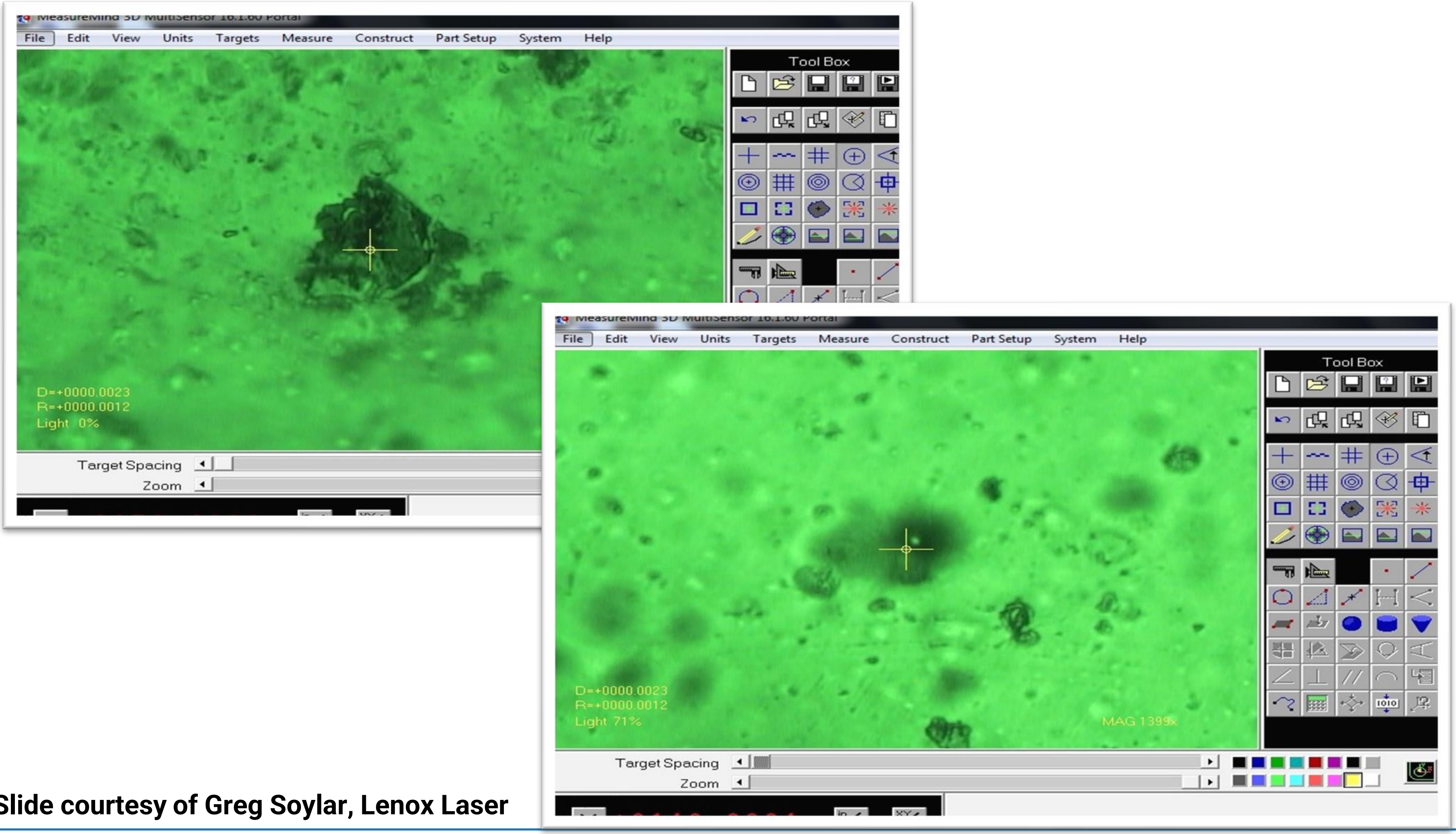
Plastic Bag (20 µm Thick)



Slide courtesy of Greg Soylar, Lenox Laser

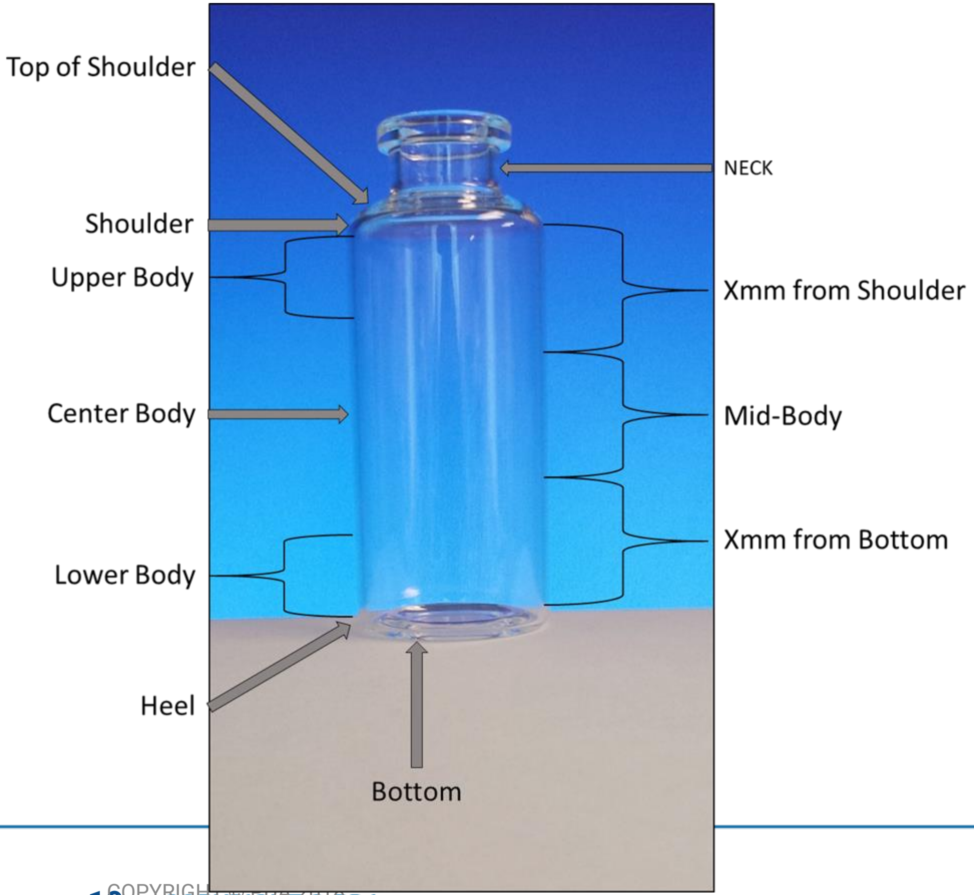
Leak Type	Advantages	Disadvantages	Experiences
Mirco-pipettes, ≥0.1 µm ID (e.g. glass)	Easy sample preparation	• Fragile & broken tips may not be detected • Difficult to determine hole size • Difficult to handle	• Too fragile for routine use • High risk of false sensitivity • Need complete seal around micropipette • Silicone oil can clog • Pinhole type defect • Material matches primary container if glass is used
Laser-drilled holes, ≥0.5 µm ID	• Wide range of sizes • Better resembles natural defects (cracks in glass; pinholes in polymer) • Newer techniques allow for defined holes; fewer cracks	• Cost • Size of laser-drilled void needs to be calibrated and represents defined path • Small hole can clog (silicone, viscous liq) • Holes can increase in size (temp changes, tension) • Variability in sizes depending on material/ wall thickness • Irregular shapes • Cannot be prepared on product	• Risk of alteration post manufacture/calibration • Specialized external supplier • Many material can be drilled • Closer to real world defects • May reuse positive controls (??) • Dirt or particulates could impact quality of holes

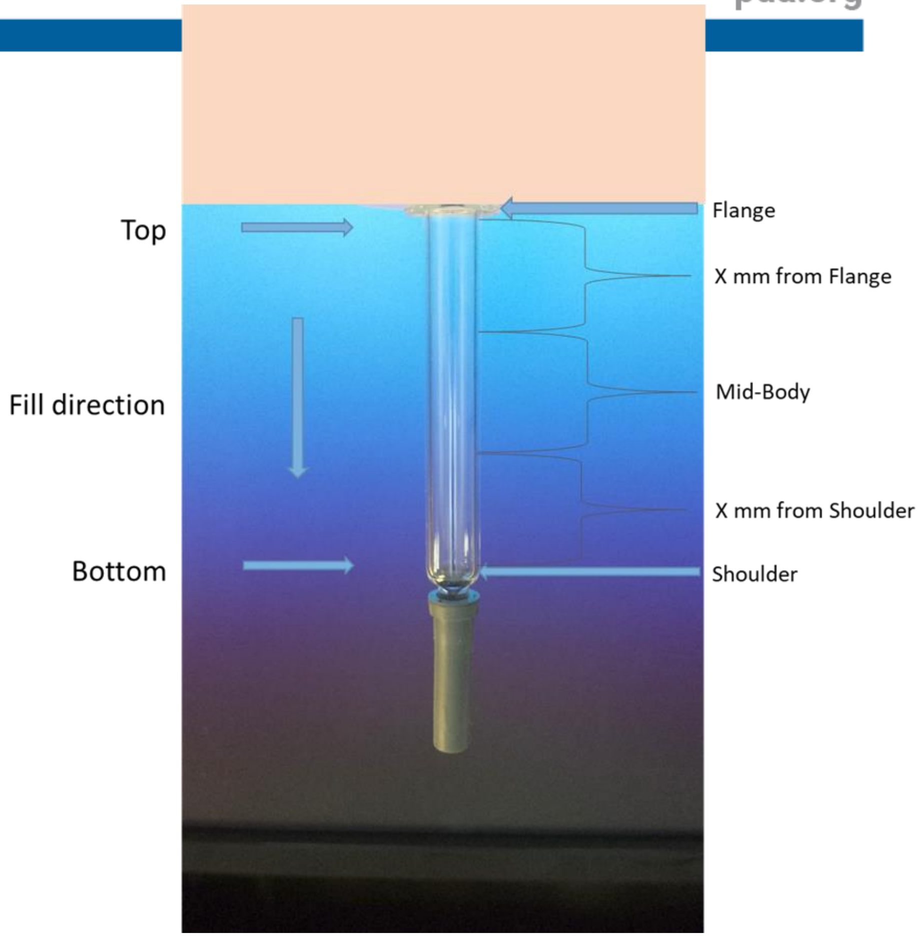
"Container Closure Integrity Testing – Practical Aspects and Approaches in the Pharmaceutical Industry"PDA J. Pharma. Sci. Technol. 2017 Mar-Apr;71(2):147-162

Leak Type	Advantages	Disadvantages	Experiences
Capillaries, ≥0.2 µm ID (e.g. fused silica)	• Robust • Easy preparation at testing location • Possible to prepare controls in specific packaging format and for multiple products • Prepared in flexible way (e.g. may contact liq and headspace)	• Length of microtube defects is usually longer than real world defects • Typically nominal diameters > 2µm available & uncertainty of actual diameter • Capillary diameter and hold diameter not comparable with regard to flow rate • Glue can create blockage	• Robust, wide size range & different materials available • Leakage rates can be fine-tuned through length of capillaries; not only using IDs • Consistent dimensions/leaks • Defined dimensions mean don't have to calibrate each lead
Micron wires, ≥10 µm ID (e.g. uncoated copper)	• Low cost • Robust	• Handling of micron wires can be difficult and size of void needs to be calibrated and represent undefined path • Holes can close up over time depending on material relaxation • No direct measurement of hole size	• Reproducible leak size with defined capping parameter and wire size • Leak size only defined when measured relative to physical phenomenon • Need to consider copper wire diameter and elastomer behavior for consistency • Actual size depends on many parameters

"Container Closure Integrity Testing – Practical Aspects and Approaches in the Pharmaceutical Industry"PDA J. Pharma. Sci. Technol. 2017 Mar-Apr;71(2):147-162

Common Hole Locations in Glass Vials





Common Hole Locations in Syringes

Which Type of Defect Should I Use?

- Use positive controls that best resemble "real-world" defects of interest to demonstrate method effectiveness.
- **Examples:** To demonstrate the method is capable of detecting 10um defects.

Defect Type of interest	Positive Controls (10um)
IV bag leaks caused by punctures/ abrasion	Laser drilled pinhole on the bag film with ~10um ID (measured by microscopic imaging)
Poor seals or micro-channels on the IV bag seams	10um capillary tube of similar length glued into the seam (the 10um ID can be microscopically verified)
Cracks in a vial glass wall (~2mm thick)	Laser drilled irregular glass defects, calibrated to be ~10um using gas flow rate per USP method

Characterization of Leaks

- Most industries uses leak flow rate to characterize a leak
  - ✓ Leak flow rate directly correlated to the material loss through the leak (e.g., compressed natural gas pipeline leaks)
  - ✓ Leakage Flow Rate is a measure of the rate of gas flow (mass or volume units) which passes through a leak path under defined conditions of temperature and/or absolute or partial pressure gradient of leaking matter that exists across the package barrier
- Gas leak flow rates are usually expressed in standard cubic centimeter per second (sccs) under standard conditions (temperature 273K, pressure 760 torr)
  - ✓ Other units are also widely used

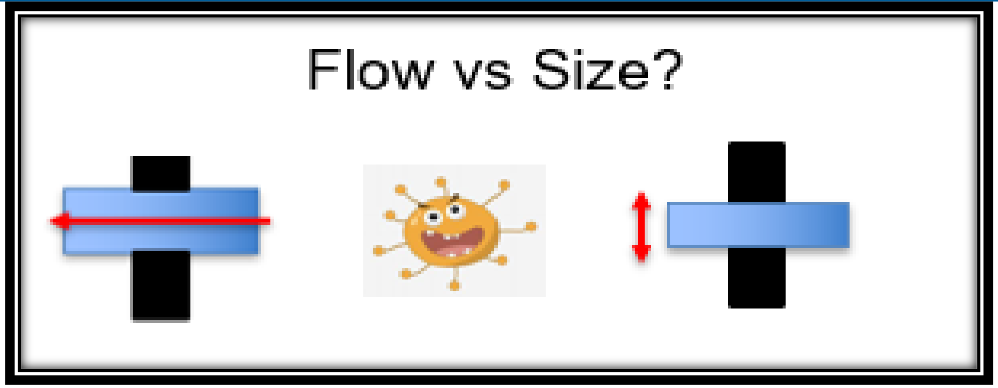
Jackson CN, Sherlock CN, Moore PO, Nondestructive Testing Handbook, 3rd ed. Vol 1 Leak Testing, American Society for Nondestructive Testing, Inc. 1998

Characterization of Leaks

Pascal Cubic Meter Per Second	Standard Cubic Centimeter Per Second	Millibar Liter Per second	Torr Liter Per Second
Pa · m^3 · s^{-1}	**Std cm^3 · s^{-1} (Alternatively, sccs)**	**mb · L ·s^{-1}**	**torr · L ·s^{-1}**
1	9.87 ($\simeq$ 10)	1.00 x 10^1	7.50

Jackson CN, Sherlock CN, Moore PO, Nondestructive Testing Handbook, 3rd ed. Vol 1 Leak Testing, American Society for Nondestructive Testing, Inc. 1998

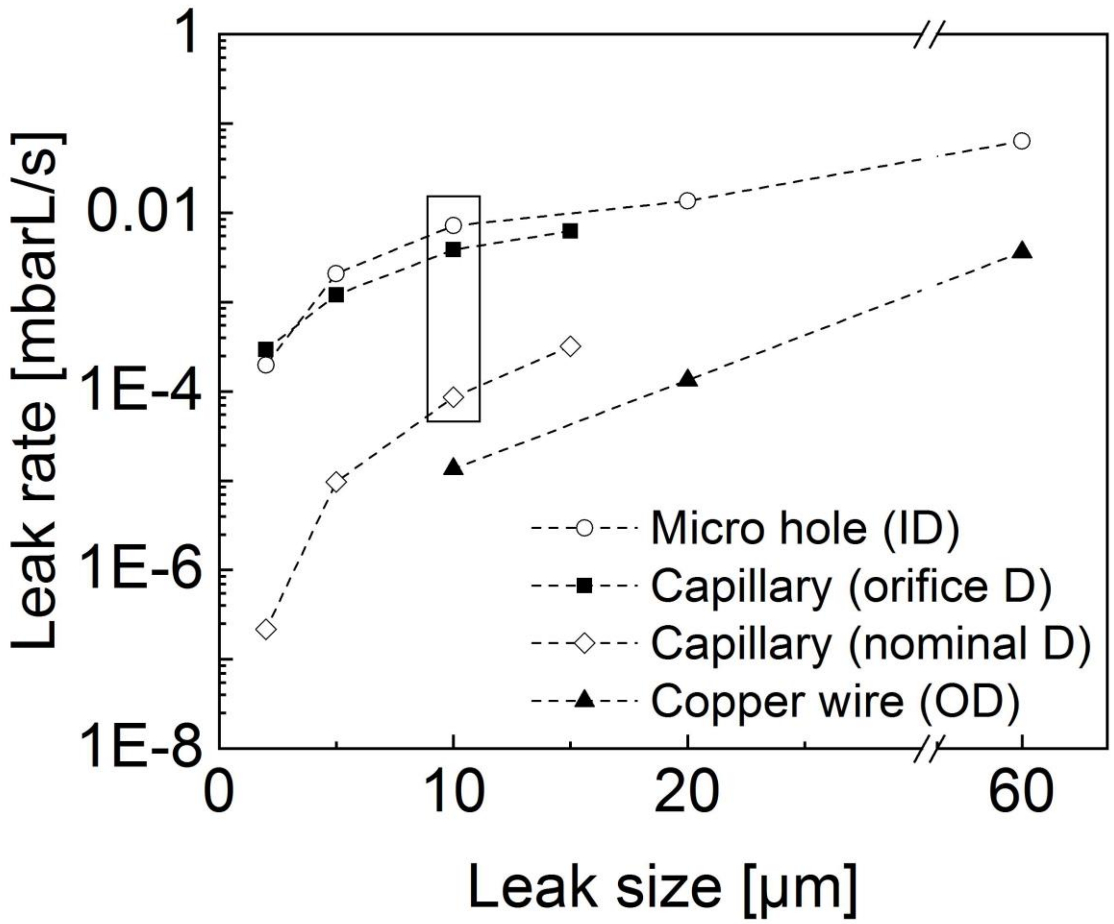
"Sizing" Leaks



"Leak size" is widely used in pharmaceutical industry as a key characteristic for leaks largely due to its close association with microbial ingress risk.

- Dimensionally sizing natural "real-world" leaks is not always practical nor precise.
  - Leaks are commonly thought of as HOLES or CHANNELS. But natural leaks are complex, multi-cavity, tortuous paths (rarely uniform)
  - Size alone does not fully define a "real-world" natural defect
- "Sizing", although not a comprehensive and precise characterization of a defect, is still useful because it allows for a rough and simplified assessment of microbial ingress risks

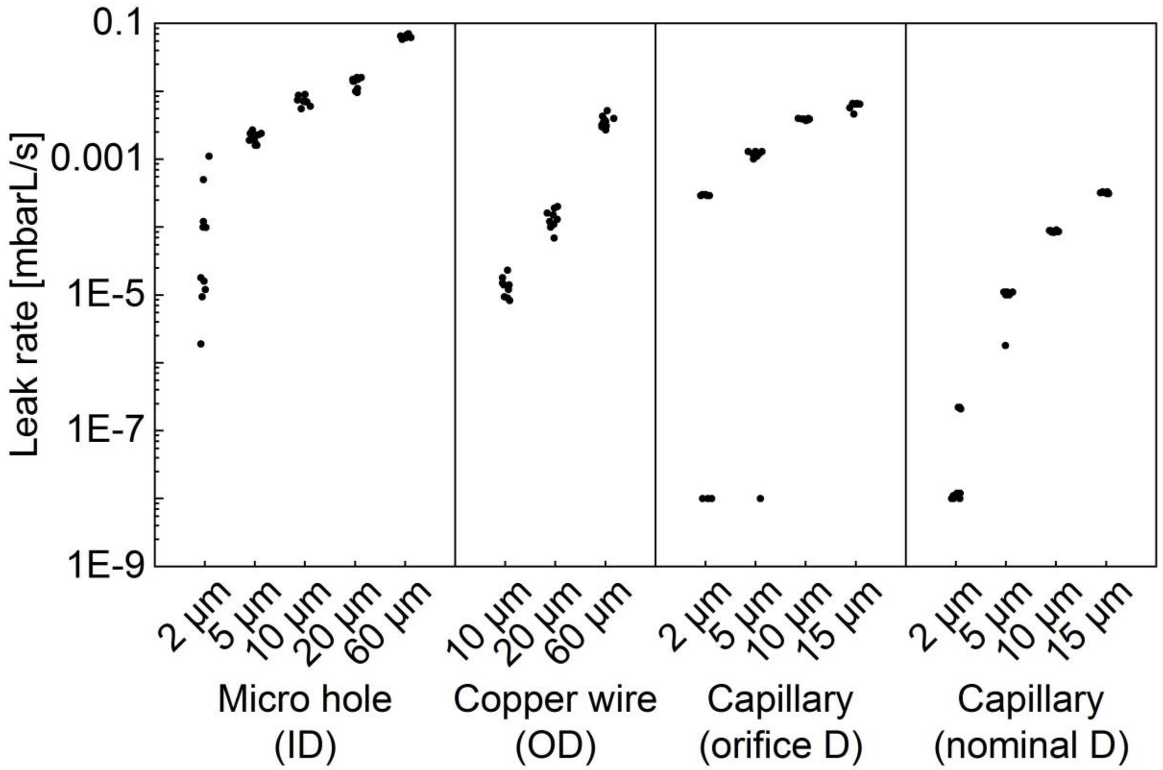
He Leak Rates for Artificial Leaks



- He leak rates increase with larger leak sizes
- Leak types show specific leak rates according to leak geometries
- 3 leak types at 10 µm significant differences in He flow rates
- Theoretical leak rates from equation mirco hole = capillary leak (orifice diameter) > capillary leaks (nominal diameter) > copper wire
- Strong dependence of glass flow rate on leak path length

Sarah S Peláez, Hanns-Christian Mahler, Christoph Herdlitschka, et al. PDA Journal of Pharmaceutical Science and Technology 2019. "Comparing Physical Container Closure Integrity Test Methods and Artificial Leak Methodologies."

Variability of Leak Rates of Artificial Leaks



- Variability increased for smaller leaks
- Artificial leaks can have significant variability in actual leak size and deviation from target
- Micro holes highest variability, especially at smallest diameter (complex and irregular shapes of micro hole channels contributes)
- Capillary leaks are consistent channels with know ID, but are quite different than real-life leaks
- Capillary leaks of very small ID can become clogged or defective (see very low leak rates), capillary leaks must be prepared with care and handled with care
- Copper wire can have kinks in the wire, become entrapped in rubber stopper wrinkles, breakage of wire; path length may vary based on stopper, leak channel size cannot be defined

Sarah S Peláez, Hanns-Christian Mahler, Christoph Herdlitschka, et al. PDA Journal of Pharmaceutical Science and Technology 2019. "Comparing Physical Container Closure Integrity Test Methods and Artificial Leak Methodologies."

Methods for "Sizing" Defects Characterization

Direct microscopic dimensional measurement

- Usually applies to simple "regular" natural defects (e.g., pin holes on an IV bag film) or positive control defects (e.g., capillary tubes)

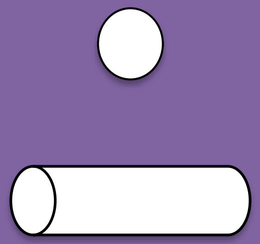
Calibration via gas diffusion rate (or headspace changes) measurement

- Gas diffusion and headspace composition modeling are usually acceptable

Methods for "Sizing" Defects Characterization

Calibration via gas leak flow rate measurement

- Usually for complex "real-world" defects (e.g., glass cracks)
- *A "nominal" size* can be obtained by comparing the gas flow rate of the defect against known leak standards (e.g., NIST-traceable leak standards)
- Two types of leak standards are commonly used – Need to specify
  - ✓ Orifice (a pin-hole of known size with essentially no depth)
  - ✓ Capillary (with known uniform ID and Length)



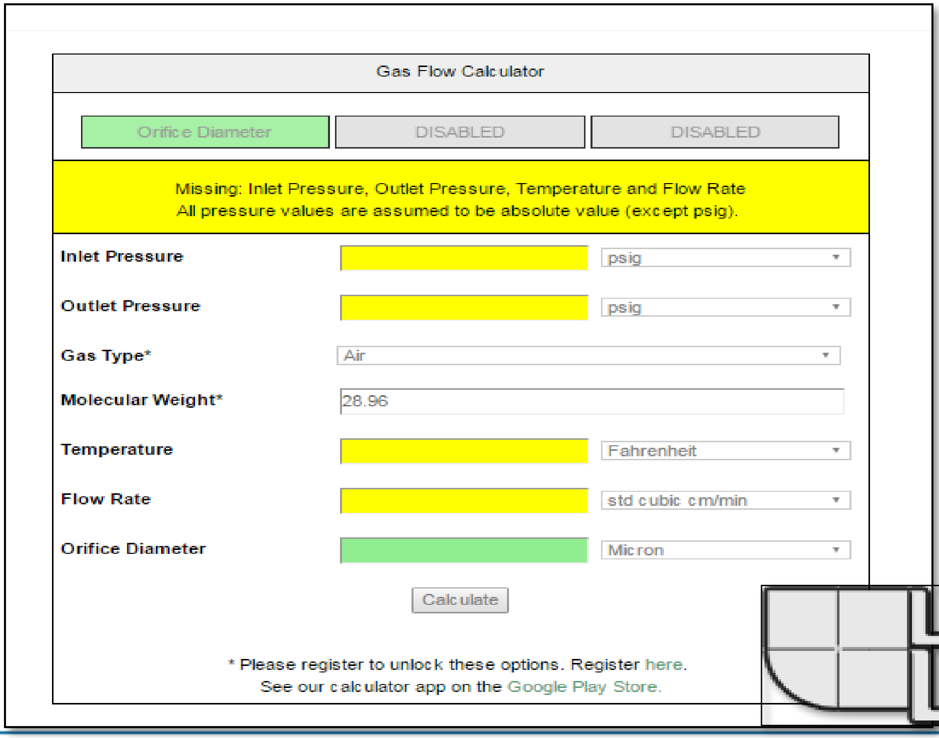
Sizing Based on Gas Leak Flow Rate – Calibration using Orifice Leak Standards

For orifice leaks (i.e., leaks with essentially no "depth", a.k.a. sharp edge defects)

Flow Rate $\propto d^2$

- Flow rate usually measured at a fully "chocked" conditions to simply the calculation.
- Methodology is included in USP<1207>.
- Nominal sizes can be easily calculated using the flow rate equation or online calculators

Calculator Examples:
https://lenoxlaser.com/resources/calculators/orifice-calculator/



Gas Leak Flow Rate vs. Orifice Size

Row	Air Leakage Rate[a] (stdcm^3/s)	Orifice Leak Size[b] (µm)
1	<1.4 x E-6	<0.1
2	1.4 x E-6 to 1.4 x E-4	0.1 to 1.0
3	>1.4 x E-4 to 3.6 × E-3	>1.0 to 5.0
4	>3.6 x E-3 to 1.4 × E-2	>5.0 to 10.0
5	>1.4 x E-2 to 0.36	>10.0 to 50.0
6	>0.36	>50.0

[A] Dry air leakage rate measured at 1 atm differential pressure across an orifice leak (i.e., leak inlet pressure of 1 atm versus outlet pressure of approximately 1 Torr) at 25 . The theoretical correlations of orifice sizes to air leakage rates were provided by Lenox Laser, Glen Arm, MD. Leakage rates are approximation ranges.

[B] Nominal diameter orifice sizes assume a leak path of negligible length. Orifice sizes are approximation ranges.

Sizing Based on Gas Flow Rate – Calibration using Capillary Leak Standards

For defects with significant length (L)

Gas Flow Model EXAMPLES

- Hagen-Poiseuille viscous flow (barometric, shallow vacuum)

$$Q = \frac{128}{\pi}\frac{d^4}{L} \times \frac{P^2{}_{IN} - P^2{}_{OUT}}{\mu}$$

- Knudsen model for molecular flow (small defects, hard vacuum)

$$\dot{m} = \frac{\pi d^3}{\sqrt{2RT}} * \frac{P^2{}_{IN} - P^2{}_{OUT}}{L}$$

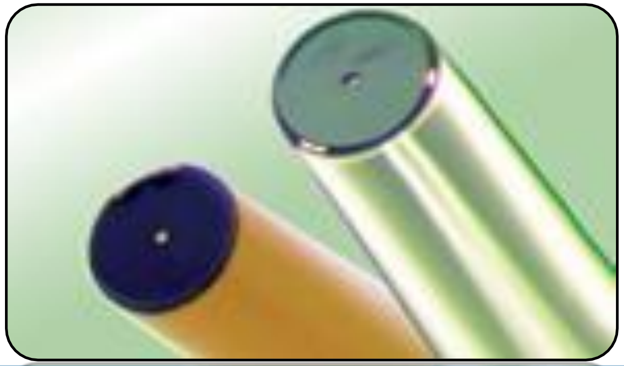
P_{in} - pressure inside package;
P_{out} - pressure inside chamber;
μ - Viscosity; T - Temperature; R - Specific Gas Constant

Flow (Q-volumetric flow, mass flow)

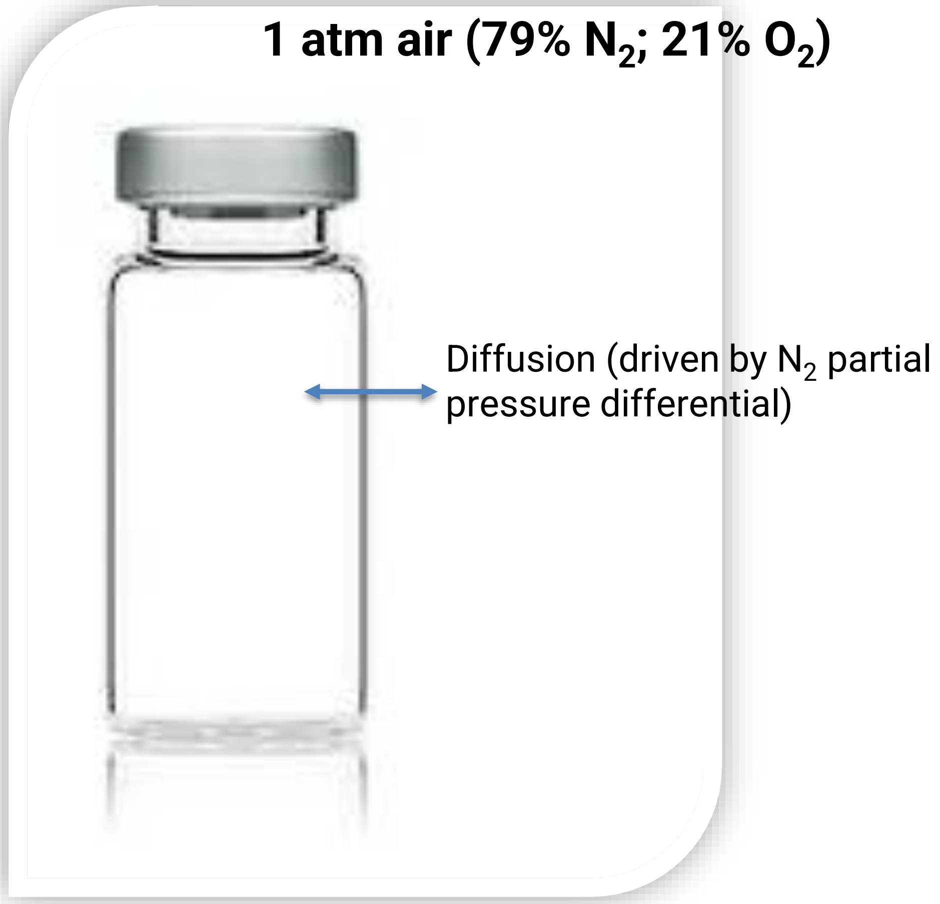
= f (**d**, Length, Pin, Pout, T)

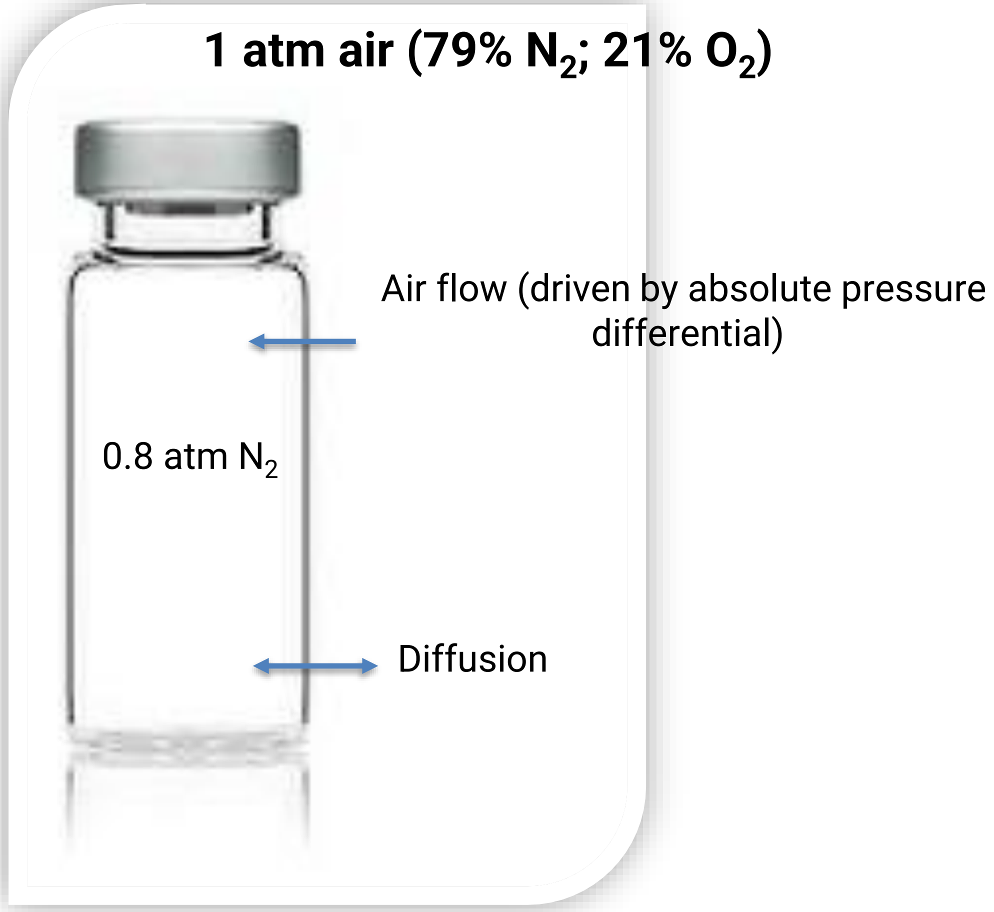
Kept constant

- Nominal sizes can be readily obtained experimentally by comparing flow rates of samples against those of known capillary standards (preferably of similar length L)

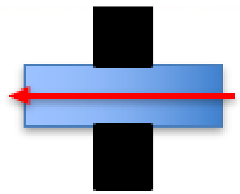


Sizing Based on Headspace Changes





Sizing Based on Gas Diffusion Rate (or Headspace Gas Concentration Change)



$$\vec{J} = -D\vec{\nabla}n$$ **Fick's 1st Law**

$$\frac{\partial P_i(t)}{\partial t} = \frac{-D \cdot A_0}{V} \frac{\partial P_i(z,t)}{\partial z}$$

$$P_{oxygen}(t) = 20.9\%(1 - \exp(-\alpha t))$$

Ingress Rate $$\alpha = \frac{D \cdot A_0}{l \cdot V} \left[s^{-1}\right]$$

USP <1207> states: “Mathematical models appropriate to leak flow dynamics may be used to predict the time required for detecting leaks of various sizes or rates.”

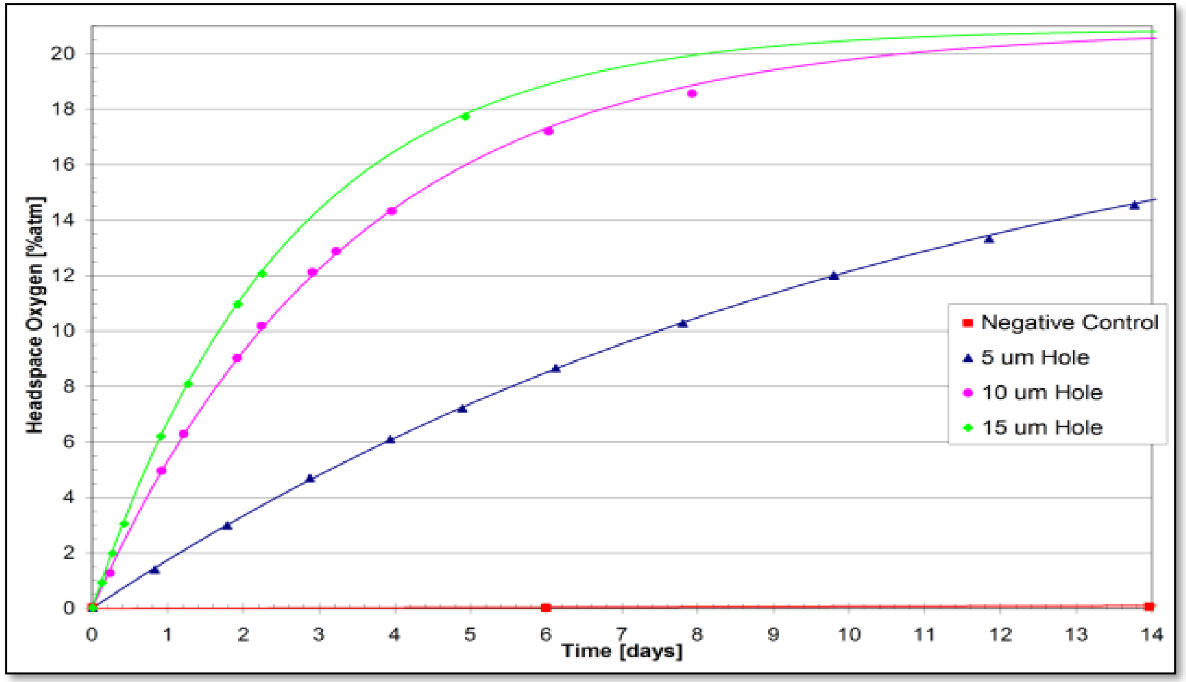
The change in oxygen will be exponential with respect to time

The Ingress Rate is a function of the Diffusion Coefficient, the container Volume and the defect cross-sectional Area and Depth

D. Duncan, Lighthouse Instruments

Example: Headspace Oxygen % vs. Leak Size

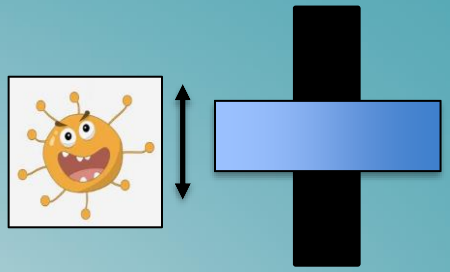
The linear regression fit (RSQ) for each line was 0.99 or higher for each set of data.



Laser-drilled holes in thin metal plates are well-defined defects. They can therefore be used to generate 'calibration data' for gas ingress dynamics through a defect into a container.

D. Duncan, Lighthouse Instruments

Reporting Leak Size



- ❑ “Nominal” size – a useful tool for communication with key stakeholders (e.g., business leaders, regulatory agency microbiology reviewers)
- ❑ Many methodologies can be used to “size” a leak – the resultant nominal sizes may not in full agreement
- ❑ No consensus methodologies haven been established yet
- ❑ When stating leak sizes (or reporting package integrity), it is important to **define the measurement approach – Be transparent!**

Reporting Leak Size - Examples

Example 1. An intentional leak size-certified in gas flow rate terms.

A laser-drilled hole = 10.3 µm, certified that the air flow rate through this defect matches that of a same diameter pre-drilled hole in a thin metal plate measured at defined pressure and temperature conditions.

- *Comment: This statement indicates that the intentional leak sample is characterized using USP 1207 methodology and therefore is suitable for use as a positive control in method development and validation studies*

Reporting Leak Size - Examples

Example 2. Unintentional leak(s) directly sized in gas flow rate terms.

A test package containing 100% helium (flooded prior to closure) demonstrated a helium leak rate of 1 x 10-7 mbarL/s, when tested using a helium mass spectrometer, at 1 atmosphere differential pressure and ambient temperature.

- *Comment: This statement indicates that resultant helium leak rate is obtained using the identical methodology by Lee Kirsch as referenced in USP 1207. Therefore, the measured leak rate can be directly compared against the MALL to demonstrate container integrity.*