Consistent Product Robustness, the Science and the Technologies for **Proven Single-Use System Integrity (SUSI)**

Case Study: Helium Integrity Test

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What Are We Going to Talk About?

Container Closure Integrity (CCI)

Filter Integrity

Single-use System Integrity (SUSI)

Leak vs. Integrity Testing & Definitions

Leak test – a test used to identify leaks not correlated to the defined barrier properties of a SUS.

Integrity Test – a test used to confirm the defined barrier properties of a SUS.

Maximum Allowable Leakage Limit – the greatest leakage rate (or leak size) tolerable for a given product package to maintain its barrier properties under its use-case conditions (e.g. prevent any risk to product safety, product quality or operator and environmental safety).

Integrity Assurance – a holistic approach of risk analysis and mitigation by means of product and process robustness, quality, and process control and integrity testing to assure that a SUS maintains its integrity prior to and during use.

Non-destructive test method – a test method that maintains the test article in a condition for further use, without impacting its quality attributes.

Destructive test method – a test method that will alter the intended use of the test article during the test and not allow further use.

Single Use Process Integrity Is a Key Industry & Regulatory Challenge

Validated single-use system integrity required to:

- Strengthen regulatory compliance
- Improve patient & operator safety
- Avoid process downtime & batch loss

Single-use system integrity testing (SUSIT) is meaningful when it correlates to liquid leaks & microbial ingress

- Lack of understanding of defect size causing liquid leaks and microbial ingress under real life conditions
- Previous testing is not correlated to leaks & microbial ingress

> Bag failures cost ~$100K to $1M per bag
>
> R. Wong, Bayer

> Leaks have been reportedly responsible for up to $20M worth of products per year at some larger facilities.
>
> Bioplan

Single Use Process Integrity Is a Key Industry & Regulatory Challenge

SUS Used in Drug Substance & Drug Product Process Steps Require Higher Assurance of Integrity

> We're very much concerned about the integrity of SUS used in final filling applications.
>
> FDA Inspector 02|2018

Regulatory Bodies Are Pushing for More Testing

European Commission

Annex1 - Manufacture of Sterile Medicinal Products *(Draft Revision V13 – Not yet published)*

8.133 Appropriate measures should be in place to **ensure the integrity** of components used in aseptic connections. The means by which this is achieved should be determined and captured in the CCS*. **Appropriate system integrity tests** should be considered when there is a risk of compromising product sterility. Supplier assessment should include the collation of data in relation to potential failure modes that may lead to a loss of system sterility.

8.135 SUS are those technologies used in manufacture of sterile products [...].

8.136 There are some specific risks associated with SUS which should be assessed as part of the CCS*. These risks include but are not limited to:

i. [...]
vi. The risk of holes and leakage.
vii. The potential for compromising the system at the point of opening the outer packaging.

FDA-ASTM Workshops – Oct 2016(1) & Apr 2018(2)

Validation for SU fill finish assemblies for sterile product manufacturing

- **Physical integrity test correlated to microbial ingress**
- Packaging integrity at the supplier, post shipping & post-installation

Microbiological challenge testing

- Identification of defect size that would allow ingress of bacteria **under process conditions**

SUS fill finish assemblies for sterile products must meet requirements to ensure flow-path sterility and integrity

- Supporting sterilization validation summary data and information on the gamma irradiation process
- **Integrity tests are also reviewed during the review of the BLA(3) and on inspection**

(1) Presentation "SUS: A Microbiology Product Quality Perspective" - Patricia F. Hughes, Ph.D.., Branch Chief (Acting), CDER/OPQ/OPF/DMA
(2) Presentation "Sterile drug product, New developments" - Patricia F. Hughes, Ph.D.., Branch Chief (Acting), CDER/OPQ/OPF/DMA
(3) Biologics License Application

Current Industry Guidance and Standards Initiatives

Annex1 - Manufacture of Sterile Medicinal Products (Revision – Not yet published)

ASTM E3244-20 Standard Practice for Integrity Assurance & Testing of SUS

ASTM E3251-20 Test Method for Microbial Ingress Testing on SUS

ASTM E3336-22 Test Method for Physical Integrity Testing of SUS

PDA TR 86 2021 Industry Challenges and Current Technologies for Pharmaceutical Package Integrity Testing

BPSA 2017 Design, Control, and Monitoring of SUS for Integrity Assurance

The Challenge Is to Ensure That No Product Leakage or Microbial Ingress Can Occur During the Process

- QbD & validation to reach consistent robustness
- Process control, quality control & integrity testing

» SU Product Robustness

- Understand liquid leakage & bacteria ingress mechanisms
- Correlate integrity testing detection limits to liquid leaks & microbial ingress under process conditions

» SU System Integrity Science

- Upon request, Implement 100% physical supplier integrity testing
- Provide point of use testing solutions

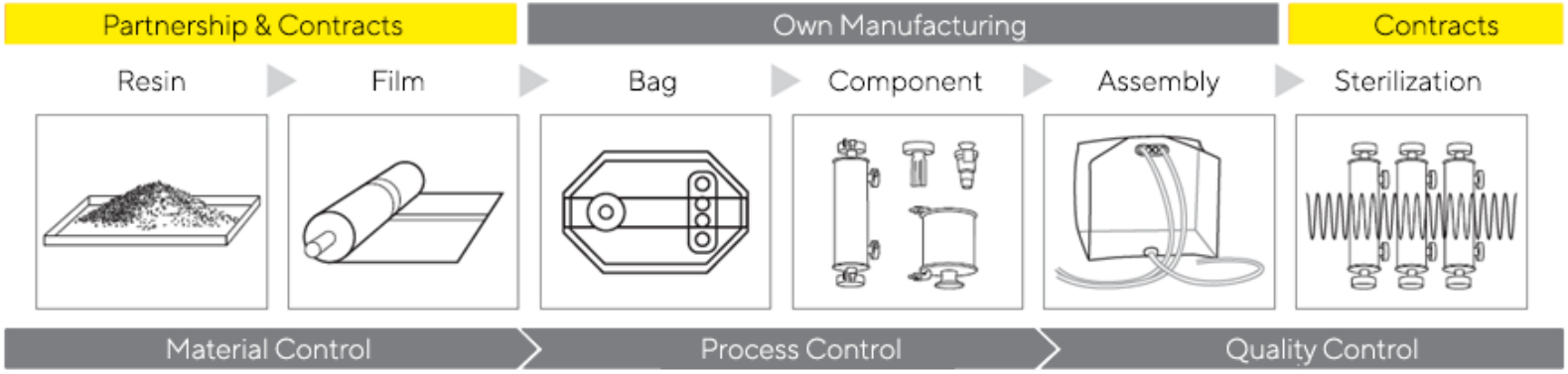
» Integrity Testing Technology

Combined with the robustness of our self-deploying bags, our integrity tests ensure that no product leakage or microbial ingress can occur during the drug manufacturing process

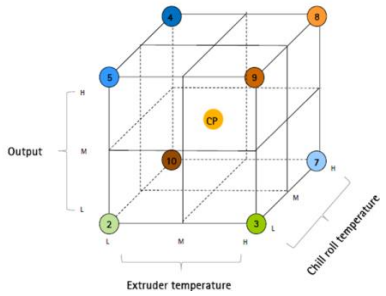
Process Control & Quality Control Ensure Integrity Along Our Entire Production Cycle

Process control reduces the risks of defects on film, seals, welds & connections
- Extrusion temperature & speed,
- Weld & seal temperature & time

Quality controls confirm the absence of leaks
- Film & seal strength integrity,
- 100% Bag Chamber Leak Test (BC-LT)



Process Control & Quality Control Ensure Integrity Along Our Entire Production Cycle



~ 3000 robustness tests

Tensile Strength: 930

Elongation at break: 930

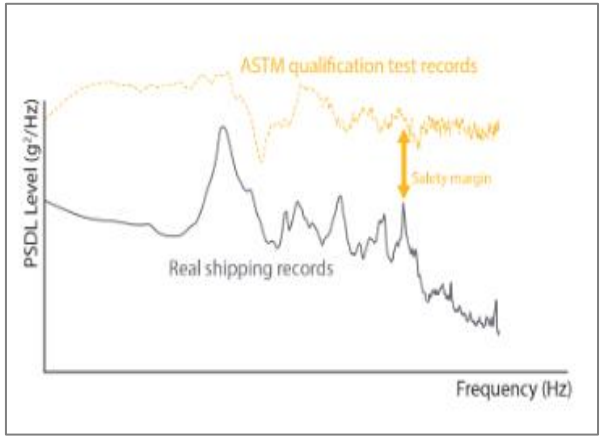
Puncture tests: 930

Flex durability: 186

Water burst tests : 26

Most stringent standards:

ASTM D4169
validation package with
real & lab. tests

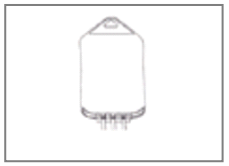


Passed ASTM Shipping Test

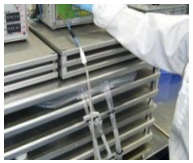
Self-deploying bag
during installation

Avoids mishandling
&
reduces bag failures

Process Control & Quality Control Ensure Integrity Along Our Entire Production Cycle



Bag chamber leak test 40 – 90 µm



Discard defects from manufacturing operations

Assembly Helium SIT 2 µm

Discard additional defects from manufacturing operations

But, why 2µm?

PoU-IT 10 µm FlexAct® BT

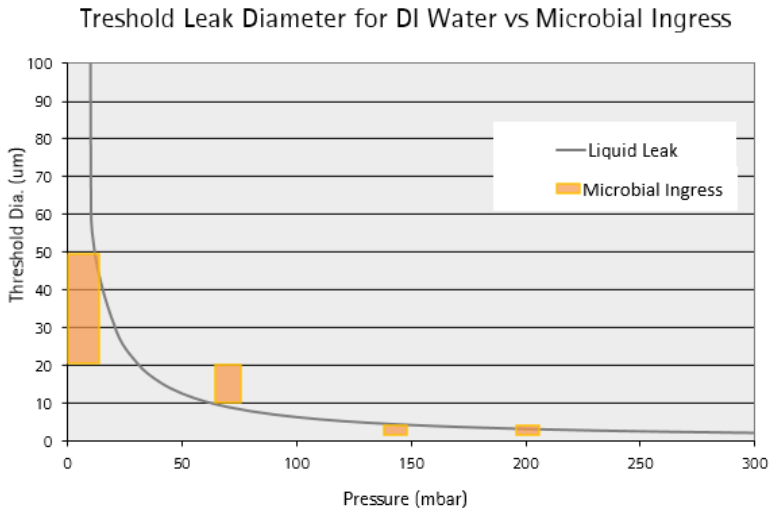
Discard additional defects from transportation & handling

Existing Data in Peer-reviewed Publications on Critical Leak Size for Sterile Package Using Micro Tubes

Gibney, 2000 $P_o > P_{atm} + \left[\left(\frac{4\sigma}{D_h} - \rho g L\right) \times 0.390\right]$

Liquid leak & bacterial ingress depend on
- Process & applications conditions
- Liquid properties
- Size of defect

Correlation between liquid leak & bacterial Ingress
- Microbial ingress cannot occur without liquid flow *(Kirsch & Morton, 1989)*



2µm is the maximum allowable leakage limit that never produce liquid leak or microbial ingress in any process conditions using micro tubes

Targets of Sartorius' Integrity Science Studies

Understand liquid leakage and microbial ingress mechanisms on film materials used in single-use biomanufacturing.

- Demonstrate the relationship between microbial ingress and liquid leak.
- Establish predictive models to determine the MALL* under any process conditions (mainly pressure).
- Establish a correlation between liquid leak/microbial ingress and physical integrity testing.
- Develop and validate the physical test methods with detection limits that guarantee the absence of liquid leak/microbial ingress in SUS.

* MALL: the greatest leakage rate (or leak size) tolerable for a given product package to maintain its barrier properties under its use-case conditions, (ASTM E3244)

Process Conditions May Impact Liquid Leak & Microbial Ingress

Hydrostatic pressure in storage

~0.7m / 2’

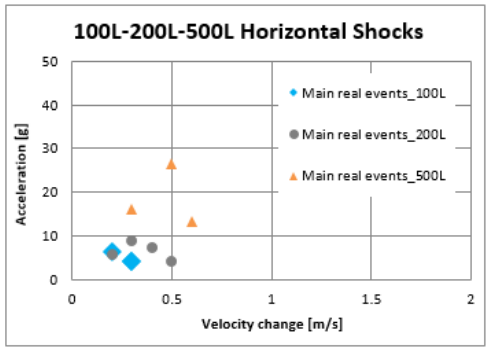
Max pressure 70mbar

Flexboy® 20 L

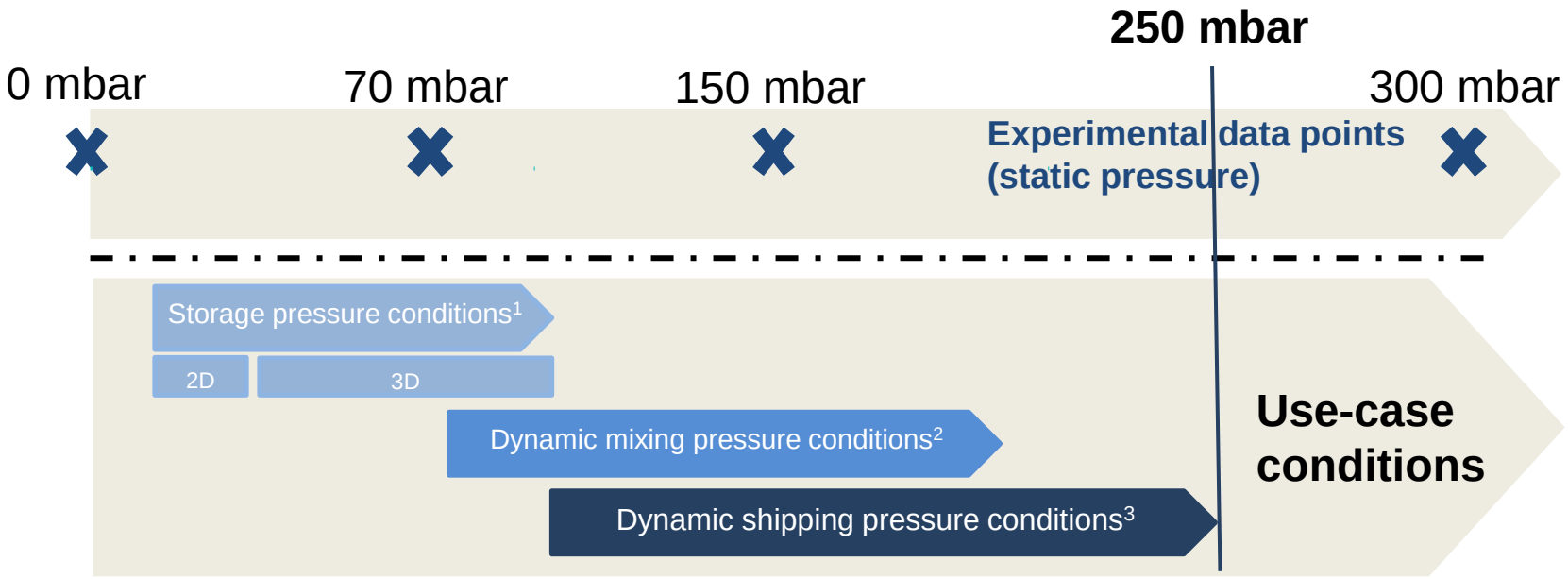
Flexsafe® 500 L

Application	Pressure range
Storage	~10 – 100 mbar
Shipping	~100 – 250 mbar

Shipping can generate up to 20g acceleration



Pressure Ranges for Integrity Science Studies



Range of pressures covered by Integrity Sciences experiments (0-300 mbar) exceeds most severe use-case conditions (max 250 mbar).

[1]Range represents static pressure due to liquid column height in the bag
[2]Range represents dynamic pressure pulses due to the liquid motion during mixing
[3]Range represents dynamic pressure pulses due to acceleration and shocks during liquid shipping

Liquid Tests on Film Show No Leak on 2µm Defects at **300mbar** Pressure

Liquid leak results for PE film at 300mbar imposed pressure

	Hole size µm	2 µm	3 µm	5 µm	10 µm
Water	Number of samples	3	3	3	3
	Number of leaks	0	2	2	3
TSB*	Number of samples	3	3	3	N/A
	Number of leaks	0	1	1	N/A

* Trypticase soy broth (growth media for BCT trials)

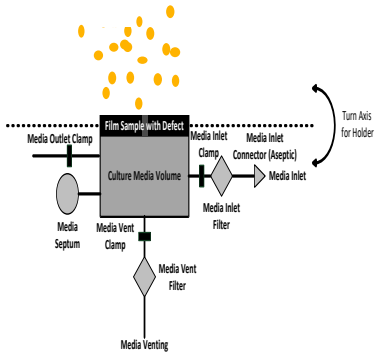
<u>Multiple module setup:</u>

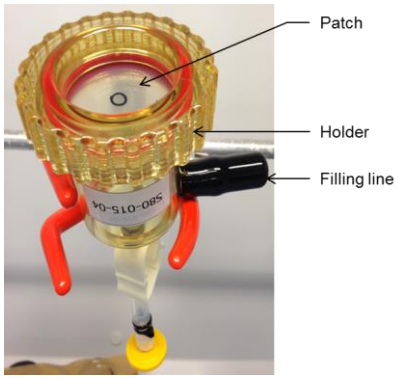
- Holders with different defect size patches
- 100ml model solution filled into each holder
- Holders connected to constant pressure source
- Indicator paper and stopwatch for leak observation

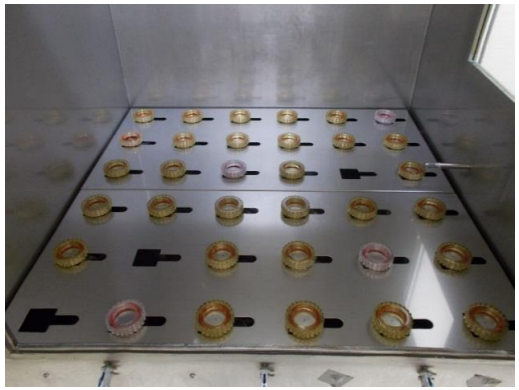
Microbial Aerosol Tests on 300 Film Samples Show No Ingress for 1µm Defects at **300mbar** Pressure

BCT results at 300mbar imposed pressure

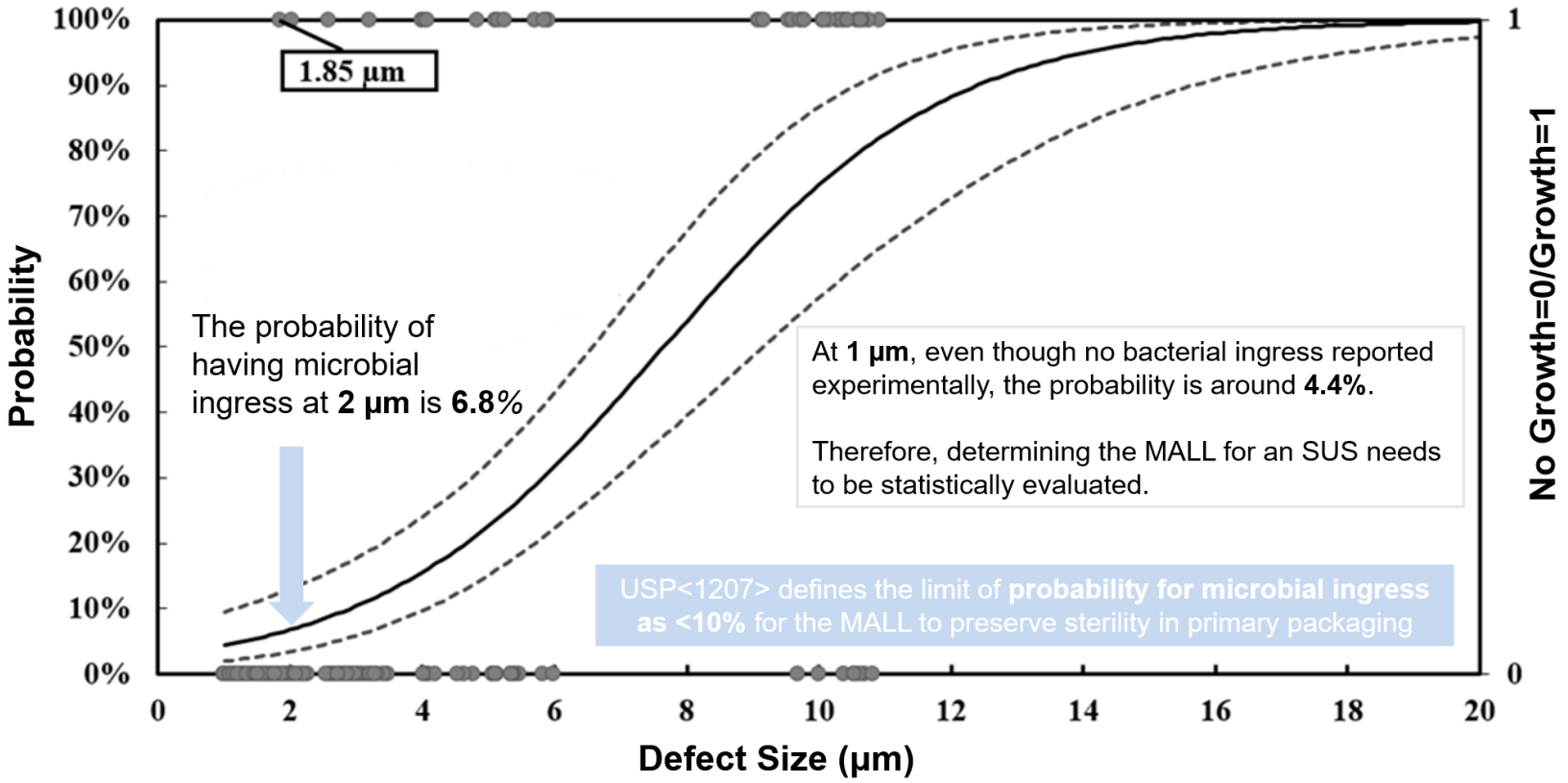
	Hole size µm	1µm	2µm	3µm	5µm	10µm
PE film	Number of samples	30	30	30	30	30
	Number of ingress	0	2	2	10	22
EVA film	Number of samples	30	30	30	30	30
	Number of ingress	0	0	9	17	13



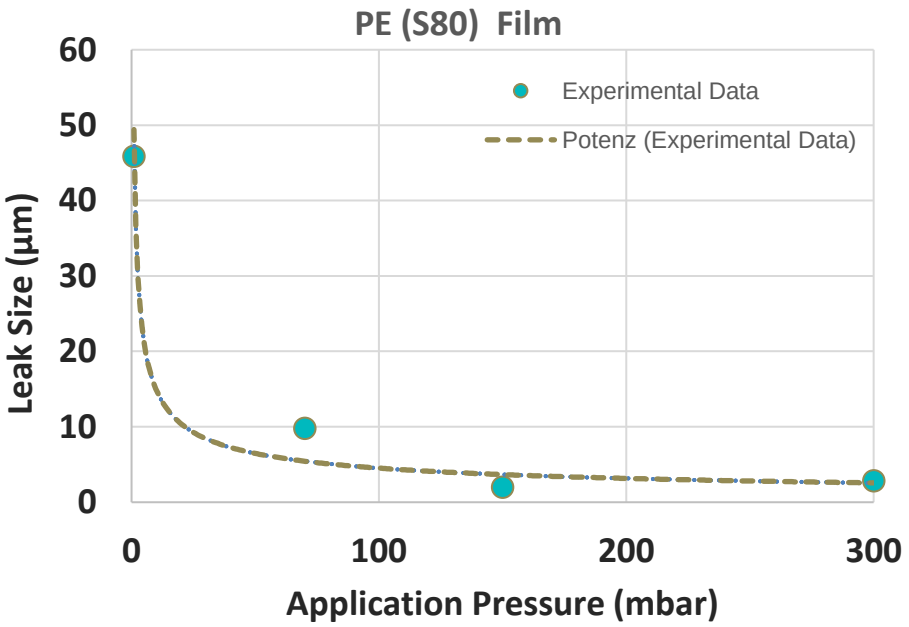




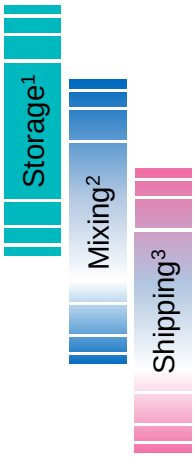
Probability for Microbial Ingress at **300 mbar** Pressure on PE Film



Predictive Model | MALL for Microbial Ingress on PE Film



Pressure* [mbar]	MALL Experiments [µm]	MALL Model [µm]
0	45.87	-
10	-	14.93
70	9.78	5.43
100	-	4.51
150	1.96	3.65
200	-	3.14
250	-	2.80
300	2.83	2.55



* Static pressure during 4h aerosolization phase

Conclusion of Experimental Data of Liquid Leak and Microbial Ingress Testing

- Results obtained on film materials fall into the existing models for sterile package using micro tubes
- Our experimental data:
  - Confirmed the **relation between microbial ingress and liquid leak**
  - Allowed creation of predictive models to determine the Maximum Allowable Leakage Limit (MALL) **under any process conditions.**
  - Identified 2µm as MALL under most severe use-case conditions, a **correlation between liquid leak/microbial ingress and our physical integrity testing was established.**

PDA Journal Publication Series:

Single-use System Integrity I: Using a Microbial Ingress Test Method to Determine the Maximum Allowable Leakage Limit (MALL) https://journal.pda.org/content/73/5/459

Single-use System Integrity II: Characterization of Liquid Leakage Mechanisms https://journal.pda.org/content/early/2020/11/16/pdajpst.2020.012088

Single-Use System Integrity III: Gas Flow Rate Through Laser-Drilled Microchannels in Polymeric Film Material https://journal.pda.org/content/early/2021/06/15/pdajpst.2021.012631

Case Study: Helium Integrity Testing

Case Study: Helium Integrity Testing

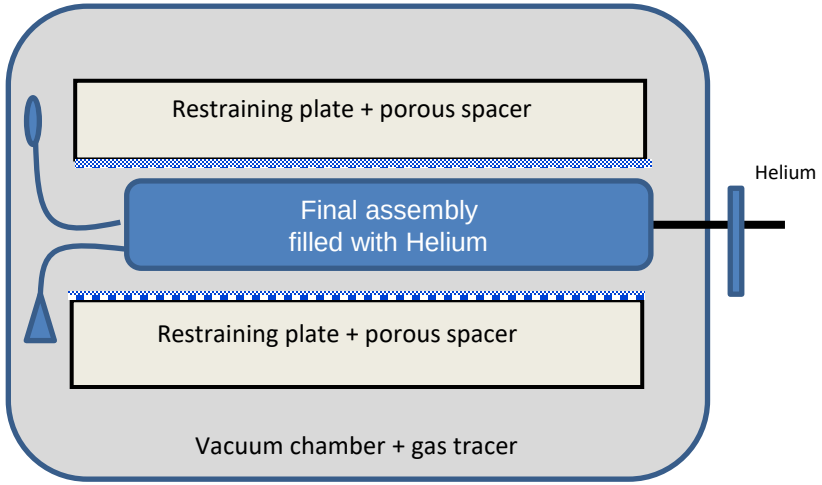
Main Requirements:

- Test of finished product (bag chamber incl. lines & connectors)
- 100% non-destructive testing (deterministic approach)
- Sensitivity that can be correlated to bacterial ingress
- Capable to be implemented in-line in routine production

Case Study: Helium Integrity Testing

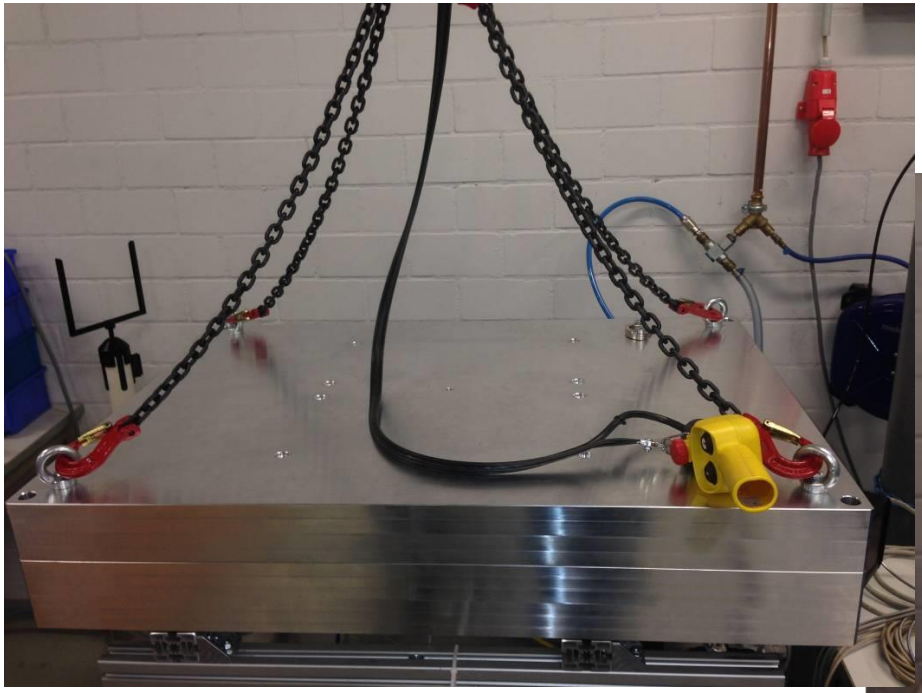
Test setup:

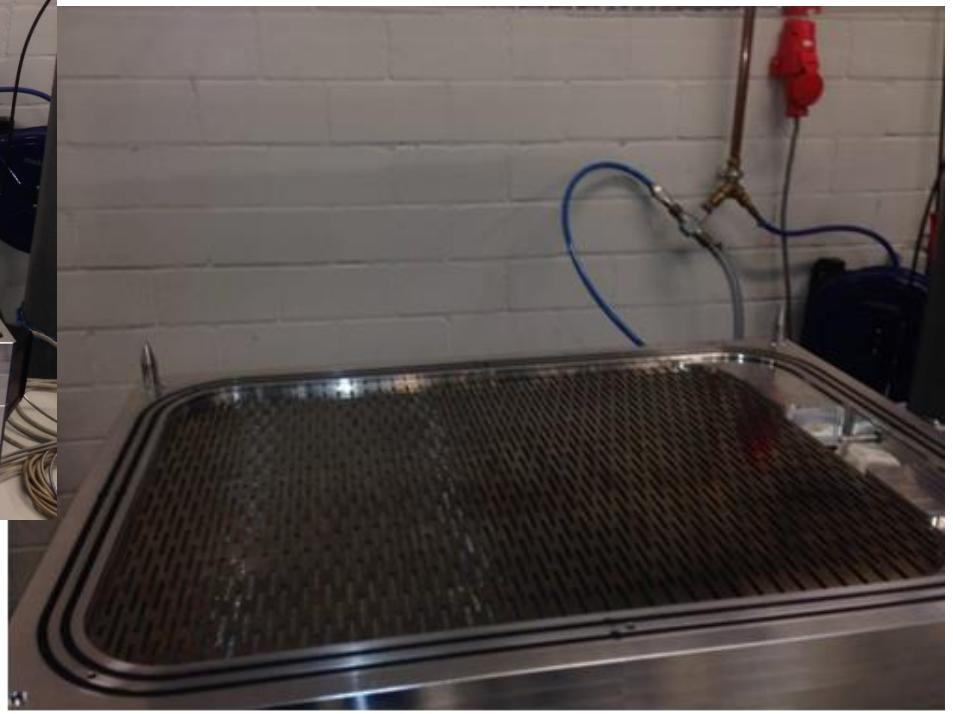
- Porous spacers avoid masking effect
- Restraining plates reduce stress on bag
- Test can be used as “Integrity Test” covering the whole SUS
- Gas leak rate can be correlated to a leak size of 2µm



small inflation volume + high test pressure = best sensitivity

Case Study: Helium Integrity Testing



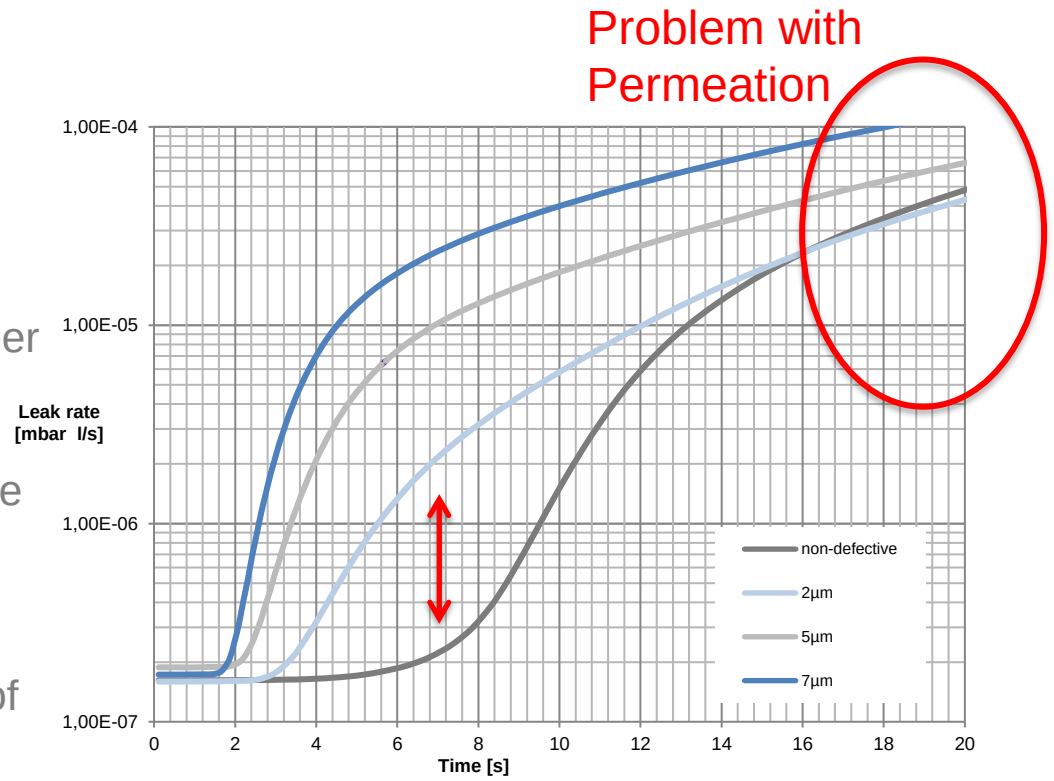


Feasibility testing on 50l test chamber

Case Study: Helium Integrity Testing

Test testing results:

- Feasibility tests done with three different test gases
- Results based on 20 samples per leak size (50l bags)
- One decade differentiation in the time frame between 4-10 s
- Helium gas tracer was the best test method to achieve a level of detection down to 2 µm



Case Study: Helium Integrity Testing

Challenges for industrialization:

- High permeability of helium through the thin bag film
- Outgassing of volatile components
- Vacuum level vs. tact time
- Helium background concentration & absorption

Case Study: Helium Integrity Testing

Solutions for industrialization:

- Machine concept with fast helium filling to measure before the start of permeation
  => dynamic leak rate evaluation
- High vacuum to get lowest background level
- High vacuum pump capacity to reach vacuum in a reasonable time frame
- 2 chamber design for alternating operation to reach target tact time
- Flexible chamber design to cover different product families

Helium Integrity Testing of 2D Bag Assemblies* Used in Critical DS & DP Applications

Helium Gas Tracer in Vacuum Chamber

*Volume: 50 mL – 50 L

- Restraining plates
  - Reduce stress on bag
  - Provide small inflation volume
  - Allow high test pressure: 300 mbar
- Porous spacer avoid masking effect of potential leaks

With low background noise and dynamic leak rate measurement helium gas tracer is the best method to achieve reliably highest sensitivity

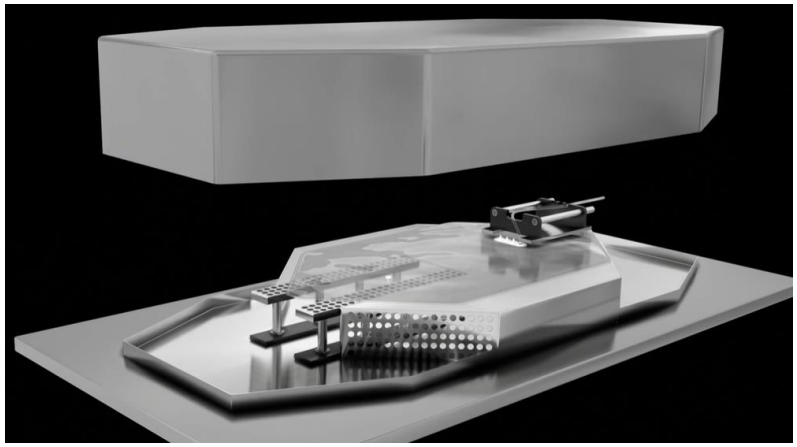
➢ Detection limit: **2 µm correlated** to microbial ingress and liquid leaks at shipping pressure conditions

Helium Integrity Testing of 3D Bag Assemblies* for Storage, Shipping & Mixing Used in Critical DS & DP Applications

Helium Gas Tracer in Vacuum Chamber

*Volume: 50 L – 650 L

- Restraining plates
  - Reduce stress on bag
  - Provide small inflation volume
  - Allow high test pressure: 300 mbar
- Porous spacer avoid masking effect of potential leaks



With low background noise and dynamic leak rate measurement helium gas tracer is the best method to achieve reliably highest sensitivity

➢ Detection limit: **2 µm correlated** to microbial ingress and liquid leaks at shipping pressure conditions

Case Study: Helium Integrity Testing

Validation Approach:

- A **reliable** test method using samples from multiple production lots
- A **reproducible**, **accurate** and **robust** test method using a 6 sigma confidence interval for the differentiation between defective and non-defective samples
- A **sensitive** test method using defective samples, calibrated on a pinhole equivalent
- A **One-for-all** validation procedure, whatever the test method is

Case Study: Helium Integrity Testing

Definition of defect:

- Aerosol microbial ingress & liquid leak studies performed on different bag film materials
- Identification of MALL for microbial ingress & liquid leak **under different process conditions**
- **2µm estimated as MALL** for worst-case (shipping) conditions -> target for the helium test validation
- Deliberate defects manufactured and confirmed with flow calibration

Case Study: Helium Integrity Testing

Definition of defect:

- Different types of defects tested: patches and capillaries
- Defects placed at worst-case positions

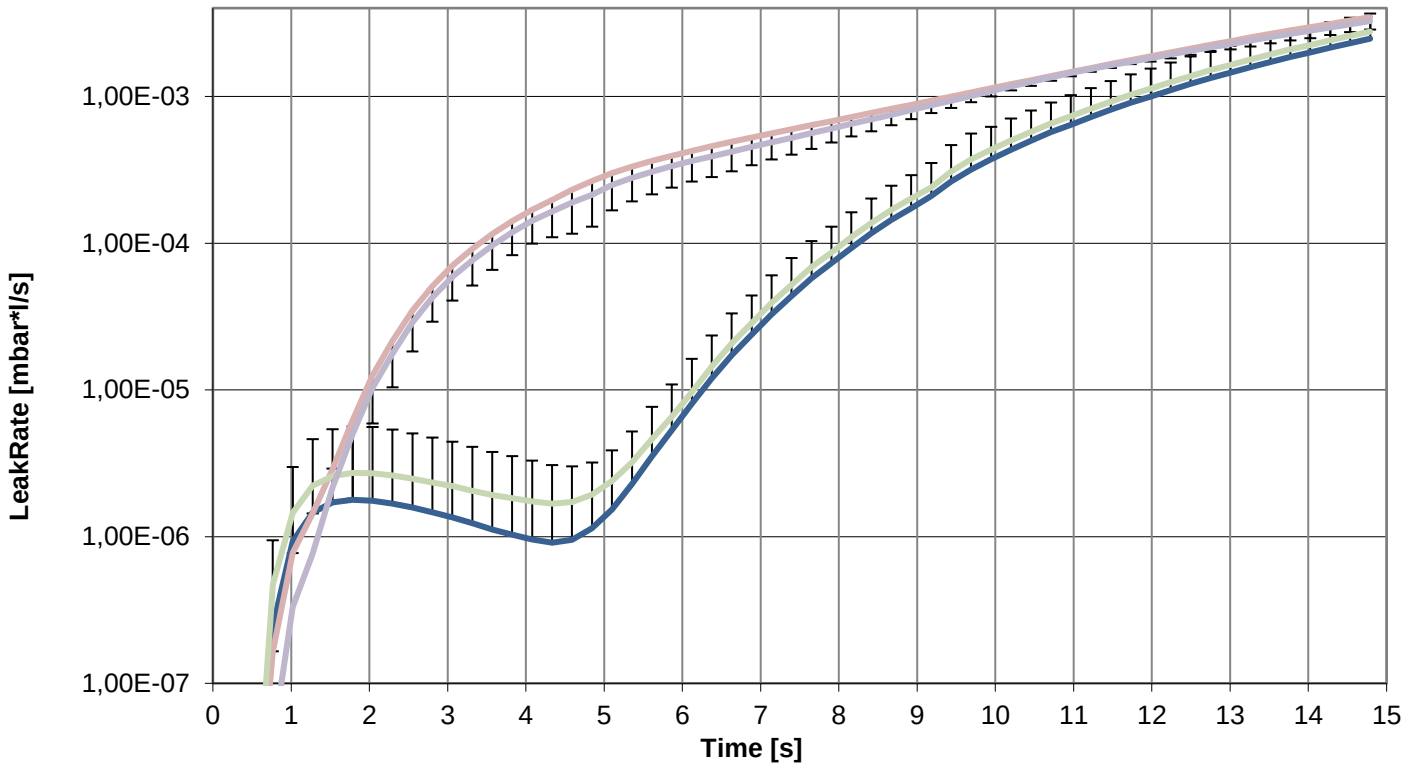
Case Study: Helium Integrity Testing

Parameter Study:

- Evaluate impact of different product designs
  - Volume
  - Components
  - Material
- Determine optimum test parameter set(s)
- Tests on a limited number of test samples

Case Study: Helium Integrity Testing

Parameter study: Results of 50l bags



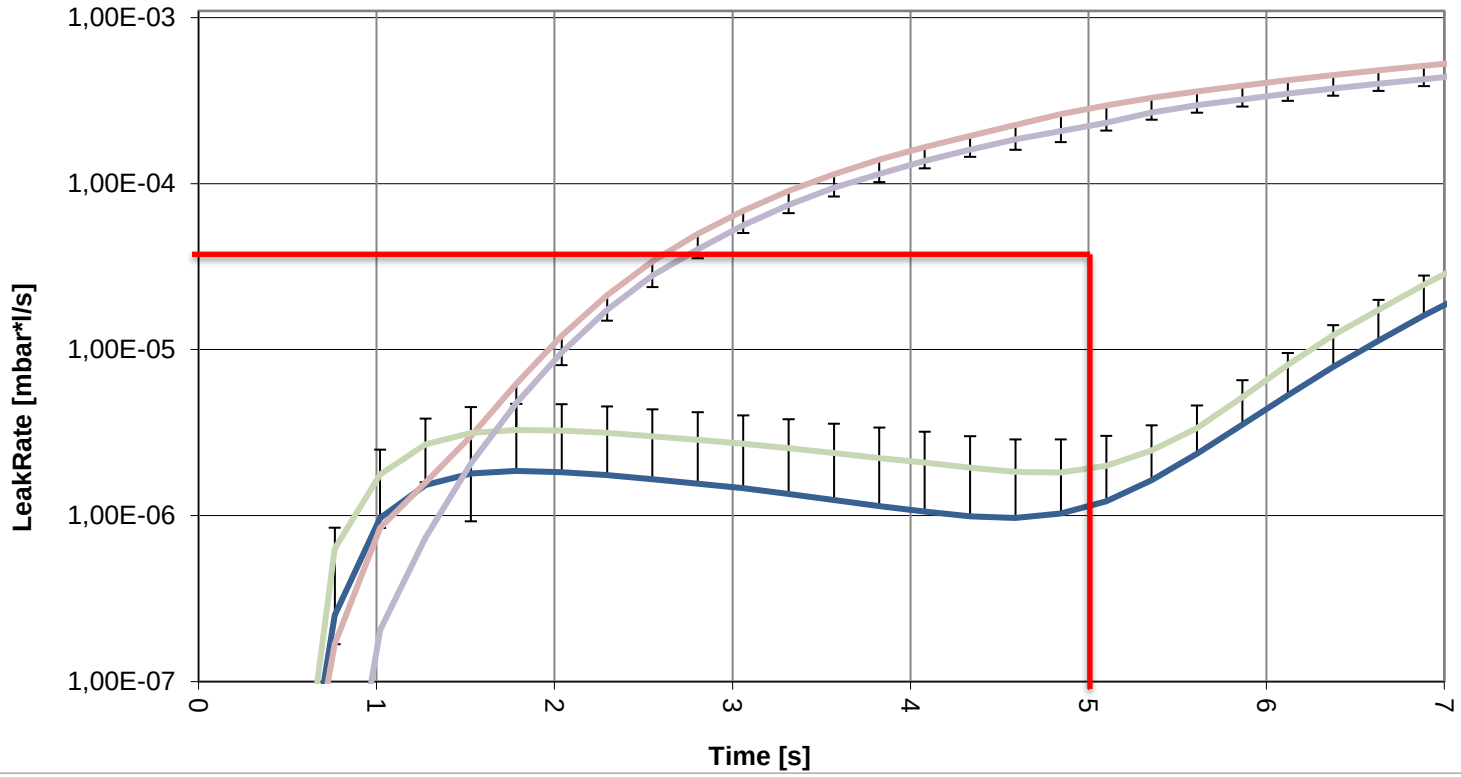
Case Study: Helium Integrity Testing

Validation Study:

- Verification of selected test parameter set(s)
- Determine pass/fail criteria (time & leak rate limit) => dynamic leak rate
- Tests on a statistically significant number of test samples

Case Study: Helium Integrity Testing

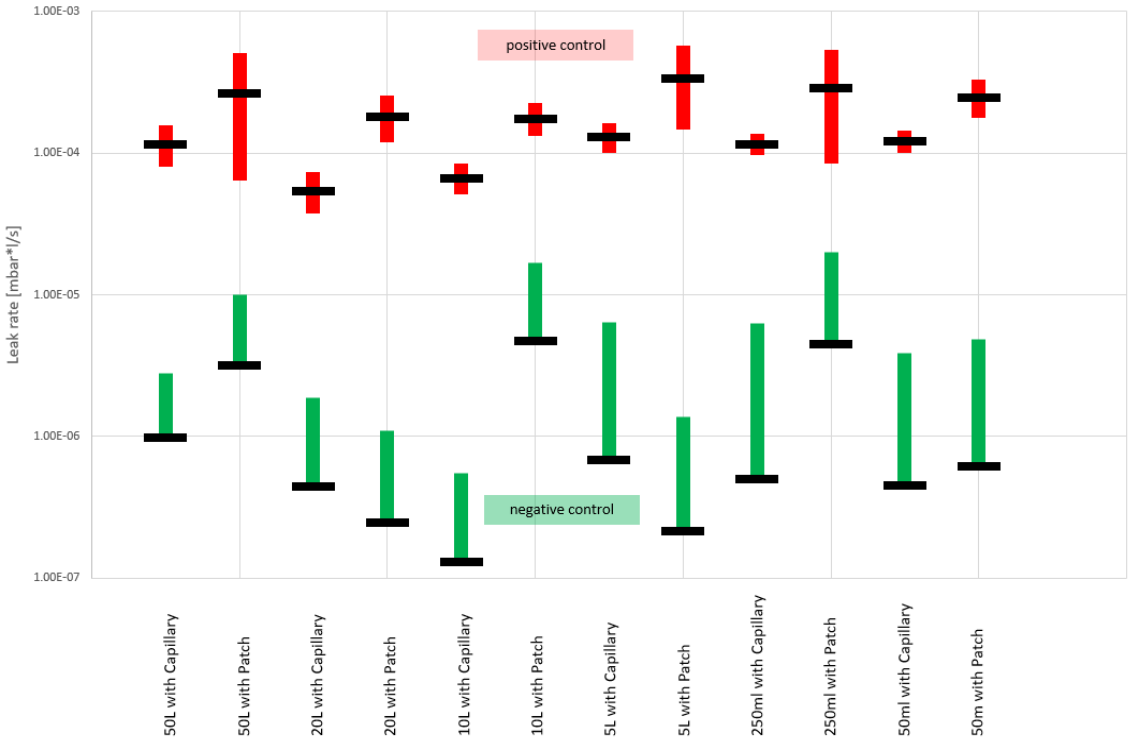
Validation study: Results of 50l bags



Case Study: Helium Integrity Testing

Validation Summary:

- Measurement of defective & non-defective samples
- Use of different types of artificial defect, like film patches & capillaries
- Defects deliberately calibrated
- Reliability proven using samples from multiple batches
- **2µm** detection validated applying a **6 sigma** confidence interval



Validation study with >380 tests to establish test specifications

Proven Integrity of Your Process Improves Patient Safety, Regulatory Compliance, Production Costs and Drug Availability

- Enhances patient and operator safety
- Prevents any risk of high value product loss at commercial phase
- Meets cGMP regulatory expectations for SU container closure integrity
- Speeds up drug product manufacturing capacity and market availability with SU proven process integrity

Thank you for your attention.
Questions?