

The Foundations for Single-Use Manufacturing. Redefined from A-Z



Single-use System Integrity

Marc Hogreve, Principal Engineer Integrity Testing

Consistent robustness and integrity testing lead to enhanced process integrity and patient safety.

Benefit from our expertise in designing robust solutions, integrity testing science and technologies to de-risk your process from liquid leaks and microbial ingress.



Single-use Process Integrity Is a Key Industry & Regulatory Challenge

Validated single-use system integrity is required to

- Strengthen regulatory compliance
- Improve patient safety

SU 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
- Current 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

Users cannot show real proof of process integrity to regulatory organizations

Current Industry Guidance and Standards Initiatives



USP<1207> 2016 Package Integrity Evaluation – Sterile Products



ASTM E55 WK64337 Standard Practice for Integrity Assurance & Testing of Single-use Systems

ASTM E55 WK64975 Test Method for Microbial Ingress Testing on Single-use Systems



NEW PDA TR on Pharmaceutical Package Integrity (to replace existing TR27)



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

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⁾ SUS: A Microbiology Product Quality Perspective – Patricia F. Hughes, Ph.D., Branch Chief (Acting), CDER/OPQ/OPF/DMA

⁽²⁾ Sterile drug product, New developments – Patricia F. Hughes, Ph.D., Branch Chief (Acting), CDER/OPQ/OPF/DMA

⁽³⁾ Biologics License Application



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

- QbD & validation to reach consistent product robustness
- Process control, quality control & integrity testing
- Understand liquid leakage & bacteria ingress mechanisms
- Correlate integrity testing detection limits to liquid leaks & microbial ingress under process conditions
- Implement 100% physical supplier integrity testing
- Provide point of use testing



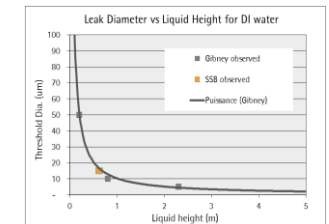
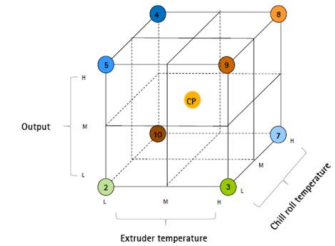
SU Product
Robustness



SU System
Integrity Science



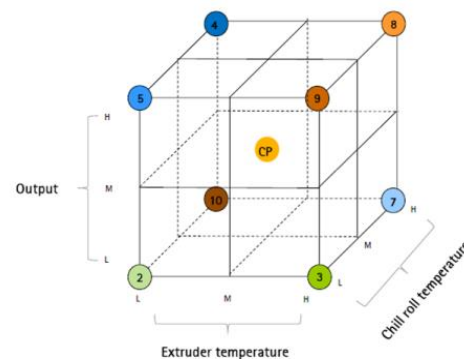
Integrity Testing
Technology



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

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

- Extrusion design space and critical process control based on 3,000 tests
- Bags visually inspected & 100% leak tested
- Liquid shipping validation with most stringent ASTM D4169 standard
- Self-inflation, strength & flexibility make SUS easy to handle & robust

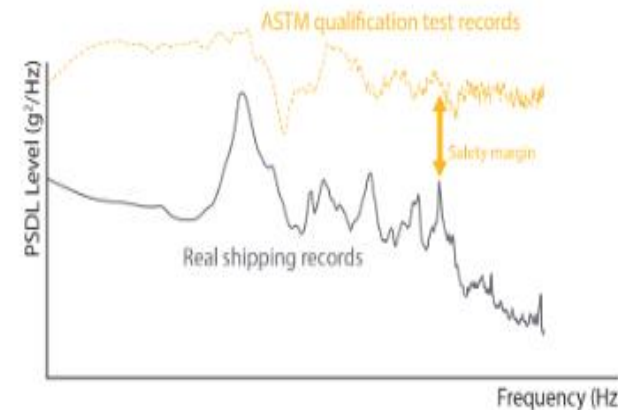


~ 3000 robustness tests
Tensile strength: 930
Elongation at break: 930
Puncture tests: 930
Flex durability: 186
Water burst tests : 26

Most stringent standards:

ASTM D4169

Validation package with
real & lab. tests



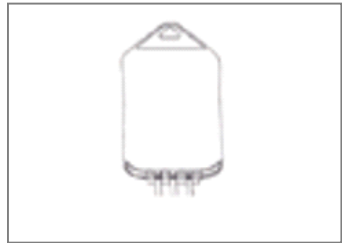
Passed
ASTM Shipping
Test

Self-deploying bag
during installation



Avoids mishandling
&
reduces bag failures

Intermediate Process Control, Quality Control & Final Product Integrity Testing



Bag chamber
leak test
40 – 90 μm



Discard defects from
manufacturing operations



Final assembly
Helium based
integrity test
2 μm



Discard additional defects from
manufacturing operations



Point-of-use
integrity test
10 μm
FlexAct® BT



Discard additional defects from
transportation & handling

Understand Liquid Leakage and Microbial Ingress Mechanisms on Film Materials Used in Single-use Biomanufacturing

- Demonstrate the relation between microbial ingress and liquid leak
- Establish a predictive model to determine the Maximum Allowable Leakage Limit (MALL*) under any process conditions
- 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: is the greatest leak size tolerable that poses no risk to product safety, USP<1207>⁽⁷⁾

Process Conditions May Impact Liquid Leak & Microbial Ingress

Hydrostatic pressure in storage



Flexboy® 20 L



Flexsafe® 500 L

~0.7m / 2'
Max pressure
70mbar

Application

Pressure range

Storage

~10 – 100 mbar

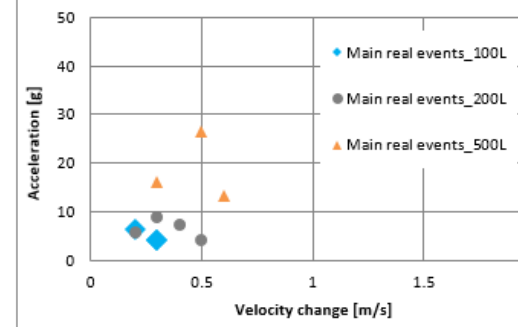
Shipping

~100 – 250 mbar

Shipping can generate up to 20g acceleration



100L-200L-500L Horizontal Shocks



Liquid Leak Study – Method

- Film patches with laser drilled holes from 2µm to 30µm
- Three model solutions:
 - Water + 0.5% methylene blue as tracer dye
 - TSB
 - 70% Ethanol
- Surface tension range from ~25 – 73 mN/m
- Four different test pressures:
 - Atmospheric pressure
 - 70mbar
 - 150mbar
 - 300mbar
- Visual inspection for up to 30 days before considering “no leak”



Multiple module setup:

- 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



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

Liquid leak results for PE film at 300 mbar 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** |
| Ethanol 70% | Number of samples | 3 | 3 | 3 | 3 |
| | Number of leaks | ongoing | ongoing | ongoing | ongoing |

* Trypticase soy broth (growth media for BCT trials)

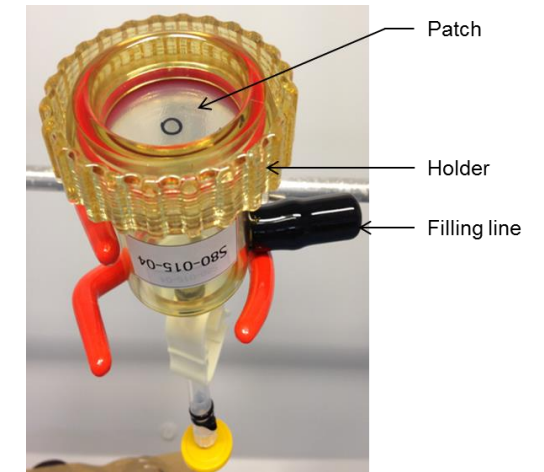
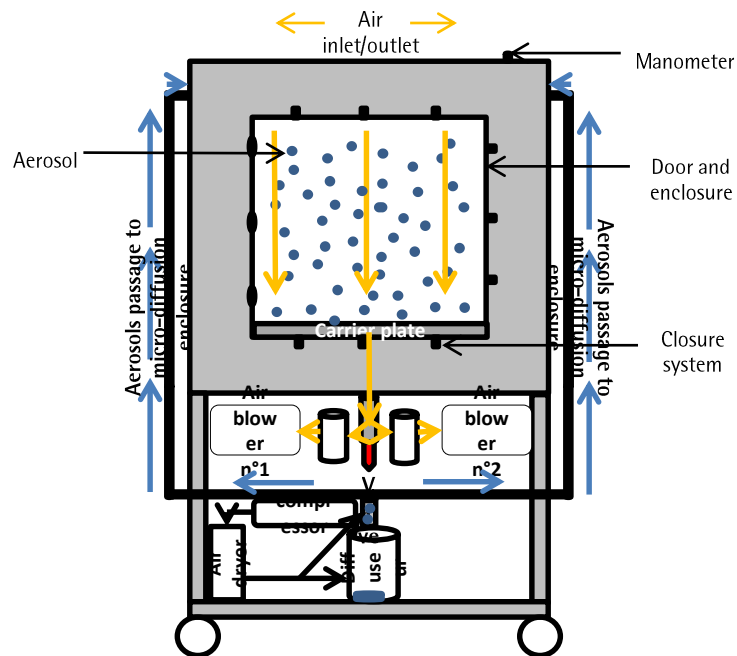
** As per DoE not required

Microbial Aerosol Study – Challenges for Testing Method Development

- Develop a homogeneous, reproducible, reliable test system with a high concentration of microorganisms still alive at the end of the aerosol cycle
- Purify and standardize the spore suspension
- Be able to test high number of samples
- Apply different pressure level inside the test article, representing SUS life cycle conditions
 - Atmospheric pressure as reference and representing small containers during storage
 - High pressure to simulate acceleration during shipping

Microbial Aerosol Study - Method

- 35 ft³ (1m³) cubical aerosol chamber using micro diffusion system on the top of the chamber allowing exposition of 36 samples per run
- Aerosol exposition at atmospheric pressure of film coupons with laser drilled holes from 1 to 100 µm using spores *B. atrophaeus* to get 10⁶ CFU/cm², Spores size distribution of 0.1-0.4 µm (measured by TEM)
- Film coupons installed in-all-in one device, integrity tested, gamma irradiated, filled with sterile TSB, exposed to microbial spores, packed, & Incubated 14 days @ 30-35°C (86-95°F)

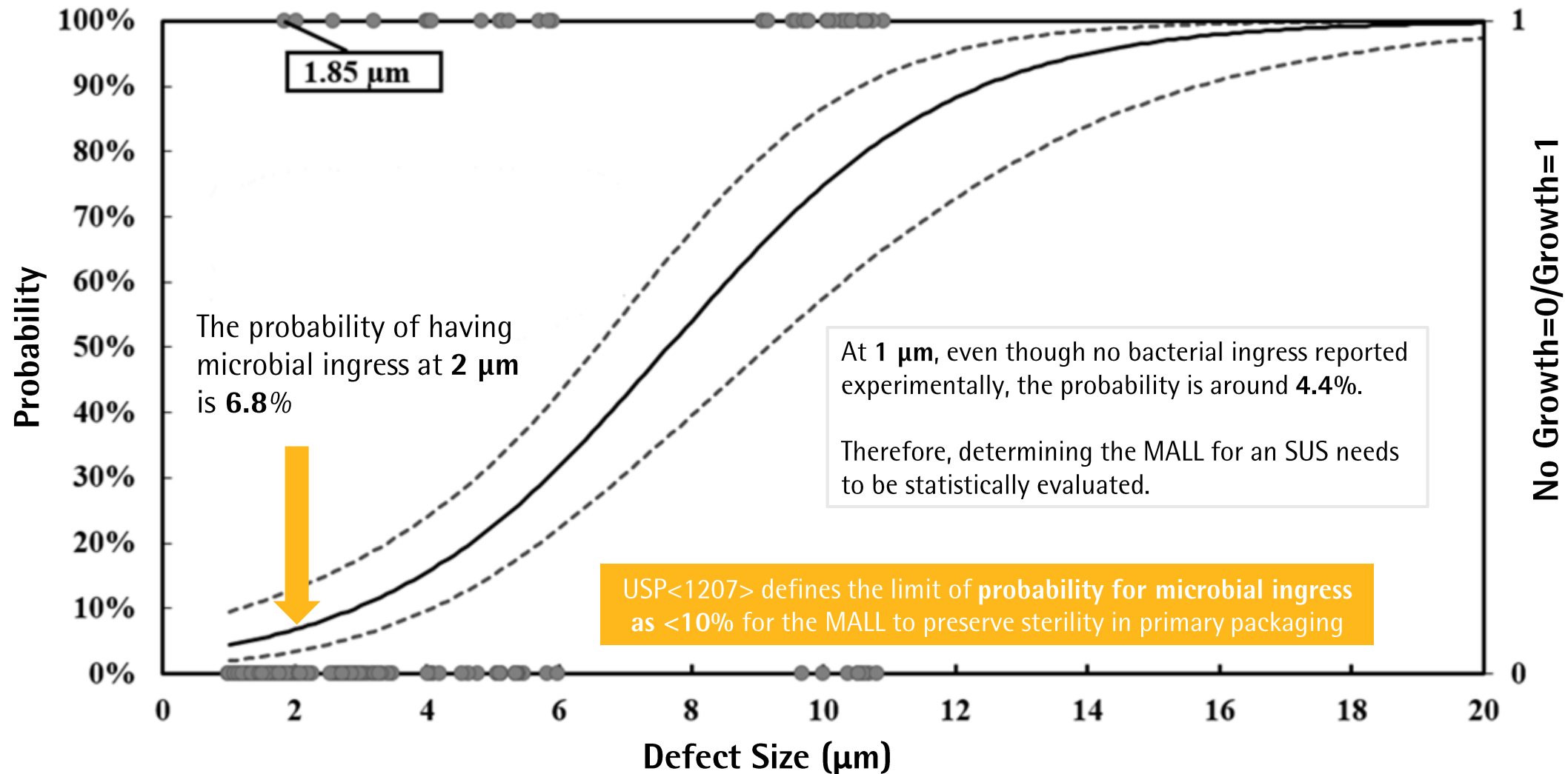


Microbial Aerosol Tests on 300 Film Samples Show No Ingress for 1 μm Defect at 300 mbar

Microbial ingress results at 300mbar imposed pressure

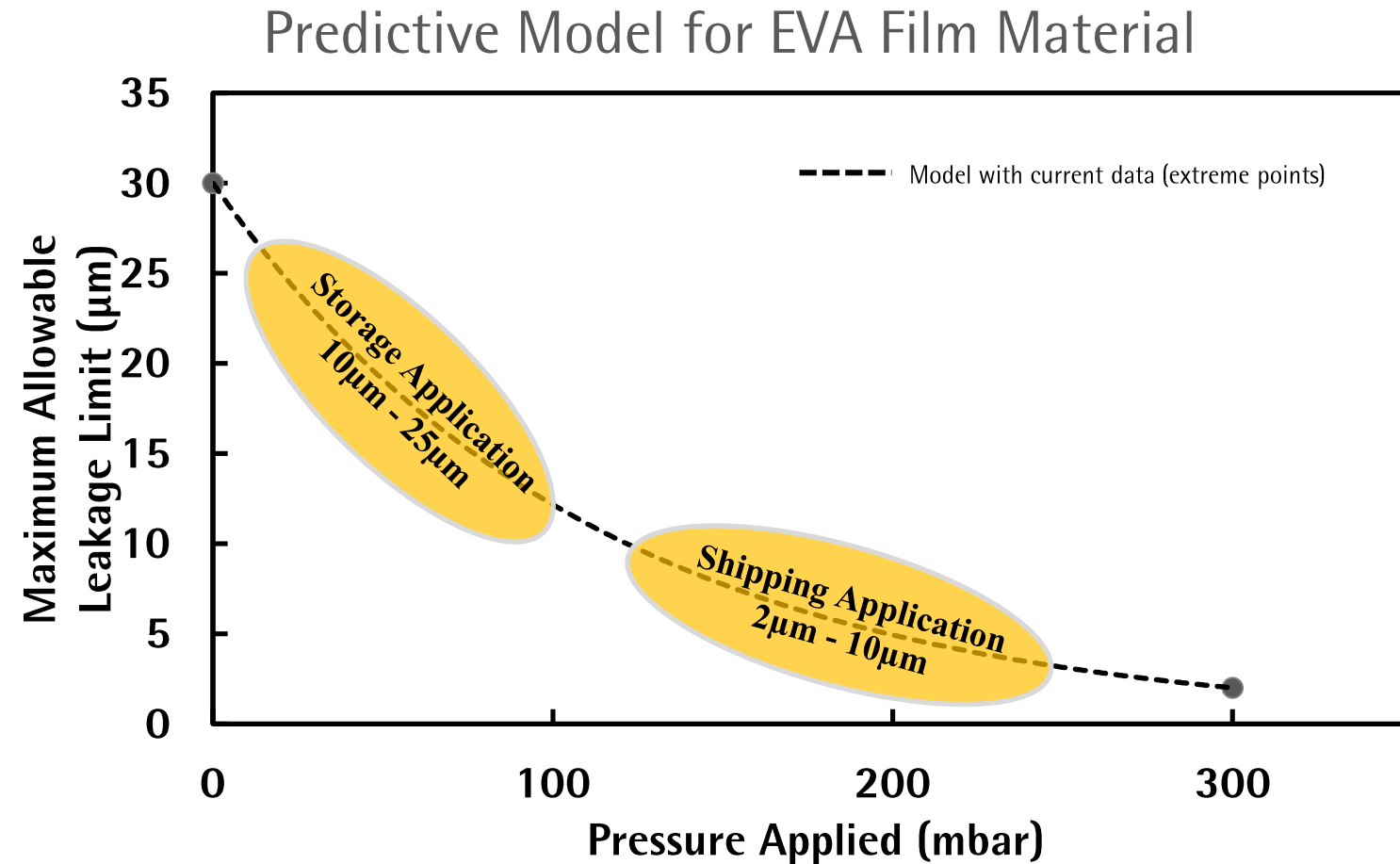
| | Hole size μm | 1 μm | 2 μm | 3 μm | 5 μm | 10 μm |
|----------|-------------------------|-----------------|-----------------|-----------------|-----------------|------------------|
| | Number of samples | 30 | 30 | 30 | 30 | 30 |
| PE film | Number of ingress | 0 | 2 | 2 | 10 | 22 |
| EVA film | 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 to Define Application-specific MALL

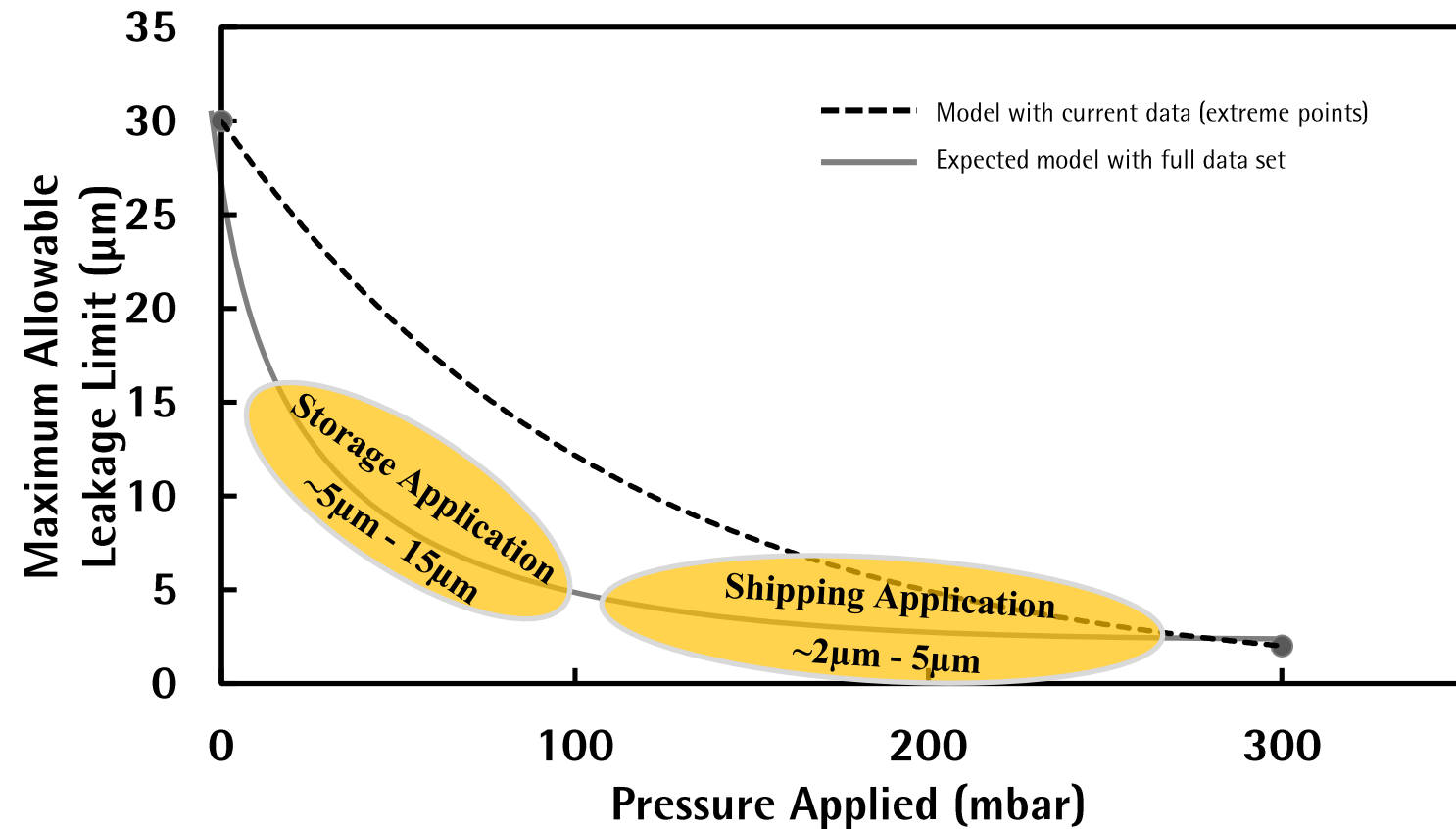
- Establishes the MALL for any process condition
- Based on observed data for liquid leaks and microbial ingress at various pressure conditions
- Each MALL is associated with a certain probability of leak/ingress



Predictive Model to Define Application-specific MALL

- Complete testing on additional data points for both film materials to increase validity of the predictive model
- Establish material-specific formula to predict any application-specific MALL
- Increase the robustness and confidence level of the predictive model by using DoE and other statistical planning tools like 3pod to design and run further experiments

Predictive Model for EVA Film Material





Interim Conclusion from Results Obtained on Liquid Leak & Microbial Ingress Studies on SUS

- Results obtained on film materials fall into the existing models for sterile package using micro tubes
- Both studies, liquid leak and microbial ingress ones, tend to confirm that leak sizes for liquid flow are not significantly different from leak sizes for sterility loss
- The conditions of use (pressure) does significantly impact the Maximum Allowable Leakage Limit (MALL)
- 2 μm is the MALL under any conditions and both, for liquid leak and microbial ingress

Integrity Testing With Detection Limits Correlated to Microbial Ingress & Liquid Leaks

Helium supplier integrity test with detection limit of 2 μ m.

- Smallest defect size detectable by integrity test
- Confirms the integrity of the complete assembly

Pressure decay test confirms the absence of defects at the point-of-use.

- Can also correlate to liquid leaks & microbial ingress

1 Bag Chamber Leak Test (BC-LT)

100% BC-LT
Pressure Decay

Detection limit
40–100 μ m



2 Final Product Supplier Integrity Test (SIT)

100% SIT
Helium Test

Detection limit
2 μ m



3 Point of Use Leak/Integrity Test (PoU-LT/IT)



Pressure Decay

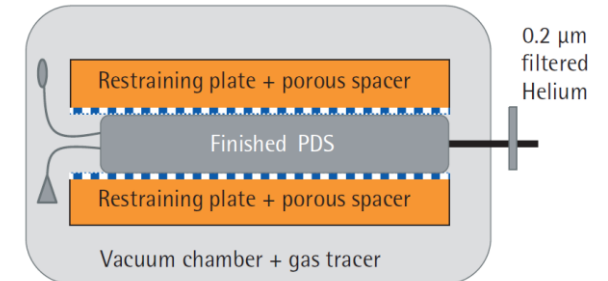
Detection limits
10 μ m on 2D bags
100–200 μ m on 3D bags
50–600 μ m on STR bags

Finished Product SIT to Test 100% of 2D Bag Assemblies Used in Critical DS & DP Applications

- **Helium gas tracer in vacuum chamber**
- 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

Bag making

1 BC-LT

Final assembly

2 SIT

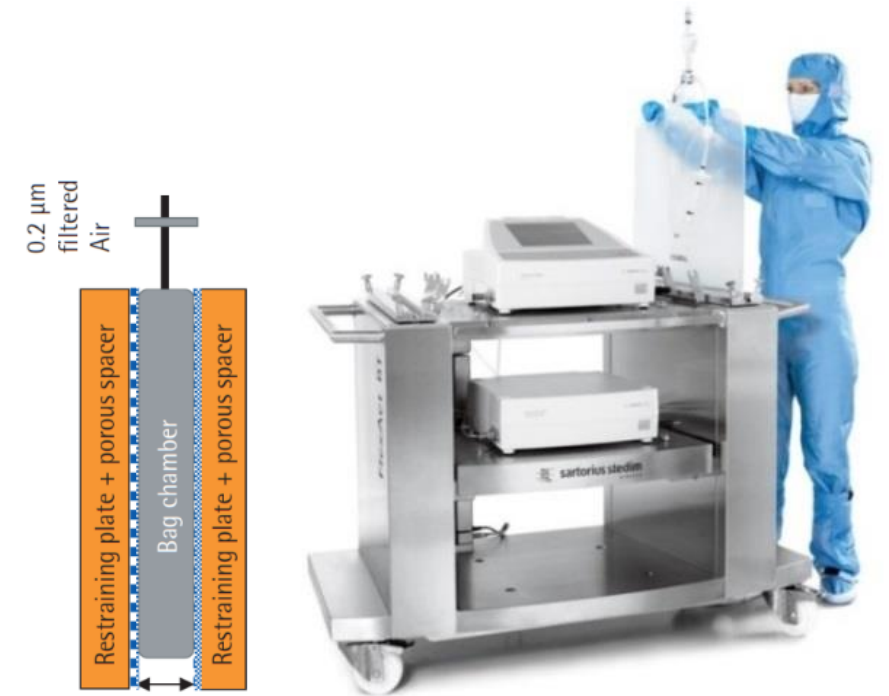
Sterilization &
shipment3 PoU-LT /
PoU-IT

PoU-IT for 2D Bags Ensures that No Defects Have Been Generated During Shipping & Handling

- **Pressure decay** with restraining plates & porous spacers
- Restraining plates
 - Reduce stress on bag
 - Provide small inflation volume
 - Allow high test pressure:
300 mbar
- Porous spacer avoid masking effect of potential leaks

Combining small volume, high test pressure and spacers provides a fast, reproducible, accurate and sensitive test

- Detection limit: **10 µm correlated** to microbial ingress and liquid leaks at storage pressure conditions



Bag making

1 BC-LT

Final assembly

2 SIT

Sterilization &
shipment3 PoU-LT /
PoU-IT

PoU-LT for 3D Bags Ensures that No Gross Defects Have Been Generated During Shipping, Handling & Installation

- **Pressure decay** with porous spacers after installation inside the final container
- Porous spacers avoid masking effect of potential leaks
- Container mechanically supports the bag
- Test is performed after installation, directly before use to detect leaks caused during complex handling & installation steps

➤ Detection limit: **100 – 200 μm**



Bag making

1 BC-LT

Final assembly

2 SIT

Sterilization &
shipment

3 PoU-LT /
PoU-IT

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

