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The science behind the integrity of single-use system: Investigating liquid leak and microbial ingress mechanisms to determine the maximum allowable leakage limit

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The Science behind the Integrity of Single-Use Systems: Investigating Liquid Leak and Microbial Ingress Mechanisms to Determine the Maximum Allowable Leakage Limit

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Introduction

Integrity is a critical quality attribute of our single-use systems as an integrity breach can have dramatic impacts on patient safety, operator safety or drug availability. Growing industry scrutiny of single-use system integrity (SUSI) is raising the need to develop good science behind reliable determination of liquid leakage and microbial ingress, as well as the appropriate physical integrity testing technologies. The results of the experiments performed to understand the mechanisms of liquid leakage and microbial ingress of SUSI are presented. A relation between liquid leakage and microbial ingress mechanisms in single- use plastic containers are confirmed. Microbial ingress testing by the aerosolization and the liquid leakage test are used to determine the maximum allowable leakage limit (MALL). Statistical analysis indicated the probability of MALL at a certain defect size for each system.

With additional experiments a correlation between a certain defect size and the corresponding gas flow rate is found. The MALL defined as a gas flow rate will link to a physical integrity testing system.

The methods provide an accurate way of predicting ingress, increasing safety down the line for drug manufacturers and patients alike.

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

Validated single-use system integrity is required to

- Strengthen regulatory compliance
- Improve patient and operator safety

Single-use system integrity testing is only meaningful when its detection limit is correlated to liquid leaks & microbial ingress

- Understanding liquid leakage and microbial ingress mechanisms on film materials used in single-use biomanufacturing
- Develop and validate the physical test methods with detection limits that guarantee the absence of liquid leak/microbial ingress in SUS

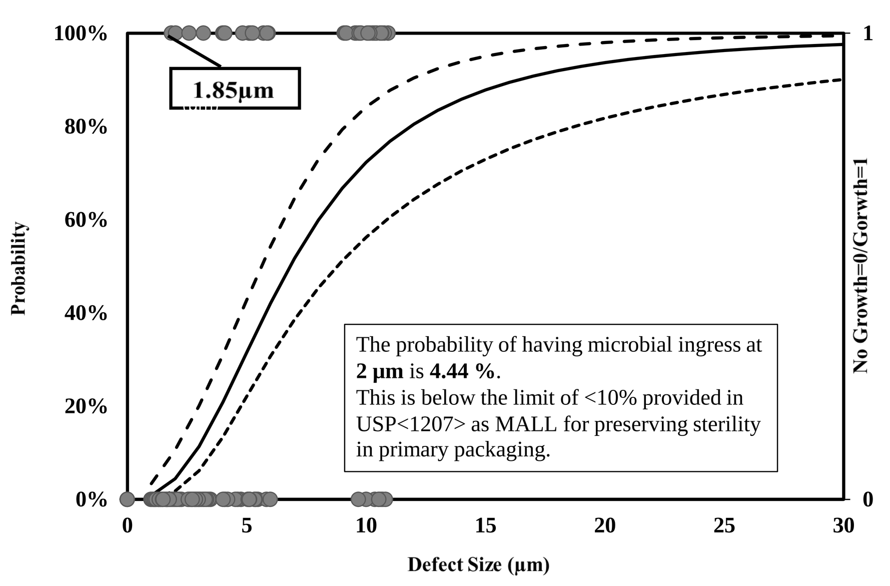
> Integrity tests are also reviewed during the review of the BLA[1] and on inspection.
>
> FDA Inspector

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

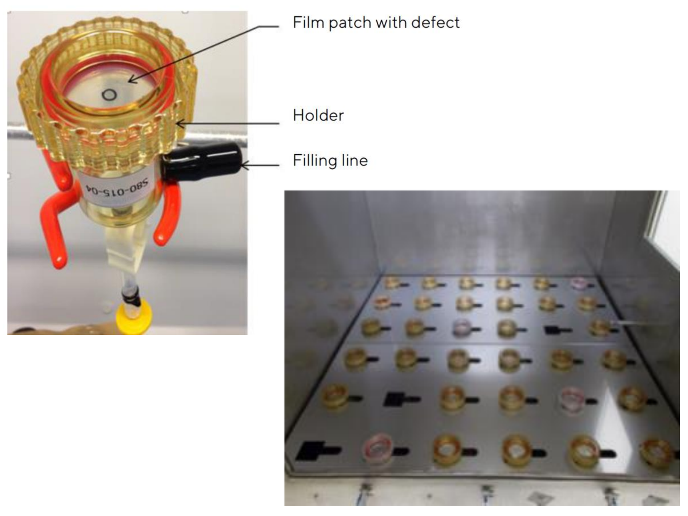
[1] Biologics License Application

Microbial Ingress Test with Aerosolization Method[1]

- Film patches from the material of the Flexboy®, Celsius® and Flexsafe® bags
- Artificial laser-drilled leak size between 1 µm and 100 µm
- Aseptic filling with media
- Pressurizing of the test assembly (0, 70, 150 and 300 mbar)
- Aerosolization with 10^6 CFU/cm[2] of *Bacillus atrophaeus*
- Incubation at 30-35 °C for 14 days and visual interpretation



The bacterial growth or absence of growth is shown with gray circles as a function of leak size for 300 mbar of applied pressure inside the patch holder with PE film. The probability of bacterial ingress expressed as a function of different leak sizes (solid line). Dashed lines show the upper and lower level of each probability with 95% confidence level.



Patch holder & aerosolization chamber

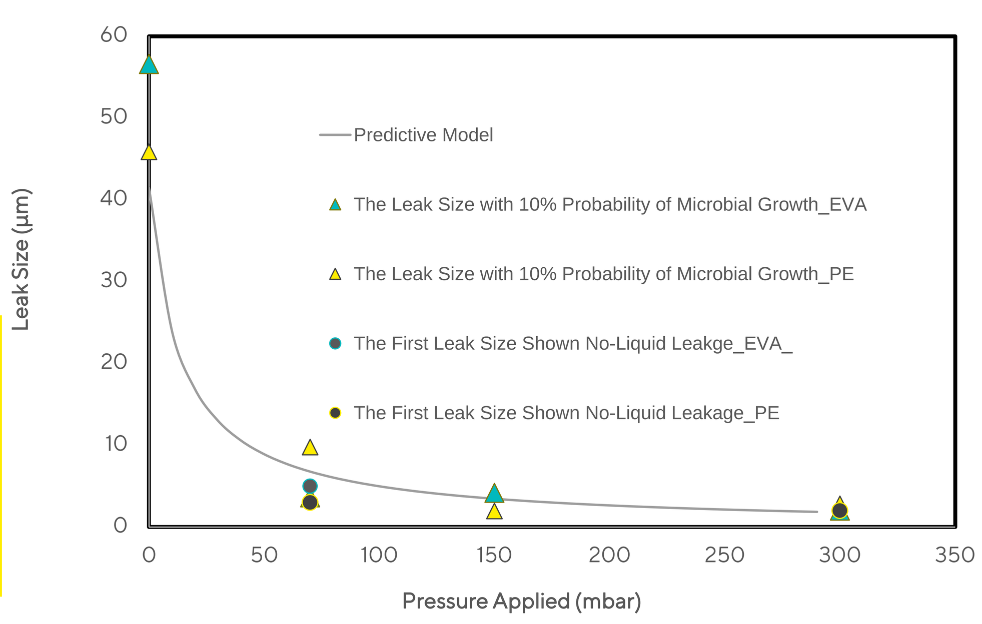
- Binomial logistic regression covers the whole range of the leak sizes
- Reporting the Microbial ingress and MALL with probability
- Predicting the probability for the leak size not tested before

Microbial Ingress and Liquid Leak

There is a direct correlation between having the liquid in the defect pathway and occurrence of microbial growth.

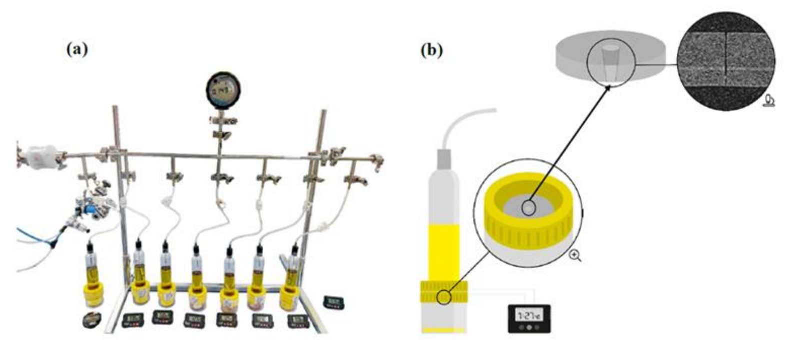
The liquid seems to act as a transport mechanism for the microbes to enter into the SUS.

- Liquid presence in the defect pathway is a prerequisite for a potential microbial contamination
- But due to the probabilistic nature of the microorganisms not every liquid leak leads to microbial ingress



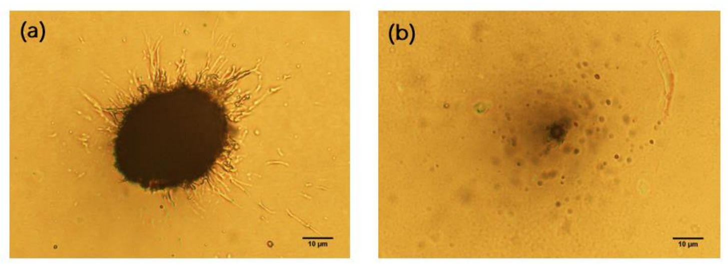
Characterization of Liquid Leakage Mechanisms[2]

- Film patches from material of the Flexboy®, Celsius® and Flexsafe® bags
- Artificial laser-drilled leak size between 2 µm and 25 µm
- Model Solution: Water & Tryptic Soya Broth
- Pressurize Test Assembly (70, 150 & 300 mbar)
- Leak detection – Type of Leak/MALL

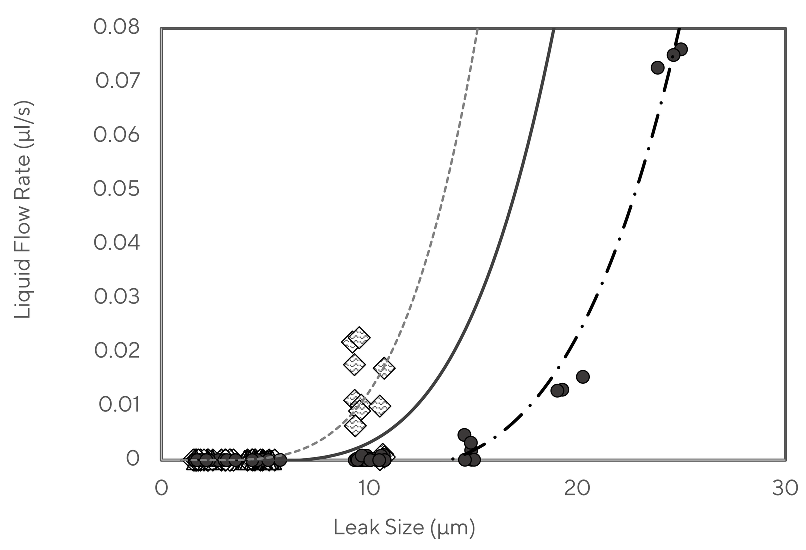


(a) Multi-unit setup for detection of liquid leakage
(b) Individual test module magnified to show patch placement with zoomed image of a microchannel.

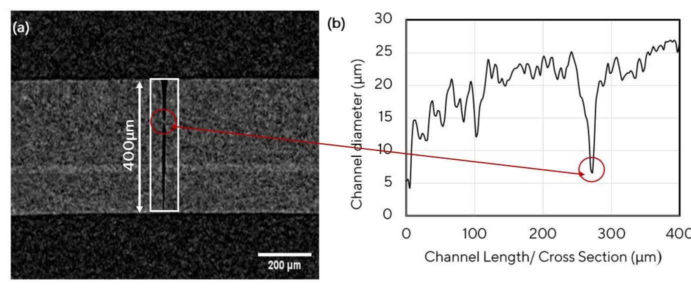
Leak Characterization



Light microscopy of PE film patches with a nominal leak size of 2 µm; there is a considerable difference between the inlet area (a) & the outlet area (b).



Experimental volumetric flow rates as function of leak size: Pressure applied of 300 mbar (rhombuses); 150 mbar (triangles) & 70 mbar (circles).
The theoretical flow rates derived from the Hagen-Poiseuille equation are shown with dashed, solid and point- dashed lines for 300 mbar, 150 mbar and 70 mbar, respectively



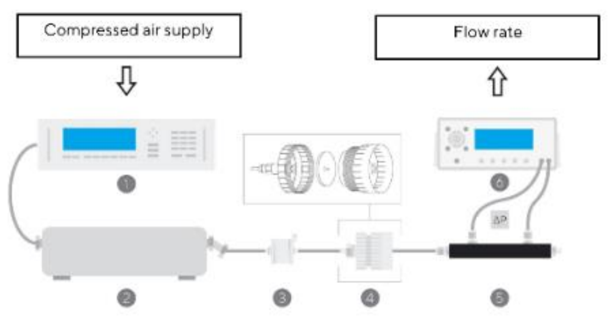
(a) Computed tomography of leak channel in a PE film with a thickness of 400 µm; (b) channel diameter as a function of film thickness

- If no liquid leakage was detected after a maximum test duration of 30 days, the leak size was reported as the maximum allowable leakage limit (MALL) for the respective combination of film material, pressure applied and model solution

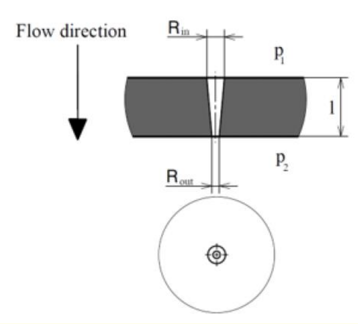
Gas Flow Rate Through Laser-Drilled Microchannels

- Film patches from the material of the Flexboy®, Celsius® and Flexsafe® bags
- Artificial laser-drilled leak size between 2µm and 100 µm
- Pressurizing of the test assembly from 50 to 1000mbar

The impact of differential pressure, leak size and leak geometry are different based on the flow regimes and shown in the empirical formulas. The relation between gas flow rate and differential pressure as an external parameter and channel geometries as internal parameters for flexible single-use system is studied.



Flow measurement test setup



Knudsen Flow

$$Q = \left(\frac{p_1}{p_2} - 1\right) \bar{c} {R_{out}}^2 \sqrt{\frac{\pi}{2}} \left(\frac{R_{in}}{R_{out}}\right)^{\kappa - 1}$$

Viscous Flow, Tube $\frac{l}{R_{in} + R_{out}} > 5$

$$Q = \left(\frac{p_1}{p_2} - 1\right)^z \bar{c} {R_{out}}^2 \sqrt{\frac{\pi}{16}} \left(\frac{R_{in}}{R_{out}}\right)^{\kappa}$$

Viscous Flow, Orifice $\frac{l}{R_{in} + R_{out}} < 4$

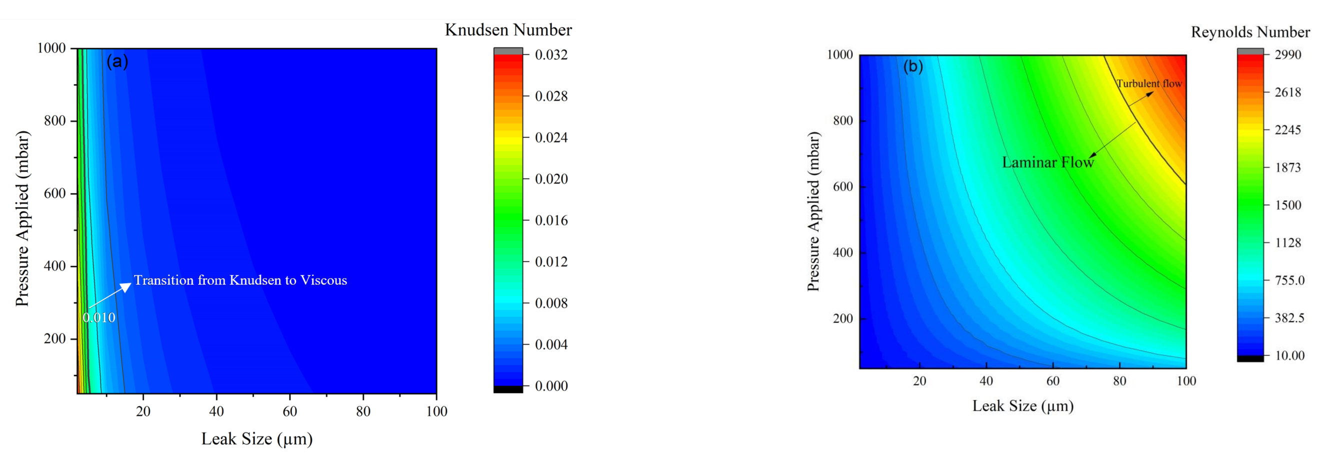
$$Q = \left(\frac{p_1}{p_2} - 1\right)^z \bar{c} {R_{out}}^2 \sqrt{2\pi} \left(\frac{R_{in}}{R_{out}}\right)^{\frac{\kappa - 1}{2}}$$

where

$p_1 = inlet\ pressure$
$p_2 = atmosphere\ pressure$
$\bar{c} = 463 \frac{m}{s}$

$z = 0{,}46 \left(\frac{l}{R_{in} + R_{out}}\right)^{0{,}3}$
$l = channel\ lengths$
$\kappa = 1{,}4$

- Finding the relation between the gas flow rate and the leak diameter for our internal validation process of integrity testing for SUSs
- Checking whether this relation between flow rate and leak size for flexible materials is like the relation applicable to rigid materials
- For single-use systems, as the concept of MALL is commonly understood as a leak size (leak diameter), micrometers are preferred to other units to express it



Contour plot of (a) the Knudsen number and (b) the Reynolds number as a function of leak size and differential pressure. (a) The blue regions correspond to viscous flow and the orange to yellow regions depict Knudsen flow. (b) For viscous flow, the blue to yellow regions cover the area in which viscous flow is laminar and the orange regions correspond to turbulent flow

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2. Aliaskarisohi, S.; Kumar, C.; Hogreve, M.; Montenay, N.; Cutting, J.; Mundrigi, A.; Paramathma, A. Single-Use System Integrity II: Characterization of Liquid Leakage Mechanisms. PDA Journal of Pharmaceutical Science and Technology, May 2021, 75 (3), 273-288 .

3. Aliaskarisohi, S.; Urbaniak,T.; Hogreve, M., Single-Use System Integrity III: Gas Flow Rate Through Laser-Drilled Microchannels in Polymeric Film Material. PDA Journal of Pharmaceutical Science and Technology, January 2022, 76 (1), 9-18.