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Developing a Test Method to Determine the Maximum Allowable Leakage Limit of Microbial Ingress for Dialysis Films

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Introduction

- The purpose of this project is to design and develop a testing-apparatus and test-method to measure the maximum allowable leakage limit (MALL) for films used by Fresenius Medical Care (FMC).
- The MALL is the greatest leak size that does not pose a risk to the product. This is the first step in developing container closure integrity testing for sterile containers.
- This test is designed using guidance from USP <1207>, a document detailing integrity assurance of packages holding sterile medical products, and 21 CFR 211.
- This test is focusing on microbial ingress rather than physical or chemical leakage.
- The test-method design is based on an aerosol microbial ingress test published in the PDA Journal of Pharmaceutical Science and Technology [1].
- A few of the key variables considered are film, microbes, channel diameters, and pressure.

Literature Methods

- Patches of the test film were laser drilled in the center to simulate a pinhole defect from 1-100 µm.
- Each patch was secured in a polypropylene patch holder and filled with tryptic soy broth.
- The test units were exposed to aerosolized *Bacillus atrophaeus* to achieve 10^6 CFU/cm^2.
- Each test unit was then subjected to 0 mbar or 300 mbar to simulate worse case conditions.
- Each unit was then incubated for 14 days and visually inspected for growth and recorded as positive or negative.

Table 1. Data from aerosol microbial ingress experiment. Positive microbial ingress was divided by the total samples to find the percent [1].

Bacterial Ingress/Total Samples		
	PE Film (400 µm thick)	EVA Film (300 µm thick)
Positive Ingress 0 mbar	44/288 (15.3%)	30/288 (10.4%)
Positive Ingress 300 mbar	36/150 (24%)	41/150 (27.3%)

Figure 1. Test unit used in aerosol microbial ingress test [1]. The unit was filled aseptically with TSB using the black line.

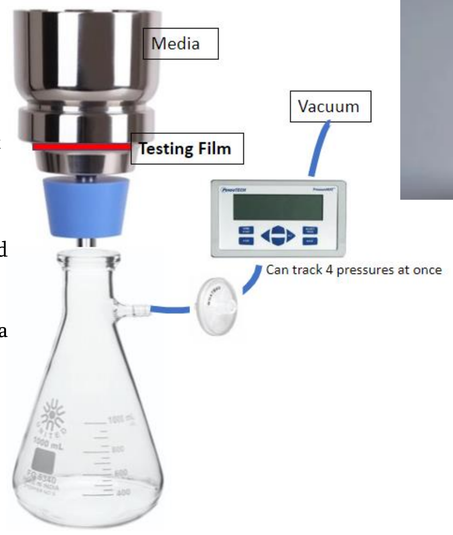

Figure 2. First test unit design. A stainless-steel filter holder would be mounted on a vacuum Erlenmeyer flask and connected to a pump and PMAT pressure sensor.

Figure 3. Centrifuge filter holder system from Millipore Sigma that will used as a test unit.

Literature Results

- For the two films that were tested, a MALL of 10-20 µm was established for controlled storage and a MALL of 2-10 µm for extreme shipping conditions.
- The probability of microbial ingress for the two films were 0.89% and 1.3% at 0 mbar and 4.4% and 13.5%. USP <1207> has a level of integrity assurance of 10% for microbial ingress.

Future Work

- The experimental test unit that will be used in future tests is depicted in Figure 2. The film with a laser cut hole at 5, 10, or 20 µm will be place in the film holder. One side will be aseptically filled with *E. coli* at a concentration of 1 x 10^8 CFU/mL.
- Pressure of the sample will be monitored and controlled using a vacuum pump and an in-line PMAT pressure monitor.
- Samples will be exposed to 0 mbar and 300 mbar and incubated at 37 C.
- A positive control will be a very compromised (3 mm hole) to validate the bacteria, a negative control with no hole to verify the experimental technique, and a non-exposed negative control to verify aseptic conditions.
- Samples will also be imaged using SEM to look at the laser cut holes before and after testing.
- Fluorescent beads of known size can be used to validate the hole size that is cut into the films.

References

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