Committee F02 on Flexible Barrier Packaging

Microbial Barrier Testing

ASTM F02 Meeting - Barcelona September 2012

Thierry Wagner
Regulatory Affairs Director
Europe, Middle East & Africa
DuPont Medical & Pharmaceutical Protection

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Aseptic Opening of a Sterile Barrier System
Transportation

Will your design survive?
Porous Sterile Barrier Systems

Porous barrier materials

- Allow the sterilisation gasses to enter and exit the package
- Allow the package to adapt to changing pressures and temperatures as well as volume changes
- Must prevent the ingress of micro-organisms in order to maintain sterility
Two Fundamental Questions

Why is Microbial Barrier Important?

- The key factor in selecting packaging materials for medical devices is the ability of the package to maintain sterility from the point of sterilization until it is opened for use.

Why is Microbial Barrier Testing Important?

- Need to understand how the packaging material will perform as a microbial barrier during handling, distribution and storage; post sterilization.
Historically: sterility viewed as absolute condition

Today: using sterility assurance level (SAL) to express probability of survivors (typically $10^{-6}$)

Before 1970: sterility test to assess sterilization efficiency

The problem: with sterility testing, there is no meaningful statement possible regarding the entire population

need 3 million tested samples to prove SAL $10^{-6}$ with 95% confidence

Sterility is defined as being free from living germs or micro-organisms
The Limitations of Sterility Testing

Let’s assume the SAL of a batch is $10^{-2}$ which is relatively high.

- With one sample, the probability to accept that batch is $1 - 10^{-2} = 99\%$
- With 2 samples $(1 - 10^{-2}) \times (1 - 10^{-2}) = 98\%$
- With $n$ samples $(1 - 10^{-2})^n$
- With 20 samples the batch is still accepted in 82% of the cases
- With 300 samples (with no false positives or negatives) batch still accepted in 5% of the cases, which is still not really acceptable.
- With a SAL of $10^{-6}$, need millions of samples to achieve a similar confidence.
Package Validation – Current Practice

- Microbial challenge testing is performed infrequently or not at all
- Physical testing of the package and a validated sterilization process is deemed sufficient evidence to indicate that the product is sterile
- But what happens to the device after sterilization during handling, distribution and storage?
- If I was a patient receiving an implantable device I would want to know, wouldn’t you?
- Microbial Barrier testing was not straightforward, until now……
Microbial Barrier Testing – An Evolution

Agenda

• Overview of the main Standard Test Methods for Microbial Barrier Testing of Porous Packaging Materials

• A Review of Filtration Theory

• Barrier Test Consortium Ltd.

• Overview of ASTM F2638
  • Methodology
  • R&R Testing Protocol and Results

• ASTM F2638 Data on breathable medical device packaging materials

• ASTM F2638 Milestones and Summary
Microbial Barrier Test Methods

DIN 58953-6:2010
Sterilization – sterile supply- sterilization paper for bags and tube packaging- test: sub clause 2.15: Testing for germ proofness with passage of air
As published by DIN (German Industry Standards)

ASTM F1608:2000
Standard test method for microbial ranking of porous packaging materials (Exposure chamber method)
As published by ASTM International, an organization based in the US that develops and publishes standards

ASTM F1608 produces a numeric result and has r & R established
# Microbial Barrier Test Comparison

<table>
<thead>
<tr>
<th></th>
<th>ASTM F1608</th>
<th>DIN 58953 Part 6</th>
<th>BS 6256 App C</th>
<th>ASTM F2638</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pro</strong></td>
<td>• Numerical result, able to rank materials</td>
<td>• Cost efficient</td>
<td>• Cost efficient</td>
<td>• Fast and safe</td>
</tr>
<tr>
<td></td>
<td>• Precision and bias statement</td>
<td>• Face velocity close to reality</td>
<td>• Quick</td>
<td>• Physical rather than microbial test</td>
</tr>
<tr>
<td></td>
<td>• Many laboratories</td>
<td>• Simulates real situation</td>
<td>• No bacteria, no special skills required</td>
<td>• Considers impact of face velocity</td>
</tr>
<tr>
<td></td>
<td>• Includes controls</td>
<td></td>
<td>• Proven over 40 year</td>
<td>• Thorough scientific documentation</td>
</tr>
<tr>
<td></td>
<td>• Well documented</td>
<td></td>
<td>• Detailed description</td>
<td>• Able to differentiate</td>
</tr>
<tr>
<td></td>
<td>• Widely accepted</td>
<td></td>
<td></td>
<td>• Validated Test method</td>
</tr>
<tr>
<td><strong>Con</strong></td>
<td>• Needs a lot of time</td>
<td>• No numerical result, only pass/fail, no differentiation</td>
<td>• High face velocity</td>
<td>• Only one apparatus available</td>
</tr>
<tr>
<td></td>
<td>• Expensive</td>
<td>• Not clear what challenge is</td>
<td>• Scale is based on different colors, difficult to judge</td>
<td>• Validatable only when additional machines available</td>
</tr>
<tr>
<td></td>
<td>• High variability</td>
<td>• High variability, prone to false positives/negatives</td>
<td>• Colors different for different materials</td>
<td>• Expensive initial investment</td>
</tr>
<tr>
<td></td>
<td>• Needs many samples to get meaningful results</td>
<td>• Documentation lacks critical details</td>
<td>• differentiation difficult/impossible</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Measures at high face velocity</td>
<td>• Probably not validatable</td>
<td>• Originally designed for face masks</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Not adequate for setting performance standards</td>
<td></td>
<td>• Not widely accepted</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Validation?</td>
<td></td>
</tr>
</tbody>
</table>
Microbial Challenges

The size of the particle challenge varies:

- from 0.002 microns diameter for viruses
- to 100 microns diameter for spores attached to dust particles.

(largest diameter particle that can remain suspended in the air for a significant length of time.)
Filtration Theory

- **Size Exclusion:** Membrane with holes of a defined maximum diameter that capture particles based upon the size of the hole and the size of the particle.

- **Depth Filtration:** Filter that retains particles through well defined mechanisms and is dependent upon the depth of the filter (basis weight), the packing density (bonding), the fiber diameter.
Filtration Theory

Predicts that there are three mechanisms that contribute to the removal of particles from an airstream flowing through a material:

- **Interception**  
  (remains constant)

- **Inertial Impaction**  
  (Larger particles at higher flow rates)

- **Diffusion**  
  (Lighter particles at slower speeds)
For any combination of filter material and flow rate, there is a particle size at which neither diffusion nor impaction dominates.

![Graph showing the relationship between log particle size and log percent penetration.](image)
For any combination of filter material and particle size, there is a flow rate at which neither diffusion nor impaction dominates.
Key Questions…

- Can we prove the hypothesis that porous sterile barrier materials follow the filtration theory? Do microorganisms behave like particles?

- If yes, can we use the filtration theory to develop a better test?
Barrier Test Consortium (BTC)

- BTC formed within the Sterile Barrier Association (SBA)
- Project Objective:
  - Develop a rapid, accurate, and affordable test method for measuring the ability of micro organisms to penetrate porous barrier materials
- Members:
  - Amcor Flexibles, Billerud, DuPont, Kimberly-Clark, Oliver Products, Perfecseal, Westfield
- Assigned ADL (Air Dispersions Ltd., Manchester, UK), a Research Organization, with the principals Alan Tallentire, Professor Emeritus and Dr. Colin Sinclair
Particle Filtration Theory

- ADL (Air Dispersions Ltd., Manchester, UK), with the principals Alan Tallentire, Professor Emeritus and Dr. Colin Sinclair

- Proved that flow rate is an important factor

- Micro-organisms behave like particles and follow the filtration theory
Particulate Barrier Test

- ADL with the principals Alan Tallentire, Professor Emeritus and Dr. Colin Sinclair

  Further proved that particle filtration testing correlates with the bacterial test [1]

Particulate Barrier Test

- Based on Tallentire’s work a test apparatus was developed working with 1 μm Polystyrene Latex spheres
- **ASTM Standard F 2638:** “Standard Test Method for Using Aerosol Filtration for Measuring the Performance of Porous Packaging Materials as a Surrogate Microbial Barrier”
  - successfully balloted in July 2007
Block Diagram of new developed Test System

- Aerosol Generator
- Sample Holder
- Challenge Particle Counter
- Filtrate Particle Counter
- Manometer
- Vacuum Generator & Filter
- Atmosphere

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Block Diagram of new developed Test System

- Challenge Particle Counter
- Filter
- Atmosphere
- Test Specimen
- Manometer
- Vacuum Generator & Filter
- Atmosphere
- Filtrate
- Particle Counter
- Aerosol Generator
- Sample Holder
Block Diagram of new developed Test System

- Challenge Particle Counter
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- Filtrate Particle Counter
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- Atmosphere
Block Diagram of new developed Test System

Apparatus measures automatically the penetration value at different flow rates

Aerosol Generator

Challenge Particle Counter

Sample Holder

Test Specimen

Manometer

Vacuum Generator & Filter

Filtrate Particle Counter

Filter

Atmosphere

Atmosphere

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<table>
<thead>
<tr>
<th>Method or Exposure</th>
<th>Face Velocity (cm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Transport</td>
<td>0.10</td>
</tr>
<tr>
<td>DIN 58953–6 Subclause 2.15 (Dry)</td>
<td>0.60</td>
</tr>
<tr>
<td>Handling</td>
<td>1.00</td>
</tr>
<tr>
<td>ASTM F 1608-00</td>
<td>~140</td>
</tr>
<tr>
<td>BS 6256 App. C (Methylene Blue)</td>
<td>&gt;1500</td>
</tr>
<tr>
<td>ASTM F 2638-12</td>
<td>~0 - 30</td>
</tr>
</tbody>
</table>
Milestones ASTM F2638

- Aug. 2007: ASTM F2638 received provisional approval pending completion of Repeatability and Reproducibility (R&R) testing for generation of Precision and Bias (P&B) statement
- Dec. 2011: Additional five test units completed
- Feb. 2012: R&R testing completed; data submitted to ASTM
- May 2012: ASTM F2638 receives full approval
- Oct 2012: Nelson Labs offers F2638 testing services
Barrier Performance

Filtration Efficiency Curves

-3 -2 -1 0 1 2
Log Face Velocity, cm/min

-3 -2 -1 0 1 2
Log % Penetration

40# Uncoated Paper
Synthetic Fiber Reinforced, Coated Paper
70# Coated Paper
55# Coated Paper
Uncoated 2FS
Uncoated 1059B
Uncoated 1073B

Standard Test Method for Using Aerosol Filtration for Measuring the Performance of Porous Packaging Materials as a Surrogate Microbial Barrier

Designation: F 2638 – 07
Breathable Materials

F 2638 Filtration Efficiency Curves

- Air Transportation 0.1 cm/min
- Handling 1.0 cm/min
Curves with F1608 Flow Reference Point

F 2638 Filtration Efficiency
Curves

% Penetration

Face Velocity cm/min

40# uncoated Paper
Synthetic Fiber
Reinforced Paper
70# coated Paper
55# coated Paper
2FS
1059B
1073B
F1608 Face Velocity
Benefits of F2638 Method vs. F1608

- Tests with polystyrene spheres vs. live spores
- No need to sterilize samples prior to testing
- No need to incubate and enumerate CFUs
- No need to maintain viable colonies of organisms
- Tests at multiple pressure differentials or flow rates
- Data generated during test is tabulated in Excel spreadsheet for fast easy data reduction
- Tests performed at conditions closer to “real package life”
Many thanks for your attention

Questions?
DIN 58953 Part 6 - Subclause 2.15 (Dry)

- **Microbiological**
  - Bacillus subtilis variant globigii spores coated on powdered quartz with a grain size of 0.04 – 0.15mm

- **Test Conditions**
  - Heat to 50 °C and cool to 10 °C five times (oven and refrigerator)
  - No vibration
  - Face velocity (theoretical) – 0.60 cm/min

- **Evaluation**
  - Max 15 CFU on the 10 samples
  - Max 5 CFU per sample
  - Test result = pass/fail
**DIN 58953-6 Wet Barrier Test**

- **Apply 5 drops with spore solution**
- **Dry drops aseptically in 6-16 hours at 22°C +/- 3**
- **Bring opposite site in contact with agar (5-6 sec)**
- **Incubation at 37°C during 16-24 hours**
- **Perform positive and sterility control of sample**

Sample
50x50 mm
Sterilized
Conditioned
23°C / 50% RH

5 drops of solution with Staphylococcus aureus

- Test 5 samples
- Zero CFU on all samples
  - yes → pass
  - no → Repeat Test with 4x5=20 samples
    - ≤ 5 CFU on all samples
      - yes → pass
      - no → fail
    - ≤ 5 CFU on all samples
      - yes → fail
      - no → pass

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BS 6256 App C - Methylene Blue test

- Methylene blue solution droplets passing through an evaporation tube to be dried into a particulate cloud, pumped through the sample at a rate of 0.03 liter/min/cm².

- The particles that pass through the sample are collected on a filter paper which is compared in colour to one which collected particles when no sample was in place.

- Pass/fail 20% of colour of filter paper without sample in place.

- Quite high velocity of the particle cloud towards the sample

- Difficult to correlate with real life situations

- Some of the more porous materials do not pass.

- No round robin documented.