Validation of Sterilizing Grade Filters

Presented by

Laura Okhio-Seaman
Sartorius Corporation
The definition of a sterilizing grade filter is one that will produce a sterile effluent after being challenged by microorganisms at a challenge level of greater than or equal to $1 \times 10^7$/cm$^2$ of effective filtration area.
Filter Qualification Tests

- Physical Tests
  - Flow rates, differential pressure, throughput
  - Sterilizability (SIP, Auto)
  - Integrity tests (bubble point, diffusive flow)

- Compendial Tests (USP, EP)
  - Particle release
  - Oxidizable substances
  - Biosafety
  - Endotoxin

- Biological Tests
  - Viability
  - Bacterial challenge test
  - Bioburden studies

- Other Tests
  - Non-volatile residue
  - Quantitative and qualitative extractables analyses in water and ethanol
  - Leachables testing
Validation of Sterilizing Grade Filters

- Demonstrates the filter retains microorganisms to produce a sterile filtrate
- Ensures the filter does not alter the product in an objectionable way
- Ensures the product does not adversely affect the filter – compatibility
- Ensures the physical parameters of the process do not adversely affect the filter or the product
Filter Qualification Tests

Viability
Brevundimonas diminuta (ATCC 19146)

Until the late 1960's, 0.45 μm-rated membranes were considered “sterilizing grade” filters, and were used successfully in the sterilizing filtration of parenterals. In the mid-1960's Dr. Frances Bowman observed a 0.45 μm “sterile-filtered” culture medium to be contaminated with a micro-organism, subsequently shown to penetrate 0.45 μm-rated membranes repeatedly in small numbers.
Viability Test

- Evaluation of potential bactericidal effects of the product solution
- Viability verified by direct inoculation into product and making serial dilutions
- Test exposure time should equal or exceed actual process filtration time
- If less than a one-log reduction (LRV) is observed, product considered non-bactericidal
- Greater than one-log reduction indicates product is bactericidal and alternatives should be considered
Viability Test Examples

<table>
<thead>
<tr>
<th>Viability Results</th>
<th>Test Exposure Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (0.1% peptone water)</td>
<td>5 min.</td>
</tr>
<tr>
<td></td>
<td>139</td>
</tr>
<tr>
<td>Not Bactericidal (example 1)</td>
<td>131</td>
</tr>
<tr>
<td>Low Bactericidal (example 2)</td>
<td>105</td>
</tr>
<tr>
<td>Strong Bactericidal (example 3)</td>
<td>0</td>
</tr>
</tbody>
</table>
Filter Qualification Tests

Bacterial Challenge
Bacterial Challenge Test

Filter Qualification by Supplier
- Challenge
- Stand. Cond.
- Bacteria/Water Suspension
- Document

Process-related Filter Validation by User/External Lab
- Challenge
- Process Cond.
- Bacteria/Product Suspension
- Document
Factors potentially affecting microbial retention include

- Filter Construction (structure, membrane polymer, pore size distribution)
- Formulation components
- Formulation properties (pH, viscosity)
- Process conditions (time, temperature, pressure differential, flow-rate)
Microbial retention studies on filter devices:

- *Brevundimonas diminuta* (ATCC 19146) has been proven to penetrate a 0.45 μm rated filter
- Spiking of the drug product with *Brevundimonas diminuta* according to ASTM 838-05
- Challenge level > 10⁷ CFUs / cm² filtration area.
- Validation Testing should simulate worst-case process conditions e.g., pressure differential, flow-rate, time, temperature
Bacterial Challenge Test

- Challenge suspension should be mono-dispersed to provide ‘worst-case’ challenge
- Optical microscopy is used to detect aggregation and clumping of bacteria
- Ultrasonic treatment reduces aggregation
- Penetration of 0.45 μm rated membranes is indicative of single-cell conditions and this membrane is used as a positive control
Microscopic investigation
Criteria: single cells, motile cells
Bacterial Challenge Test

- ASTM 838-05 (2005) uses *B. diminuta* as the standard challenge organism
- 2004 Aseptic Processing Guidance suggests the use of native bioburden isolate when appropriate
Microbiological challenge tests using one lot of low Bubble Point (close to manufacturer's minimum specification) membranes should be used.*

Example:
Minimum Bubble Point Value: 46 psi
Release criteria of filter vendor: 50 psi
Low BP Range: 46 – 50.6 psi

* At or near filter manufacturers minimum integrity test value (10%), 1999 PDA / FDA Joint conference, PDA Newsletter December 1999
The purpose of the test is to validate the retention efficacy of a particular membrane material. A small 47mm membrane disc can be used.

To more closely simulate process conditions and to determine the integrity of process filters, a small area 150 or 300 cm² filter device capsule can be used.

Full sized 10” filter cartridges require large volumes of product (25–30 liters) to perform the challenge testing.
Either 0.45 or 0.2 micron-rated filter membrane can be used for recovery membranes

Studies indicate that 0.45 micron-rated membranes might be more efficient than 0.2 for this purpose

Cellulose Nitrate is the preferred membrane polymer – high affinity for proteins
Pressure Differential and Flow Rate

- Maximum process pressure differentials (\(\Delta P\)) across the filter should be used in the challenge studies.
- Process flow-rate should be achieved in challenge studies.
- If pressure and flow rate cannot be simultaneously achieved, the user should determine which is more relevant and develop a rationale to support the decision.
- Regulators tend to prefer higher pressure data rather than flow-rate.
Manual BCT Set-up
BCT Set-up for Small Scale Pleated Filters
“Big Bertha” rig
Sartorius AG
Possible BCT Strategies

Determine & Document viability of *B. diminuta* in Product under Process Conditions

Is *B. diminuta* viable in product under process conditions?

- **Viable**
  - Direct inoculation of *B. diminuta* in product under process conditions
  - Modify process (adjust temperatures, etc.)
  - Modify formulation (adjust pH, remove bactericidal component or product surrogate)
  - Change from *B. diminuta*; use bacteria isolated from formulation or environment

- **Not Viable**
  - Use product for time period that the challenge organism is viable
  - Precondition filter with Product – followed by Microbial Challenge
BCT Strategies – Method 1  (Direct Inoculation)

Determine & Document viability of *B. diminuta* in Product under Process Conditions

Direct inoculation of *B. diminuta* in product under process conditions

Is *B. diminuta* viable in product under process conditions

Viable

Direct inoculation of *B. diminuta* in product under process conditions
## BCT Strategies – Method 1 (Direct Inoculation)

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Test Exposure Times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min.</td>
</tr>
<tr>
<td>Standard (0.1% peptone water)</td>
<td>129</td>
</tr>
<tr>
<td>Product Solution</td>
<td>132</td>
</tr>
</tbody>
</table>

→ **BCT by direct inoculation**
BCT Method 2 (Process Modification)

Determine & Document viability of *B. diminuta* in Product under Process Conditions

Direct inoculation of *B. diminuta* in product under process conditions

Is *B. diminuta* viable in product under process conditions?

**VIALE**

**NOT VIALE**

Modify process (adjust temperatures, etc.)

Modify formulation (adjust pH, remove bactericidal component or product surrogate)

Change from *B. diminuta* Use bacteria isolated from formulation or environment

Use product for time period that the challenge organism is viable

Precondition filter with Product – followed by Microbial Challenge
Temperature of $\geq 40^\circ$C supports mortality rate of *B. diminuta*

Check viability of test bacteria in the product solution at lower temperature

Direct inoculation into product solution might be possible at 35°C
BCT Strategies Method 3
(Time Period Challenge)

Determine & Document viability of *B. diminuta* in Product under Process Conditions

Direct inoculation of *B. diminuta* in product under process conditions

Is *B. diminuta* viable in product under process conditions?

- **VIA BLE**
  - Modify process (adjust temperatures, etc.)
  - Use product for time period that the challenge organism is viable

- **NOT VIA BLE**
  - Modify formulation (adjust pH, remove bactericidal component or product surrogate)
  - Precondition filter with Product – followed by Microbial Challenge
  - Change from *B. diminuta* Use bacteria isolated from formulation or environment
BCT Strategies – Method 3  
(Time Period Challenge)

### BCT by direct inoculation

Contact time bacteria / test solution max. 30 min.

<table>
<thead>
<tr>
<th>Solutions</th>
<th>TEST EXPOSURE TIMES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min.</td>
</tr>
<tr>
<td>Standard (0.1% peptone water)</td>
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<tr>
<td>Product Solution</td>
<td>111</td>
</tr>
</tbody>
</table>
BCT Strategies – Method 4

(Determination & Document viability of *B. diminuta* in Product under Process Conditions)

- **Direct inoculation of *B. diminuta* in product under process conditions**

- **Is *B. diminuta***
  - **Viable** in product under process conditions?

- **NOT VIABLE**
  - **Modify process** (adjust temperatures, etc.)
  - **Modify formulation** (adjust pH, remove bactericidal component or product surrogate)
  - **Change from *B. diminuta***
    - Use bacteria isolated from formulation or environment

- **Use product for time period that the challenge organism is viable**

- **Precondition filter with Product – followed by Microbial Challenge**
Determine & Document viability of *B. diminuta* in Product under Process Conditions

- **Direct inoculation of *B. diminuta* in product under process conditions**

- **Is *B. diminuta* viable in product under process conditions?**
  - **Viable**
    - Modify process (adjust temperatures, etc.)
    - Use product for time period that the challenge organism is viable
  - **Not Viable**
    - Modify formulation (adjust pH, remove bactericidal component or product surrogate)
    - Change from *B. diminuta* Use bacteria isolated from formulation or environment
    - Precondition filter with Product – followed by Microbial Challenge

**BCT Strategies – Method 5 (Preconditioning)**
BCT Strategies – Method 5 (Preconditioning)

<table>
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<th>Solutions</th>
<th>TEST EXPOSURE TIMES</th>
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<td></td>
<td>5 min.</td>
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</tr>
<tr>
<td>Product Solution</td>
<td>0</td>
</tr>
</tbody>
</table>

Indirect BCT by separate filtration of test solution and bacteria
Is *B. diminuta* viable in product under process conditions?

**Viable**
- Direct inoculation of *B. diminuta* in product under process conditions

**Not Viable**
- Modify process (adjust temperatures, etc.)
- Modify formulation (adjust pH, remove bactericidal component or product surrogate)
- Change from *B. diminuta* Use bacteria isolated from formulation or environment

Use product for time period that the challenge organism is viable

Precondition filter with Product – followed by Microbial Challenge
BCT with bioburden isolate

- Reproducible growth and size of bacteria is required

- Growth in required concentration for BCT must be possible
Filter Integrity
Integrity Test Correlation

Destructive Methods

For producers; applied to determined limits:

Bacteria-Challenge-Test (according to ASTM)

Non-Destructive Methods

For users; applied to every sterilizing filter before and after each filtration:

- Bubble Point Test
- Diffusion Test
- Multipoint Diffusion Test
- Pressure Decay Test
- Water Intrusion Test (WIT)

Direct correlation of diffusion, bubble point, and intrusion-limit values to bacteria-challenge test.
Integrity Test Correlation

5.3 Diffusion Test Limits

5.3.1 Cartridges 0.2 µm (10"/250 mm)

Note:
Since most of the filters tested during the validation stage had low diffusion values and produced a sterile filtrate, the following data is a sampling from all filters tested during the validation testing indicating results near the diffusion/sterile filtrate limits.

<table>
<thead>
<tr>
<th>Lot Number</th>
<th>Diffusion [m/min]</th>
<th>Bacterial [CFU]*</th>
<th>Filtered Quality</th>
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<td>930441</td>
<td>11.5</td>
<td>1.12 x 10^3</td>
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</tr>
</tbody>
</table>

* CFU – Colony Forming Units
Bacterial Challenge Test
**Product Wet Integrity Testing**

**Bubble Point**
Filter membrane is wetted with the product. Pressure is applied on the upstream side of the filter. The pressure at which a stream of air bubbles is detected downstream of the filter is known as the Minimum Bubble Point of the filter.
Product Specific Integrity Test – Bubble Point

Flush with Water
Test a, b, c
Calculate average, \( WBP_{\text{avg}} \)

Flush with Product
Test a, b, c
Calculate average, \( PBP_{\text{avg}} \)

\[ PBP_{\text{avg}}/WBP_{\text{avg}} \]

\[ \text{e.g. } 46 \text{ psi} \times 36 \text{ psi}/49 \text{ psi} \]

\[ PBP_{\text{min}} = 46 \text{ psi} \times 0.73 \]

\[ PBP_{\text{min}} = 33.79 \text{ psi} \]

\[ PBP_{\text{min}} = WBP_{\text{min}} \times \text{Correction Factor} \]
Product Wet Integrity Testing

Diffusive Flow

Depends mainly on the solubility of the test gas in the wetting fluid in addition to temperature and test pressure. The filter membrane pores are wetted with fluid and a gas pressure less than the BP is applied. Due to differential pressure, gas diffuses through the fluid in the pores and is quantified as downstream flow in mL/min.
Flush with Water

Test a, b, c with \( \text{MTP}_{WW} \)

\[ \text{TP}_{PW} = \text{MTP}_{WW} \times \frac{\text{PBP}_{avg}}{\text{WBP}_{avg}} \]

Calculate average, \( \text{DF}_{WW} \)

Flush with Product

Test a, b, c with \( \text{TP}_{PW} \)

Calculate average, \( \text{DF}_{PW} \)

\[ \text{DF}_{PW} = \text{DF}_{WW} \times \text{Correction Factor} \]
Integrity Test Failure

TR #26 includes a Trouble Shooting Guide in case of Integrity Test Failures:

Determines when a filter has to be classified as failed

- Filter fails first time → Measurements & Actions
- Filter fails second time → Wetting with solvent
- Filter fails third time = Filter failed
Guidance
Final Guidance released on September 29, 2004, which replaces the 1987 Guidance

Describes the integrity test requirements of liquid and air filters, as well as the validation requirements of liquid filters
Post approval Change Guidance for chemical entities (Revision).

Describes which changes in filtration steps are considered moderate (CBE 30) or major (Post Approval Supplement) changes.
Warning letters and inspection guides are always good to review – learn from the mistakes and from the training.

Listings are posted on FDA website as prescribed by the FOI Act.
EC cGMP Guidance, Revision of Annex 1.

Describes sterilization by filtration, integrity test needs, some validation topics.

Important document to know for exporting.
Released ISO Guidance, part 2. on filtration.

Describes filtration requirements, validation and integrity test needs thoroughly for liquid and air filters.

A good document to have, with thorough descriptions.

The most thorough and descriptive document on the topic of liquid sterilizing filtration.

This document is a must have, must read and must understand as it is used by QC and regulators worldwide.
Conclusion

Viability Testing
Bacterial Challenge Testing
Chemical Compatibility
Analysis of Extractables
Product Integrity Testing
Plant and Process Surveys
Systems and Integrity Tester Validation
Process Related Validation Studies
Validation of Sterilizing Grade Filters

END