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Application Note

Retention of Process-Contaminant Organisms by Durapore® 0.22 µm Filters

A Study on Bacterial Retention to Determine the Worst Case Challenge Organism

Background

Incidents of microbial penetration of membrane filters rated as 0.2 µm or 0.22 µm sterilizing-grade have been cited in the literature.[1,2,3] Passage has been attributed to (1) grow-through; (2) the ability of organisms to create deformable L=forms, and; (3) exceeding the inherent clearance capabilities of a given filter. Of specific concern are "small" microorganisms such as *Burkholderia cepacia*, *Ralstonia pickettii*, *Pseudomonas luteola* and *Pseudomonas fluorescens*.

The goal of these studies was to determine the sizes of a range of bacteria that could be possible contaminants of bioprocess fluid streams and to assess their retention on Durapore® 0.22 µm membrane filters. The organism sizes were compared to *Brevundimonas diminuta*, the standard bacterium used for microbial retention studies of sterilizing grade filters. Results showed that all organisms were larger than *B. diminuta* and that as Durapore® 0.22 µm membrane provided complete retention of *B. diminuta* in process-simulation testing, it can reasonably be inferred that we would predict similar complete retention of other bacterial contaminants.

Small Organisms and Their Sizes

Table 1 provides a list of bacteria, many of which were isolated from process streams and submitted for evaluation. Each isolate was cultured with an aim of producing small cells and was examined by scanning electron microscopy to measure cell length and width.

These data support the generally accepted premise that when cultured appropriately, *B. diminuta* is the smallest bacterium relevant for most pharmaceutical applications and is therefore a suitable organism for filter microbial retention studies. When prepared in a saline lactose broth (SLB) medium, *B. diminuta* cells have a mean length 0.68 µm and a mean width of 0.31 µm. **Biopharmaceutical manufacturers should assess their product bioburden and if justified as worst case compared to *B. diminuta*, use this microorganism for the product-specific bacterial retention test.**[6,7,8,9]

Table 1. Sizes of Various Organisms

Organism	Source	Culture Medium	Length (µm)	Width (µm)
Brevundimonas diminuta	ATCC® 19146™	SLB	0.68	0.31
Burkholderia cepacia	ATCC® 35254™	DI H_2O	1.11	0.46
Burkholderia cepacia	ATCC® 25416™	SLB	1.15	0.42
Burkholderia cepacia	Process isolate	Saline	1.00	0.43
Pseudomonas fluorescens	Process isolate	SLB	0.90	0.53
Pseudomonas fluorescens	Process isolate	SLB	1.17	0.46
Pseudomonas fluorescens	Process isolate	DI H_2O	1.02	0.22
Pseudomonas luteola	Process isolate	SLB	0.72	0.39
Pseudomonas luteola	Process isolate	DI H_2O	0.86	0.33
Stenotrophomonas maltophilia	Process isolate	DI H_2O	0.88	0.44
Stenotrophomonas maltophilia	Process isolate	Product	1.40	0.52
Ralstonia pickettii	CDC-Anderson	DI H_2O	1.37	0.48
Pseudomonas pseudoalcaligenes	Process isolate	RPMI	1.06	0.32
Pseudomonas stutzeri	Process isolate	SLB	1.22	0.50
Comamonas testosteroni	Process isolate	SLB	0.99	0.38
Xanthomonas maltophilia	Process isolate	DI H_2O	1.28	0.37
Bacillus cereus	Process isolate	Media Fill	1.19	0.36

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Process Contamination Concerns

Contamination episodes usually raise questions regarding retention of small organisms with 0.2 µm or 0.22 µm sterilizing-grade filters. If there is a product contamination, the source of contamination needs to be determined by a thorough investigation. For example but not limited to:

- Is the sample truly contaminated or is there a sample handling/QC testing issue?
- Is aseptic processing achieved and maintained? For example, has the sterilization cycle (steaming or autoclaving) been validated, were the SOPs for the sterilization cycle followed? Is the filter integral?
- Is the downstream piping compromised in any way, e.g., non-sanitary valve?
- Is the organism truly retained or not retained by the 0.2 µm or 0.22 µm sterilizing-grade filter?

Retention of Small Organisms with Durapore® 0.22 µm Filters

The bacterial retention results of small microorganisms with hydrophilic Durapore® 0.22 µm membrane are shown in **Table 2**. The results shown are for informational purposes only. Bacterial retention tests with process specific organisms for validation of sterilizing filter performance should be performed under worst case process conditions.

Table 2. Retention Test Results for Small Organisms with Durapore® 0.22 µm Membrane

Challenge Organism	Challenge Fluid	LRV	No. of Tests
B. cepacia	SLB	>9.35	15
P. fluorescens	SLB	>8.45	3
P. fluorescens	Product	>8.50	3
R. pickettii	SLB	>9.67	15
P. stutzeri	SLB	>8.34	3
P. stutzeri	Product	>8.52	3

Table 2 shows that under the test operating conditions, Durapore® 0.22 µm membrane is completely retentive of these small microorganisms which were previously believed to be smaller than *B. diminuta*.

Bacterial Passage Concerns During Extended Processing

Bacterial "grow-through" is a potential explanation for the presence of microorganisms downstream of 0.2 µm or 0.22 µm pore size-rated filters that have been in use for an extended period. Grow-through refers to the phenomenon of bacterial penetration into a porous filtration membrane, followed by cell division within the membrane pore structure if the environment is sufficiently nutritive, and ultimate release of small "daughter" cells to the downstream side of the membrane. Limited studies in the literature have demonstrated the occurrence of bacterial grow-through with various types of polymer membranes, although not consistently.[4,5] To mitigate this concern, regulatory agencies state that appropriate processing time limitations be set forth and validated for sterilizing filtration steps.[6,7,8,9]

Support Services

Our single-use and filter validation services team can help you throughout your filter validation process:

- By performing filter bacterial retention under your worst case process conditions with the standard *B. diminuta* or with your bioburden process isolate.
- By providing consultancy support and assessing whether your bioburden process isolate is a suitable candidate for the filter bacterial retention test.

References

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