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Pharma & Biopharma
Manufacturing &
Testing Services

Application Note

Retention of Small Organisms by 0.22 µm Durapore® Filter

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

Background

Incidents of microbial penetration of membrane filters rated as "0.2 or 0.22 µm sterilizing grade" have been cited in the literature.[1,2,3] Passage has been attributed to (1) growthrough; (2) the ability of organisms to create deformable L=forms, and; (3) exceeding the inherent titre reduction of a given filter. Of specific concern are "small" microorganisms such as *Burkholderia cepacia*, *R. pickettii*, *P. luteola* and *P. fluorescens*, which have been isolated from process streams.

Several experiments were performed to examine the size of the organisms, and the ability of these organisms to penetrate Durapore® 0.22 µm PVDF membrane. Results show that the organisms are larger than *Brevundimonas diminuta* (*B. Diminuta*) the industry-standard organism for evaluating microbial retention of sterilizing grade filters, and show that, in process-simulation testing, 0.22 µm Durapore® membrane will provide complete retention of these organisms.

Small Organism and Their Sizes

In **Table 1** is a listing of bacteria, many of which were isolated from actual 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 lengths and widths.

In every case, *B. diminuta* was smaller than any of the other organisms. These data support the generally accepted conclusion that appropriately cultured *B. diminuta* is the smallest bacterium that is relevant to most pharmaceutical applications, and is therefore the challenge organism used in filter validation studies. When prepared in a saline lactose broth (SLB) medium, *B. diminuta* cells are deaggregated and very small, with a mean length 0.68 microns (0.14 micron standard deviation) and a mean width of 0.31 microns (0.04 micron standard deviation). **However, if a smaller bacterium is found in a given process stream, the biopharmaceutical company is required to perform validation studies with the smaller challenge organism.**[4,5,6,7]

Table 1. Sizes of Various Organisms

Organism	Reference	Media	Length (µm)	Width (µm)
Brevundimonas diminuta	ATCC 19146	SLB	0.68	0.31
Burkholderia cepacia	ATCC 35254	DI H2O	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 H2O	1.02	0.22
Pseudomonas luteola	Process isolate	SLB	0.72	0.39
Pseudomonas luteola	Process isolate	DI H2O	0.86	0.33
Stenotrophomonas maltophilia	Process isolate	DI H2O	0.88	0.44
Stenotrophomonas maltophilia	Process isolate	Product	1.40	0.52
Ralstonia pickettii	CDC-Anderson	DI H2O	1.37	0.48
Pseudomonas pseudoalcaligenes	Process isolate	RPMI	1.06	0.32
Pseudomonas stutzeri	Process isolate	SLB	1.22	0.50
Comamonas testoseroni	Process isolate	SLB	0.99	0.38
Xanthamonas maltophilia	Process isolate	DI H2O	1.28	0.37
Bacillus cereus	Process isolate	Media Fill	1.19	0.36

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Small Organism Contamination Concerns

Contamination episodes usually raise questions regarding retention of small organisms with a 0.2 µm or 0.22 µm sterilizing-grade filter. If there is a product contamination, the source of the contamination needs to be determined and the following issues need to be investigated:

- 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 abided by?
- Was 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 0.22 µm hydrophilic Durapore® membrane are shown in **Table 2**.

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

Challenge Organism	Media	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 operating conditions studies, 0.22 µm Durapore® membrane is completely retentive of these small microorganisms which have been thought to be potentially smaller than *B. diminuta*.

Bacterial Concerns During Extended Processing

"Growthrough" is one hypothesis used to explain the presence of microorganisms downstream of 0.2 µm or 0.22 µm rated filters that have been in service for an extended period of time. Although there is no reliable evidence to support this hypothesis, the possibility of growthrough is of concern to regulatory agencies. Regulatory agencies state that appropriate time limitations be set forth for sterilizing filtration steps.[4, 5, 6, 7]

Bacterial Retention During Extended Processing with Durapore® 0.22 µm Filters

Durapore® filters have been studied under simulated conditions of extended processing. Studies have shown[8] that Durapore® filters do not permit growthrough when tested with *B. diminuta* under the following conditions:

- Up to 96 hours of continuous processing (10^7 cfu/cm^2)
- Up to 7 days of intermittent processing (10^4 cfu/cm^2)
- Up to 5 days with *B. diminuta* suspended in specific aqueous pharmaceuticals (10^7 cfu/cm^2).

Support Services

Our Validation Services laboratories can perform:

- Filter retention testing for process isolates in specific drug products
- Filter retention testing under extended processing conditions

References

1. Christian, D.A. et al., "The penetration of membranes by organisms growthrough and its related problems., Ultrapure Water. May/June 1986. pp. 39-44
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4. FDA Guidance for Industry. Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice.
5. European Guidelines to Good Manufacturing Practice, Volume 4 Medicinal Products for Human and Veterinary Use, ANNEX 1 Manufacture of Sterile Medicinal Products
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