An evaluation of microbial recovery and retention for sterile Analytical Membrane Bands

The purpose of this study was to evaluate Thermo Scientific™ Analytical Membrane Bands for microbial recovery and retention according to standards outlined in ISO 7704 and ASTM F838, respectively. The results of this study show that the performance of the membrane filters exceeded the requirements for microbial recovery and retention according to both standards.

Introduction

Membrane filters are routinely used in the laboratory to determine microbial counts during water quality testing. ISO 7704:1985 is a standard for evaluation of membrane filters used for enumerating microorganisms in water samples. More specifically, the ISO 7704 requirements state that membrane filters with colony forming unit (CFU) counts ≥80% of the control plate counts are considered acceptable. To determine if the sterile Analytical Membrane Bands meet the ISO 7704 standard for water quality testing, the filters were tested for microbial recovery according to this criteria.

Membrane filters, specifically those with a 0.2 µm pore size, are also routinely used in laboratories to sterilize solutions such as buffers, water, and antibiotics. ASTM F838 is a standard test method that determines if the membrane will retain microbes and prevent them from passing through during liquid filtration. This test method can be used to evaluate any membrane filter system used for sterilization-grade filtration. *Brevundimonas diminuta* is typically used as the challenge organism because of its small size and uniform growth compared to other microorganisms.

Materials and methods

To evaluate sterile Analytical Membrane Bands for microbial recovery, three challenge organisms were used. *Escherichia coli* (ATCC, Cat. No. 11775) and *Klebsiella aerogenes* (ATCC, Cat. No. 35029) were used to test the 0.2 µm and 0.45 µm pore–size membrane filters, and *Saccharomyces cerevisiae* (ATCC, Cat. No. 7754) was tested used to test the 0.8 µm pore–size membrane filters. For each organism tested, 10 test plates and 5 control plates with the same concentration of challenge organisms were prepared. After filtration of the challenge organism, the membranes were placed on the appropriate agar medium. To allow for microbial growth, control plates and plates containing challenged membrane filters were incubated at 28–32°C for approximately 24 hours. After incubation, the CFUs were counted on all plates and percent recovery was determined.

$$\text{Percent recovery} = \frac{\text{mean CFUs of challenged membrane filters}}{\text{mean CFUs of control plates}} \times 100\%$$

To evaluate sterile Analytical Membrane Bands for microbial retention, *B. diminuta* was grown to 10 x 10^6 CFU/mL in saline lactose broth (SLB). Replicates (n = 32) of the culture were passed through the 0.2 µm membrane filters. The filtrate was kept and incubated for 7 days at 30°C ± 2°C, then evaluated for microbial growth by looking for turbidity of the SLB solution, which indicates growth.

Results

All membrane filter pore sizes with each challenge organism passed the acceptance criteria (percent recovery of ≥80%) according to the ISO 7704 standard for microbial recovery (Table 1). Specifically, *E. coli* recovery on the 0.2 µm filters and 0.45 µm filters was 111% and 108%, respectively. *K. aerogenes* recovery on the 0.2 µm filters and 0.45 µm filters was 108% and 109%, respectively. *S. cerevisiae* recovery on the 0.8 µm filters was 102%.

Table 1. Microbial recovery on filters of different pore size from testing according to ISO 7704 criteria.

Filter pore size	Challenge organism	Average percent recovery (n = 10)	Acceptance criteria	Result
0.2 µm	*E. coli*	111%	≥80%	Pass
	K. aerogenes	108%	≥80%	Pass
0.45 µm	*E. coli*	108%	≥80%	Pass
	K. aerogenes	109%	≥80%	Pass
0.8 µm	*S. cerevisiae*	102%	≥80%	Pass

Microbial retention testing of 32 samples of *B. diminuta* at a concentration of 10 x 10^6 CFU/mL using 0.2 µm filters resulted in no turbidity in the SLB solution filtrates after 7 days of incubation at 30°C ± 2°C (Table 2).

Table 2. Microbial retention from testing according to the ASTM F838 standard test method.

Filter pore size	Challenge organism	Turbid samples/ total samples	Acceptance criteria	Result
0.2 µm	*B. diminuta*	0/32	0	Pass

Discussion

Membrane filters perform to ISO 7704 standards for the three organisms tested (*E. coli*, *K. aerogenes*, *S. cerevisiae*). Each organism exceeded the requirement of 80% recovery on their respective pore sizes.

The 0.2 µm filter was capable of retaining all of the challenge organisms. Out of 32 samples tested, zero exhibited microbial growth after 7 days of incubation at 30°C ± 2°C. Therefore, 0.2 µm membranes passed microbial retention testing according to the ASTM F838 standard test method.

Additional product criteria

Non-fiber releasing membrane

This product was manufactured using a mixed cellulose esters microporous membrane, which meets the criteria for "nonfibrous releasing" filters as defined in 21 CFR 210.3 (b)(6) of the Food Additives Amendment of the U.S. Federal Food and Drug Act.

Water quality testing

The product has been tested for bacterial recovery as described in Standard Methods for Examination of Water and Wastewater [23rd Ed., Part 9020, Section 5i] established by the American Public Health Association (APHA), American Water Works Association (AWWA) and the Water Environment Federation (WEF). Filters have been tested for microbiological recovery in accordance with ISO 7704 procedures and exceed the requirements of the standard.

Sterilizing grade

For *B. diminuta*, a reduction of 7 logarithmic units is achieved using the 0.2 µm membrane filters.

Microbial recovery

- 0.2 µm and 0.45 µm pore sizes: meet ISO 7704 for recovery of *E. coli* (ATCC, Cat. No. 11775) and *K. aerogenes* (ATCC, Cat. No. 35029)
- 0.8 µm pore size: meets ISO 7704 for recovery of *S. cerevisiae* (yeast) (ATCC, Cat. No. 7754)

Sterilization validation

Product has been sterilized by gamma irradiation and accepted for release per sterility assurance level (SAL) of 10^{-6} as defined in ISO 11137-1:2006.

Note: These filter products are designed for analytical and research purposes only and are not intended for the sterilization of liquids, manufacturing applications, or clinical applications. This product is not intended for use in direct patient care or diagnostic procedures.

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