Technical Brief

Extended bacterial retention testing of Millipore Express®0.2 µm filters in a continuous flow system

Introduction

Membrane filter manufacturers utilize the ASTM® method as a standard method for establishing a sterilizing filter claim on their products[1]. The ASTM method requires the filter and device samples be challenged with and retain a high concentration of bacteria when tested at a high differential pressure over a short filtration time (typically less than <5 minutes). While this method sets a useful standard for the industry by providing a quantitative assessment of filter performance, it is limited in that it demonstrates performance under only one set of conditions that is unlike typical filter operating parameters.

This additional study examines the performance of Opticap® XL 150 capsule filters with Millipore Express® 0.2 µm membrane with gram-negative, non-fermenting bacterium under extended continuous flow. Flux was maintained at approximately 1,230 LMH over 4, 8, and 16 hours to simulate potential extended duration processing conditions.

Objective

Demonstrate extended bacterial retention effectiveness and capabilities of KGEPG015FF1 Opticap XL 150 pleated filter devices when challenged with *Brevundimonas diminuta* ATCC® 19146 at a minimum level of 1.0 x 10^7 colony forming units per centimeter squared (cfu/cm^2) using a peristaltic pump and continuous fluid flow over 4, 8, and 16 hours.

Methods And Materials

Test Filters

Pre-gamma sterilized Opticap XL 150 capsule filters with Millipore Express 0.2 µm membrane (KGEPG015FF1) provide high flow rates and sterility assurance. These filters are recommended for the sterile filtration of buffers, pH adjusters, cleaning solutions and other low-fouling aqueous based streams where the key performance needs are high permeability, process efficiency and bacterial retention.

Test System

There were four different individual test systems for each experiment. One test system design was used for each bacterial retention test. A schematic diagram of an individual test system is in Figure 1. The test system used a peristaltic pump, tubing, and stainless steel components. Calibrated pressure transmitters and a precise electronic data acquisition system were used to record the continuous flow pressure and ensure there was no system backpressure.

Culture Preparation

The *B. diminuta* challenge culture was prepared in a two-stage cultivation process using saline lactose broth (SLB) method based on current ASTM F838 and Merck Millipore validated methods. A 25 mL volume of the *B. diminuta* SLB culture was then added to 1,475 mL of sterile water and mixed to re-suspend the cells. *B. diminuta* cell counts remained stable over time ensuring consistent cell counts during the retention tests.

Bacterial Challenge Test

Test system components (excluding pressure transmitters and data acquisition components) were autoclave sterilized at 121 °C before testing. The 4, 8 and 16 hour endpoints experiments were conducted separately using four Opticap XL 150 filter capsules per experiment. Each of the sixteen Opticap XL 150 devices tested had been previously sterilized by gamma radiation and subjected to an additional sixty minute autoclave sterilization cycle at 121 °C. This additional autoclave sterilization cycle was conducted to increase stress on the device and ensure a rigorous test. Pre-use, post gamma sterilization filter integrity tests were performed using an Merck Millipore Integritest® 4 testing system.

Following assembly, a single pass/re-circulation challenge method was used to minimize the total volume of test fluid. A vessel containing the *B. diminuta* challenge was connected to the inlet side of each individual test system. A sample of the challenge was removed and stored at room temperature for the duration of the eight hour and sixteen hour study. Upon test completion, the sample was serially diluted and spread plate enumerated to ensure challenge viability and minimum challenge cfu/cm^2 requirements were met. Previous study data supported end of challenge cell counts for the four hour study.

The challenge was slowly fed through each individual test system to eliminate any trapped air. Next, the pump speed was met. To create a more difficult filtration test scenario, the challenge suspension flow rate was set for 450 mL/min and processed through the test device and assay filters into a collection vessel. Once the challenge flask was empty, it was disconnected from the test system. The recirculation vessel, containing sterile water, was attached to the inlet side of the test system and the fluid was re-circulated continuously for the entire test duration. This ensured continuous flow over the bacteria/test membrane layer for the required test time.

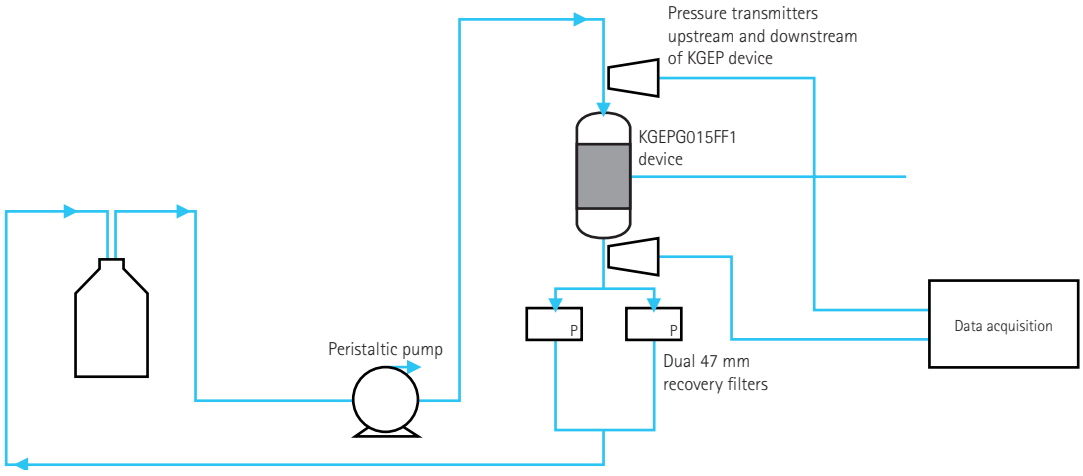

Figure 1. Test systems schematic design

Recovery Assay

The test filter effluent was expected to produce an absence of bacterial passage. For this reason, the entire effluent volume was assayed via the membrane filter method utilizing 0.45 µm mixed esters of cellulose assay filters (Merck Millipore HAWG04700). To accommodate the continuous flow volumes, two parallel assay filters were incorporated downstream of each test filter (see Figure 1). The fluid flow through the recovery filters was continuous throughout the bacterial challenge test. Upon test completion, both assay filters were aseptically removed from the holders and individually plated onto Trypticase Soy Agar plates. The assay filters were observed for presence or absence of growth after a minimum of 48 hours and 7 days.

Upon test completion, the assay filters were aseptically removed from the test system and were post-use integrity tested using an Merck Millipore Integritest 4 system.

Results

After 7 days of incubation, there was no growth detected downstream of the four Opticap XL 150 capsule filters with Millipore Express 0.2 µm membrane test filters that were tested at each time point (for a total of sixteen tested filters). A review of the pressure graphs for each showed a pressure profile consistent with peristaltic pump driven flow and confirmed there were no reverse pressure forces during the experiments.

Calculations

Total Challenge (cfu/filter) = Innoculum Concentration in cfu/mL x Challenge Volume in mL

$$\text{EFA Challenge (cfu/cm}^2\text{)} = \frac{\text{Total Challenge (cfu/filter)}}{\text{Effective Frontal Area (cm}^2\text{/filter)}}$$

All other test acceptance criteria for each experiment including; challenge organism viability at test completion and minimum challenge level of greater than 1.0×10^7 cfu/cm^2 at experiment start and end were met indicating the studies were well executed.

Conclusion

The results from these experiments demonstrate Opticap XL 150 capsules with Millipore Express SHF 0.2 µm membrane were fully retentive when challenged with *B. diminuta* at a challenge level of at least 1.0×10^7 colony forming units/cm^2 using a peristaltic pump and continuous fluid flow of 450 mL/min for at least sixteen hours.

References

(1) American Society for Testing and Materials. Standard test method for determining bacterial challenge of membrane filters utilized for liquid filtration. ASTM F838-05. ASTM International: West Conshohocken, PA. 2005.

Summary Table 1

	Sample No.	Device Serial No.	Device Lot No.	Initial EFA Challenge (cfu/cm^2)	Final EFA Challenge (cfu/cm^2)	Result	LRV
Study 1: 4 Hour Bacterial Challenge	4-A	001	C9JN16885	2.8×10^7	n/a	Retentive	>9.8
	4-B	002	C9JN16885	2.9×10^7	n/a	Retentive	>9.8
	4-C	003	C9JN16885	3.0×10^7	n/a	Retentive	>9.8
	4-D	013	C9JN16885	2.9×10^7	n/a	Retentive	>9.8
Study 2: 8 Hour Bacterial Challenge	8-A	002	C9CN99813	2.7×10^7	2.9×10^7	Retentive	>9.7
	8-B	009	C9CN99813	3.1×10^7	2.8×10^7	Retentive	>9.8
	8-C	014	C9CN99813	3.0×10^7	2.4×10^7	Retentive	>9.8
	8-D	022	C9CN99813	3.0×10^7	2.7×10^7	Retentive	>9.8
Study 3: 16 Hour Bacterial Challenge	16-A	004	C9JN16885	2.2×10^7	2.1×10^7	Retentive	>9.7
	16-B	007	C9JN16885	2.7×10^7	2.3×10^7	Retentive	>9.7
	16-C	022	C9JN16885	2.2×10^7	2.2×10^7	Retentive	>9.7
	16-D	023	C9JN16885	2.2×10^7	2.2×10^7	Retentive	>9.7

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