Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Technical Brief

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

Introduction

Millipore Express® filters are designed to deliver sterility assurance and offer biomanufacturers high-performance filtration to support every step of their journey. Like many membrane filter manufacturers, we use an ASTM® test method for establishing a sterilizing filter claim on our filtration products[1]. In this method, the filter is challenged with a high concentration of bacteria at a high differential pressure over a short filtration time (typically less than 5 minutes). While the method sets a useful industry standard, it is limited in that it evaluates filter performance under only one condition that is not representative of typical filter operating parameters.

The goal of this technical brief is to describe microbial retention performance of sterilizing-grade Millipore Express® SHF (Sterile High-Flux) filters under continuous flow operations for up to 16 hours processing duration.

Methods And Materials

Test Filters and System

Millipore Express® SHF 0.2 µm filters are recommended for sterile filtration of low-fouling aqueous streams where the key performance needs are high permeability, process efficiency and bacterial retention.

A schematic of the test system is shown in **Figure 1**. The test system used a peristaltic pump, tubing, and stainless steel components connected to an Opticap® XL 150 capsule containing a Millipore Express® SHF membrane. 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. Each of the filters was sterilized by both gamma irradiation and autoclaved at 121 °C for 60 minutes before testing, to represent a worst-case sterilization scenario for device stress. Pre-use, post-gamma sterilization filter integrity tests were performed using an automated integrity testing system.

Culture Preparation

The *Brevundimonas diminuta* ATCC® 19146 challenge culture was prepared in a two-stage cultivation process using saline lactose broth (SLB) method based on current ASTM® F838 and our own validated methods. The *B. diminuta* SLB culture was then mixed with 1475 mL of sterile water to achieve the target challenge of 1.0×10^7 colony forming units per centimeter squared (cfu/cm^2).

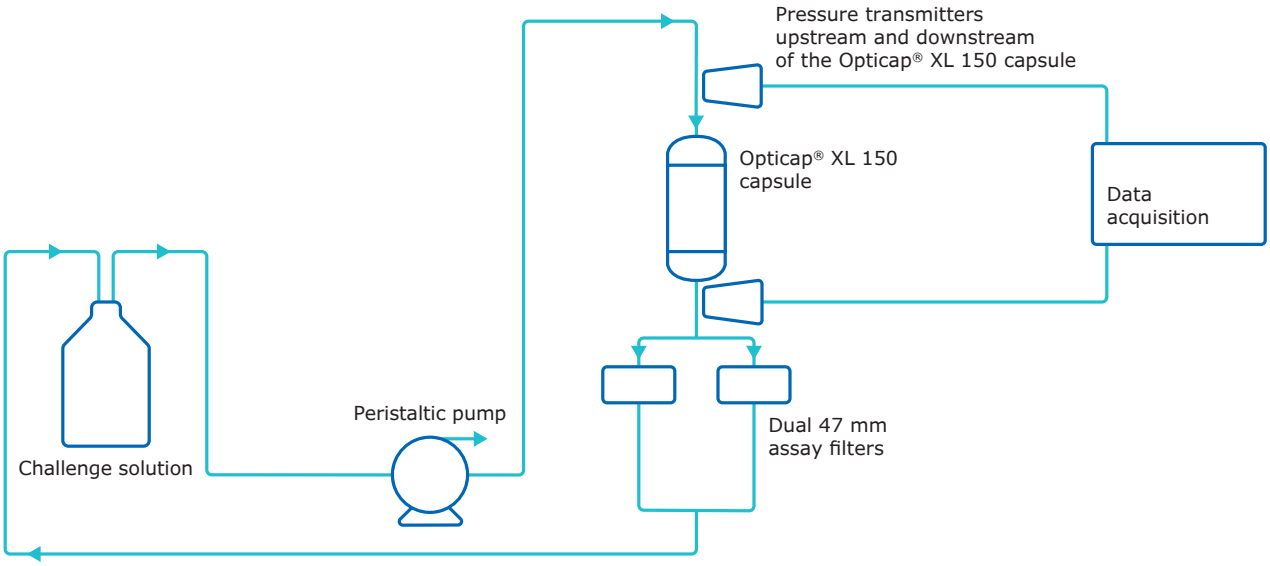

Figure 1. Test systems schematic design

Bacterial Challenge Test

Test system components (excluding pressure transmitters and data acquisition components) were autoclaved at 121 °C before testing. Test endpoints of 4, 8 and 16 hour were evaluated separately using four replicate filter capsules for each study.

A single pass/re-circulation challenge method was used to minimize the total volume of test fluid required. A vessel containing the *B. diminuta* challenge was connected to the inlet side of each individual test system.

The challenge suspension was slowly fed through each individual test system to eliminate trapped air. Next, the flow rate was set for 450 mL/min (1230 LMH) and the challenge suspension was processed through the filter capsule 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 test duration. This ensured continuous flow over the Milipore Express® SHF test membrane for the required test time.

A sample of the challenge suspension was removed and stored at room temperature for the duration of the 8-hour and 16-hour experiments to confirm viability.

Recovery Assay

To determine if bacteria passed through the Millipore Express® SHF capsule filters, filter effluent was processed over two 47-mm MF-Millipore™ 0.45 µm membrane discs (assay filters) throughout the duration of each test. Two filters were needed to accommodate the large volumes processed, and their integrity was confirmed after use. Upon test completion, assay filters were removed from their holders, plated onto Trypticase Soy Agar, incubated, then observed for presence or absence of growth after a minimum of 48 hours.

Table 1

	Sample No.	*Initial EFA Challenge (cfu/cm²)	* Final EFA Challenge (cfu/cm²)	Result	LRV
Study 1: 4 Hour Bacterial Challenge	4-A	2.8×10^7	n/a	Retentive	>9.8
	4-B	2.9×10^7	n/a	Retentive	>9.8
	4-C	3.0×10^7	n/a	Retentive	>9.8
	4-D	2.9×10^7	n/a	Retentive	>9.8
Study 2: 8 Hour Bacterial Challenge	8-A	2.7×10^7	2.9×10^7	Retentive	>9.7
	8-B	3.1×10^7	2.8×10^7	Retentive	>9.8
	8-C	3.0×10^7	2.4×10^7	Retentive	>9.8
	8-D	3.0×10^7	2.7×10^7	Retentive	>9.8
Study 3: 16 Hour Bacterial Challenge	16-A	2.2×10^7	2.1×10^7	Retentive	>9.7
	16-B	2.7×10^7	2.3×10^7	Retentive	>9.7
	16-C	2.2×10^7	2.2×10^7	Retentive	>9.7
	16-D	2.2×10^7	2.2×10^7	Retentive	>9.7

***Calculations**

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

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

Results and Discussion

Under the standard conditions of the ASTM® F838 test for sterilizing filters, Millipore Express® SHF filters show quantitative retention of the test organism. This means no bacteria are detected downstream of the filter on recovery filters after challenge with at least 1×10^7 cfu/cm² of *B. diminuta*.

The goal of these studies was to better understand microbial retention performance of Millipore Express® SHF filters under continuous flow, extended duration processing conditions.

Table 1 summarizes the results of the studies. For all tests, the bacterial challenge exceeded the minimum standard of 1×10^7 cfu/cm². In addition, there was no negative impact of extended duration testing on organism viability. All filters showed complete retention with no bacteria detected downstream of the sterilizing Millipore Express® SHF filters. This level of retention resulted in calculated log reduction values (LRV) of at least 9.7 logs.

The results of these tests confirm sterilizing performance from Millipore Express® SHF membrane filters is maintained under long duration, constant flow conditions up to processing times of 16 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.

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

To Place an Order or Receive Technical Assistance

Please visit
EMDMillipore.com/contactPS

For additional information, please visit
EMDMillipore.com

TB3376ENEM Ver. 2.0
33515
01/2021