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Sponsor:
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Microbial Retention GLP Report

Test Article:	SS-2 Filter Part #XMSS1706003 Serial #1706883CI0001
Purchase Order:	1125
Study Number:	964705-S01
Study Received Date:	15 May 2017
Testing Facility:	Nelson Laboratories, LLC, a Business Unit of Sterigenics International 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: 801-STP0103 Rev 04 Customer Specification Sheet (CSS) Number: 201703404 Rev 02

Summary: This report describes the results of testing in accordance with the approved protocol for the evaluation of bacterial retention characteristics of membrane filters.

The test filter was challenged with a suspension of *Brevundimonas diminuta*, American Type Culture Collection (ATCC) #19146, containing at least 1×10^7 colony forming units (CFU) per cm^2 of test filter membrane effective filtration area (EFA). The sterility of the complete apparatus was tested before the challenge. The filter was challenged at a pressure of 30 pounds per square inch gauge (psig). The effluent was collected and quantified using 0.45 µm assay membranes or spread plate techniques.

EFA:	2,040 cm^2
Pore Size:	0.2 - 0.5 µm
Liquid used in Testing:	USP purified water
Test Filter Identification:	SS-2 Filter Serial #1706883CI0001
Size Control Filter Identification:	Lot #R4EA68020

The filter was effective in retaining the bacterial challenge as demonstrated by zero CFUs present on the assay membranes.

The culture size control results indicate that the size and the degree of aggregation of the challenge organisms represented an acceptable challenge to the test filters. The absence of counts of the challenge organism on the negative control rinse plate indicates that the apparatus was appropriate for use in the challenge.

All test method acceptance criteria were met.

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY

Study Director Kari Hewitt, B.S.

20 Jul 2017
Study Completion Date

964705-S01

Results:

Microbial Challenge:

Filter Identification	Flow Rate @ 30 psig	Total Challenge (CFU)	Challenge (CFU/cm^2)	Filtrate Count (CFU)	Rinse Count (CFU)	LRV[a]
Test Filter	3 L/ 29 sec	3.9×10^{10}	1.9×10^{7}	<1	<1	>10.6
0.45 µm Positive Control	50 mL/ 8 sec	1.3×10^{8}	1.1×10^{7}	9.1×10^{5}	N/A	2.16

LRV = log reduction value

[a] Data may be expressed with more significant figures than valid when rounding would imply higher log reduction than measured.

Test Method Acceptance Criteria: Challenge organism suitability is demonstrated by passage of organisms through an appropriate sized membrane filter, if applicable. This test serves as the positive control. The negative rinse must be negative for the challenge organism.

Procedure:

Preparation of the Challenge in Saline Lactose Broth (SLBR): Approximately 200 mL of sterile soybean casein digest broth (SCDB) was inoculated with at least one colony from a stock culture of *B. diminuta* growing on soybean casein digest agar (SCDA) and incubated at 30 ± 2°C for 24-30 hours. Approximately 4-6 mL of the broth culture was added to each 1 L volume of sterile SLBR. The inoculated SLBR was mixed and incubated at 30 ± 2°C for 24-30 hours to provide at least 1.0×10^{7} CFU/cm^2 of test filter membrane EFA. The challenge suspension titer was determined by making serial 1:10 dilutions in 0.1% peptone water (PEPW), plating a 0.1 mL aliquot in triplicate onto SCDA or equivalent media and incubating at 30 ± 2°C for 7 days. The average number of colonies formed on the plates multiplied by the serial dilution factor and the plating volume factor was recorded as the challenge titer.

Culture Control: Concurrent with the challenge, a 10 mL aliquot of the challenge suspension was removed and passed through a 0.45 µm filter at 30 psig. This served as a positive control. The effluent was enumerated using a spread plate technique onto SCDA or equivalent media and incubated at 30 ± 2°C for a minimum of 7 days.

Negative Control: Prior to the microbial challenge, the filter was rinsed. A portion of this rinse fluid was collected in a sterile container and then quantified to verify the sterile condition of the complete apparatus. The rinse was assayed as indicated in the assay procedure. The sterile integrity of the apparatus was not broken between the rinse step and the challenge.

Microbial Challenge: For the challenge, the test system was closed. A sufficient volume of the challenge suspension was added to the pressure vessel to meet the guideline of at least 1×10^{7} CFU/cm^2 of test filter membrane EFA. The pressure vessel was pressurized to approximately 1-5 psig to begin filling the test filter with the *B. diminuta* challenge and to bleed off the trapped air.

The pressure was increased to 30 psig and the downstream valve opened and a calibrated timer was started to initiate filtration. The challenge flow rate was determined using the volume of filtrate over the filtration time. The filtrate was collected in an attached sterile collection vessel.

Assay Procedure: The filtrate was passed through 0.45 µm assay membranes and plated onto SCDA or equivalent media or plated using spread plate techniques. All plates were incubated at 30 ± 2°C for a minimum of 7 days.

Calculations:
The total challenge titer was calculated using the following equation:

$$\text{Challenge titer} = (\text{CFU/mL}) \times \text{Total Challenge Volume (mL)}$$

The challenge titer/cm^2 of EFA (CFU/cm$^2_{[EFA]}$) was calculated using the following equation:

$$CFU/\,cm^2_{[EFA]} = \frac{Total\ Challenge\ Titer}{Effective\ Filtration\ Area\ (cm^2)}$$

The LRV was calculated using the following equation:

$$LRV = Log_{10} \frac{Number\ of\ Organisms\ in\ Challenge}{Number\ of\ Organisms\ in\ Filtrate}$$

When the filtrate is sterile, a one (1) is substituted in the denominator and the LRV is expressed as greater than (>) the calculated value.

Justification: The selection of *B. diminuta* as the challenge organism is based on its historical acceptance within the industry. When grown under stress or starvation conditions, many *B. diminuta* will pass through 0.45 µm membranes, so *B. diminuta* represents a severe bacterial challenge to the filter. The organism's low pathogenicity also favors the use of *B. diminuta* in laboratory studies.

The test procedure complies with many of the key aspects of ASTM F838 "Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration," and the Health Industry Manufacturers Association (HIMA) test method "Microbiological Evaluation of Filters for Sterilizing Liquids".

Challenge conditions include high pressure, high flow rates, and a high bacterial concentration per cm^2 of EFA. Growth parameters, temperatures and media were as specified by ASTM and HIMA and are detailed in the protocol.

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Quality Assurance Statement

Compliance Statement: The test was conducted in accordance with the USFDA (21 CFR Parts 58, 210, 211, and 820) Regulations. This final report reflects the raw data.

Activity	Date
Study Initiation	28 Jun 2017
Phase Inspected by Quality Assurance: Plate Counts	14 Jul 2017
Audit Results Reported to Study Director	14 Jul 2017
Audit Results Reported to Management	17 Jul 2017

Scientists	Title
Eric Clark	Supervisor
Kari Hewitt	Study Director

Data Disposition: The study plan, raw data and final report from this study are archived at Nelson Laboratories, LLC or an approved off-site location.

J. B. Thompson
Quality Assurance

20 Jul 2017
Date