Nelson Labs
A Sotera Health company

Sponsor:
ION Clairety, LLC

Bacterial Filtration Efficiency (BFE) at an Increased Challenge Level GLP Report

Test Article:	SW6-2020
Study Number:	1377658-S01
Study Received Date:	05 Jan 2021
Testing Facility:	Nelson Laboratories, LLC 6280 S. Redwood Rd. Salt Lake City, UT 84123 U.S.A.
Test Procedure(s):	Standard Test Protocol (STP) Number: STP0009 Rev 14
Deviation(s):	None

Summary: This test procedure was performed to evaluate the BFE of test articles at an increased challenge level. A suspension of *Staphylococcus aureus*, ATCC #6538, was delivered to the test article at a challenge level of greater than 10^4 colony forming units (CFU). The challenge was aerosolized using a nebulizer and delivered to the test article at a fixed air pressure and flow rate of 6 liters per minute (LPM). The aerosol droplets were generated in a glass aerosol chamber and drawn through the test article into all glass impingers (AGIs) for collection. The challenge was delivered for a one minute interval and sampling through the AGIs was conducted for two minutes to clear the aerosol chamber. The mean particle size (MPS) control was performed at a flow rate of 28.3 LPM using a six-stage, viable particle, Andersen sampler for collection.

This test procedure was modified from Nelson Laboratories, LLC (NL), standard BFE procedure in order to employ a more severe challenge than would be experienced in normal use. This method was adapted from ASTM F2101. NL has not performed a validation using the flow rate performed in this testing; however, adequate controls are included to verify the reliability of this study. All test method acceptance criteria were met.

Challenge Flow Rate:	6 LPM
Area Tested:	~40 cm^2
Side Tested:	Either Side
Challenge Level:	8.7 x 10^4 CFU
MPS:	~3.2 µm
Test Monitor Results:	Acceptable

James Luskin electronically approved — 09 Feb 2021 20:56 (+00:00)
Study Director — James Luskin — Study Completion Date and Time

Results:

Test Article Number	Total CFU Recovered	Filtration Efficiency (%)
1	9.5×10^2	98.9
2	9.0×10^2	99.0
3	2.0×10^3	97.8
4	1.1×10^3	98.8
5	1.8×10^3	97.9

The filtration efficiency percentages were calculated using the following equation:

$$\% BFE = \frac{C - T}{C} x\ 100$$

C = Challenge Level
T = Total CFU recovered downstream of the test article

Test Method Acceptance Criteria: The average BFE positive control challenge level shall be ≥1 x 10^6 CFU when the flow rate is ≥30 LPM. The average MPS of the challenge aerosol at 1 cubic foot per minute (CFM) (28.3 LPM) shall be maintained at 3.0 ± 0.3 µm. Other Challenge levels and MPS averages may be used as approved by the sponsor.

Procedure:

Culture Preparation: Approximately 100 mL of soybean casein digest broth (SCDB) was inoculated with *S aureus*, ATCC #6538, and incubated with mild shaking for 24 ± 4 hours at 37 ± 2°C. To determine the MPS of the challenge aerosol, the culture was diluted in peptone water (PEPW) to an appropriate concentration in order to yield counts within the limits of the Andersen sampler.

AGI Preparation: In a laminar flow hood, a 30 mL aliquot of PEPW was dispensed into each AGI.

Challenge Procedure: The bacterial culture suspension was aerosolized using a nebulizer and delivered to the test article at a constant flow rate and fixed air pressure. The aerosol droplets were generated in a glass aerosol chamber and drawn through the test article into AGIs. The challenge was delivered for a one minute interval and the vacuum and air pressure were allowed to run for an additional minute in order to clear the aerosol chamber. Positive control runs were performed (no filter medium in the air stream) prior to the first test article, after every 5-7 test articles, and after the last test article to determine the average number of viable particles being delivered to each test article. The MPS of the challenge aerosol was determined using a six-stage Andersen sampler.

Assay Procedure: The titer of the AGI assay fluid was determined using standard spread plate and/or membrane filtration techniques.

Spread Plating: An aliquot of the test article assay fluid was dispensed onto a Tryptic soy agar (TSA) plate and spread using a sterile rod.

Membrane Filtration: A sterile filter funnel was placed on a manifold. A sterile 0.45 µm membrane was aseptically removed from the packaging and centered over the base of the funnel. An appropriate volume of the test article assay fluid was transferred into the sterile filter funnel. The vacuum was applied in order for the assay fluid to be filtered under light suction. The membrane was then rinsed to ensure that all organisms were impinged onto the membrane. The membrane was removed from the filter funnel and placed onto the surface of a TSA plate.

All plates were incubated at 37 ± 2°C for 48 ± 4 hours prior to counting.