Nelson Labs
A Sotera Health company

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Viral Filtration Efficiency (VFE) at an Increased Challenge Level GLP Report

Test Article:	1: 01VFE123 2: 02VFE121 3: 03VFE073 Lot# of all is 001
Study Number:	1249646-S01
Study Received Date:	12 Dec 2019
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: STP0010 Rev 14 Customer Specification Sheet (CSS) Number: 202000304 Rev 01
Deviation(s):	None

Summary: This test procedure was performed to evaluate the VFE of test articles at an increased challenge level. A suspension of ΦX174 bacteriophage was delivered to the test article at a challenge level of greater than 10^7 plaque-forming units (PFU) to determine the filtration efficiency. The challenge was aerosolized using a nebulizer and delivered to the test article at a fixed air pressure and flow rate of 150 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 an 10 minute interval and sampling through the AGIs was conducted for 11 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. The VFE at an Increased Challenge Level test procedure was adapted from ASTM F2101.

This test procedure was modified from Nelson Laboratories, LLC (NL), standard VFE test procedure in order to employ a more severe challenge than would be experienced in normal use. 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:	150 LPM
Area Tested:	Entire Test Article
Side Tested:	Entrance Region Side
Challenge Level:	1.2×10^7 PFU
MPS:	2.9 µm
Test Monitor Results:	Acceptable

Study Director James W. Luskin

29 JAN 2020
Study Completion Date

1249646-S01

Results:

Test Article	Total PFU Recovered	Filtration Efficiency (%)
01VFE123	30	99.99976
02VFE121	<1[a]	>99.9999919
03VFE073	<1[a]	>99.9999919

[a] There were no detected plaques on any of the assay plates for this test article.

The filtration efficiency percentages were calculated using the following equation:

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

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

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

Procedure:

Challenge Procedure: The viral 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 an glass aerosol chamber and drawn through the test article into AGIs. Approximately one third of the effluent air was collected for quantification during testing; therefore, the plate count results for the controls and test articles were multiplied by three in order to reflect the entire quantity of air passing through the test article. The challenge was delivered for a 10 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 run, 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.

Plaque Assay Procedure: The titer of the AGI assay fluid was determined using standard plaque assay techniques. Approximately 2.5 mL of molten top agar was dispensed into sterile test tubes and held at 45 ± 2°C in a waterbath. An aliquot of the assay fluid from the test article was added to the sterile test tubes along with approximately 0.1 mL of an *Escherichia coli* culture. The contents were mixed and poured over the surface of bottom agar plates. The agar was allowed to solidify on a level surface and the plates were incubated at 37 ± 2°C for 12-24 hours.

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	16 Jan 2020
Phase Inspected by Quality Assurance: Counting Procedure	23 Jan 2020
Audit Results Reported to Study Director	28 Jan 2020
Audit Results Reported to Management	28 Jan 2020

Scientists	Title
Sarah Smit	Supervisor
James Luskin	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.

C [signature]
Quality Assurance

30 Jan 2020
Date