NELSON LABORATORIES

Bacterial Filtration Efficiency (BFE) and Differential Pressure (Delta P) GLP Report

Test Article:	AntiVirus - Respilon Lot: 20160601
Study Number:	906436-S01
Study Received Date:	25 Jul 2016
Test Procedure(s):	Standard Test Protocol (STP) Number: STP0004 Rev 13

Summary: The BFE test is performed to determine the filtration efficiency by comparing the upstream bacterial control counts to downstream test article counts. A suspension of *Staphylococcus aureus* was aerosolized using a nebulizer and delivered to the test article at a constant flow rate and challenge delivery. The challenge delivery is maintained at 1.7 - 2.7 x 10^3 colony forming units (CFU) with a mean particle size (MPS) at 3.0 µm ± 0.3 µm. The aerosol droplets were drawn through a six-stage, viable particle, Andersen sampler for collection. This procedure allows a reproducible bacterial challenge to be delivered to test materials. This test method complies with ASTM F2101-14 and EN 14683:2014, Annex B.

The Delta P test determines the breathability by measuring the differential air pressure on either side of the test article using a manometer, at a constant flow rate. The Delta P test was designed to comply with MIL-M-36954C, Section 4.4.1.2 and complies with EN 14683:2014, Annex C.

All test method acceptance criteria were met.

Test Side:	Inside
BFE Area Tested:	~40 cm^2
BFE Flow Rate:	28.3 Liters per minute (L/min)
Delta P Flow Rate:	8 L/min
Conditioning Parameters:	85 ± 5% relative humidity (RH) and 21 ± 5°C for a minimum of 4 hours.
Test Article Dimensions:	~155 mm x ~170 mm
Positive Control Average:	2.4 x 10^3 CFU
Negative Monitor Count:	<1 CFU
MPS:	2.9 µm

ANAB ACCREDITED TESTING LABORATORY

Study Director — Trang Truong, B.S.

26 Oct 2016
Study Completion Date

906436-S01

Results:

Test Article Number	Percent BFE (%)	Delta P (mm H_2O/cm^2)	Delta P (Pa/cm^2)
1	99.9	4.9	47.7
2	UFA[a]	4.8	47.3
	99.9	N/A	N/A
3	99.9	5.5	53.5
4	UFA[a]	5.2	51.5
	99.9	N/A	N/A
5	99.9	4.9	47.9

[a] Due to a tear during BFE testing the initial test article was deemed unsuitable for analysis (UFA). A new test article was tested for BFE in its place.

The filtration efficiency percentages were calculated using the following equation:

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

C = Positive control average
T = Plate count total recovered downstream of the test article
Note: The plate count total is available upon request

Test Article Preparation: The test articles were conditioned for a minimum of 4 hours at 21 ± 5°C and 85 ± 5% RH, prior to BFE and Delta P testing.

Test Method Acceptance Criteria: The BFE positive control average must be 1.7 - 2.7 x 10^3 CFU. Other positive control averages may be used as approved by the sponsor.

The average MPS of the challenge aerosol must be maintained at 3.0 ± 0.3 µm.

The Delta P test flow rate must be maintained at 8 liters per minute throughout the testing.

Procedure:

BFE: A culture of *S. aureus*, ATCC #6538, was diluted in peptone water (PEPW) to a precise concentration to yield challenge level counts of 1.7 - 2.7 x 10^3 CFU per test article. The bacterial culture suspension was pumped through a nebulizer at a controlled flow rate and fixed air pressure. The constant challenge delivery, at a fixed air pressure, formed aerosol droplets with a MPS of approximately 3.0 µm. The aerosol droplets were generated in a glass aerosol chamber and drawn through a six-stage, viable particle, Andersen sampler for collection. Test articles, positive controls, and reference material received a one minute challenge followed by a one minute vacuum cycle.

The Andersen sampler, a sieve sampler, impinged the aerosol droplets onto six soybean casein digest agar (SCDA) plates based on the size of each droplet. The agar plates were incubated at 37 ± 2°C for 48 ± 4 hours and the colonies formed by each bacteria laden aerosol droplet were counted and converted to probable hit values using the positive hole conversion chart provided by Andersen. These converted counts were used to determine the average challenge level delivered to the test articles. The distribution ratio of colonies for each of the six agar plates was used to calculate the MPS of the challenge aerosol.

Delta P: The Delta P test simply measured the differential air pressure on either side of the test article using an incline, "U" tube, or digital manometer. Testing was conducted at a flow rate of 8 L/min (volumetric). At least one reference material is included with each set of test articles.

The Delta P values were reported in mm water/cm^2 of test area and calculated using the following equation:

$$Delta\ P = \frac{\bar{M}}{Test\ Area}$$

Where: $\bar{M}$ = Average mm water or Pa of test replicates.

The test article holder used in the Delta P test has a test area of 4.9 cm^2.

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	04 Oct 2016
Phase Inspected by Quality Assurance: Delta P Testing	12 Oct 2016
Audit Results Reported to Study Director	20 Oct 2016
Audit Results Reported to Management	21 Oct 2016

Scientists	Title
Adam Meese	Supervisor
Trang Truong	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.

Quality Assurance

26 OCT 2016
Date