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FINAL REPORT

VIRAL FILTRATION EFFICIENCY

PROCEDURE NO. SOP/ARO/014H.1

LABORATORY NO. 270138

SUBMITTED BY:

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VIRAL FILTRATION EFFICIENCY

LABORATORY NUMBER:	270138
PROCEDURE NUMBER:	SOP/ARO/014H.1
SAMPLE SOURCE:	Refil
SAMPLE IDENTIFICATION:	Half Mask REFIL 651
DEVIATIONS:	None
DATA ARCHIVE LOCATION:	Sequentially by lab number
SAMPLE RECEIVED DATE:	09 Aug 2004
LAB PHASE START DATE:	20 Aug 2004
LAB PHASE COMPLETION DATE:	23 Aug 2004
REPORT ISSUE DATE:	25 Aug 2004
TOTAL NUMBER OF PAGES:	7

REFERENCE:

MIL-M-36954C. 1975. Headquarters, Defense Personnel Support Center, Philadelphia, PA.

Andersen 2000 Inc. 1976. Viable (Microbial) Particle Sizing Samplers Operating Manual. Andersen 2000 Inc., Atlanta, GA.

ASTM F2101-01. 2001. Test Method for Evaluating the Bacterial Filtration Efficiency (BFE) of Medical Face Mask Materials, Using a Biological Aerosol of *Staphylococcus aureus*. American Society for Testing and Materials, West Conshohocken, PA.

ACCEPTANCE CRITERIA:

The VFE control average must be 2200 ± 500 PFU. A VFE run with a control average of less than 1700 PFU shall be unacceptable. Challenges greater than 2700 PFU, but less than 3000 PFU, are, in our experience, valid. Acceptance of runs with control averages exceeding 2700 PFU shall be at sponsor's approval. The mean particle size (MPS) of the challenge aerosol should be maintained at 3.0 ± 0.3 μm. The average % VFE for the reference material should be within the upper and lower control limits established for the VFE test for the testing to be acceptable.

INTRODUCTION:

This procedure was performed to determine the filtration efficiency of various filtration materials, employing a ratio of the challenge to effluent, to determine percent virus filtration efficiency (%VFE). The challenge used in this procedure is the bacteriophage φX174, which is commonly used in various types of laboratory testing of barrier and filtration materials. This test procedure allows a reproducible challenge to be delivered to the test samples. This procedure has been used with little or no modifications for many years and provides a reference for comparison of similar test samples.

The VFE test provides a number of advantages over other filtration efficiency tests. The mean particle size (MPS) of the challenge aerosol is tightly controlled. The concentration and the particle size distribution are determined using a six-stage viable particle Andersen sampler, permitting stage by stage analysis. All aerosols are contained so that there are no biosafety problems. A large number of test materials can be evaluated in a relatively short period of time. The VFE test procedure was adapted from the Military standard MIL-M-36954C and ASTM F2101.

SAMPLE PREPARATION:

VFE test samples were conditioned for a minimum of 4 hours at 21 ± 5°C and 85 ± 5% relative humidity prior to testing.

TEST PROCEDURE:

The stock bacteriophage φX174 was prepared by inoculation of φX174 into a log phase culture of *E. coli* C. The culture was shaken at 37 ± 2°C until bacterial turbidity cleared. The virus stock was centrifuged to remove large cellular debris and then filtered through a 0.2 μm membrane filter to remove remaining host cell debris. The stock culture was stored at 2-8°C.

The titer of the bacteriophage was calculated and the titer adjusted before use to yield challenge density that was within 2200 ± 500 PFU per test sample.

The bacteriophage suspension was pumped through a Chicago 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 droplets were collected in a glass aerosol chamber and drawn through a six stage, viable-particle, Andersen sampler. The flow rate through the test sample and Andersen sampler was maintained at 28.3 Lpm (1 CFM).

The Andersen sampler, a sieve sampler, impinges the aerosol droplets onto one of the six agar plates based on size. The agar plates used for assays consisted of 31 mL of bottom agar overlayed with 3 mL of top agar containing *E. coli* C. After the challenges, agar plates were incubated at 37 ± 2°C for 12-24 hours. The plaques formed by each bacteriophage-laden particle were then counted and converted to probable hit values using the published conversion chart of Andersen.

The test samples were challenged by placing them between the aerosol chamber and the Andersen sampler. The filtration efficiency was calculated as a percent difference between test sample runs and runs without a test sample in place using the following equation:

$$\%VFE = \frac{Plaques\ without\ filter - Plaques\ with\ filter}{Plaques\ without\ filter} \times 100$$

The procedure produces a more severe challenge to most filtration materials than would be expected in use. Our purpose with this procedure is not to optimize the efficiency, but to consistently measure, as accurately as possible, the differences between material, or difference in the same material over time, thereby alerting the manufacturer to significant trends or changes which can then be dealt with promptly.

To insure our own efficiency at performing this procedure, several quality assurance steps are observed:

1 - The control average, the plaques formed without a filter in place, must fall within 2200 ± 500 PFU for the test to be valid.

2 - We test at least one reference material sample with every set of tests. Statistical evaluation of these data are recorded on control charts. The reference material results must be within the upper and lower control limits (±3 standard deviations) established for the test.

3 - Actual test results are statistically analyzed to alert us to unusual variations which may indicate a need for retesting before data are reported.

STATEMENT OF UNCERTAINTY:

Due to the large number of data points available for the standard reference material used in the Viral Filtration Efficiency Test, the Type B uncertainty factors have been determined to be incorporated into the Type A uncertainty.

A statistical analysis of the VFE data resulted in the following:

Viral Filtration Efficiency (VFE) Mean = 98.9% VFE
Standard Deviation = 0.50% VFE

The combined uncertainty for the VFE test is 0.085% VFE and the expanded is 0.17% VFE at a confidence level of 95%.

It should be noted that the statistical analysis was conducted on data from Nelson Laboratories' standard reference material with a mean of 98.9% VFE. It is expected that test materials submitted for VFE testing which have a VFE higher than 98.9% would have a combined uncertainty and an expanded uncertainty less than the uncertainty values reported here. Conversely, test materials with VFE values of less than 98.9% would be expected to yield a combined uncertainty and an expanded uncertainty greater than the uncertainty values reported here.

Test samples were not collected by the laboratory and therefore the representative nature of the samples is not included in the uncertainty assessment.

RESULTS:

Results are summarized in Table 1.

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26 Aug 2014

Study Completion Date

jtp

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TABLE 1. Results

UNIT NUMBER	SAMPLE IDENTIFICATION	PERCENT VFE
1	Half Mask REFIL 651	>99.9%*
2	Half Mask REFIL 651	99.8%
3	Half Mask REFIL 651	>99.9%*
4	Half Mask REFIL 651	>99.9%
5	Half Mask REFIL 651	>99.9%*

CONTROL AVERAGE: 2217 PFU

MEAN PARTICLE SIZE: 2.8 μm

* There were no detected plaques on any of the Andersen sampler plates for this sample.

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