



FINAL REPORT

VIRUS FILTRATION EFFICIENCY TEST (VFE)  
AT AN INCREASED CHALLENGE LEVEL

PROCEDURE NO. SOP/ARO/018F.1

LABORATORY NO. 286728

PREPARED FOR:

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AT AN INCREASED CHALLENGE LEVEL

LABORATORY NUMBER:	286728
PROCEDURE NUMBER:	SOP/ARO/018F.1
SAMPLE SOURCE:	Superior Felt & Filtration / Hollingsworth & Vose
SAMPLE IDENTIFICATION:	See Table 1 P.O. #50080
DEVIATIONS:	None
DATA ARCHIVE LOCATION:	Sequentially by lab number
SAMPLE RECEIVED DATE:	11 Mar 2005
LAB PHASE START DATE:	16 Mar 2005
LAB PHASE COMPLETION DATE:	17 Mar 2005
REPORT ISSUE DATE:	21 Mar 2005
TOTAL NUMBER OF PAGES:	6

REFERENCE:

U.S. Department of Defense. 1975. MIL-M-36954C. Military Specification Mask, Surgical, Disposable.

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

INTRODUCTION:

This report describes the procedure and results of the virus filtration efficiency (VFE) at increased challenge level testing. This procedure was performed to determine the filtration efficiency of the test materials using a ratio of the challenge to effluent to determine percent efficiency. This procedure allowed a reproducible aerosol challenge to be delivered to each of the test materials. This test procedure was modified from Nelson Laboratories, Inc., standard VFE test and employed a more severe challenge than would be expected in normal use.

JUSTIFICATION:

This VFE test provides a number of advantages over other filtration efficiency tests. The use of all glass impingers (AGIs) in the collection process allowed a high concentration of challenge to be delivered to each test material. The aerosol challenge particle size can be tightly controlled by monitoring the airflow and challenge flow through the nebulizer. The aerosol particles can be sized using a six-stage viable particle Andersen sampler.

The  $\phi$ X174 bacteriophage has a diameter of 27 nm (0.027  $\mu$ m) and, therefore, provides a severe challenge to the test filter.

#### ACCEPTANCE CRITERIA:

The mean particle size (MPS) of the challenge aerosol must be maintained at  $3.0 \pm 0.3 \mu$ m.

The average % VFE for the reference material must be within the upper and lower control limits established for the VFE test.

#### CHALLENGE PROCEDURE:

The stock bacteriophage  $\phi$ X174 was prepared by inoculation of  $\phi$ X174 into a log phase culture of *E. coli* C. The culture was shaken at  $37 \pm 2^\circ\text{C}$  until bacterial turbidity cleared. The virus stock was centrifuged to remove large cellular debris and then filtered through a 0.2  $\mu$ m membrane filter to remove remaining host cell debris. The stock culture was stored at 2-8 $^\circ\text{C}$ .

The challenge suspension was pumped through a 'Chicago' nebulizer using a peristaltic pump at a controlled flow rate and fixed air pressure. The constant challenge delivery formed aerosol droplets of defined size. The challenge level was adjusted to provide a consistent challenge of greater than  $10^6$  plaque forming units per test sample.

The aerosol droplets were generated in a glass aerosol chamber and drawn through the sample holder and into all AGIs in parallel. Each AGI contained 30 mL aliquots of sterile PEPW to collect the aerosol droplets. The aerosol challenge flow rate was maintained at 30 Lpm.

The challenge was delivered for a 1 minute interval and sampling through the AGIs was conducted for 2 minutes to clear the aerosol chamber. Control runs (no media in sample holder) were performed after every 5-7 test samples to determine the number of viable particles being generated in the challenge aerosol.

The AGI fluid was assayed using standard plaque assay techniques. All plates were incubated at  $37 \pm 2^\circ\text{C}$  for 12-24 hours.

#### STATEMENT OF UNCERTAINTY:

Due to the large number of data points available for the standard reference material used in the Viral Filtration Efficiency Test at Increased Challenge Level, 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 @ 30 LPM = 99.83%  
Standard Deviation = 0.11% VFE

The combined uncertainty for the VFE test @ 30 LPM is 0.012% VFE and the expanded uncertainty is 0.024% 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 VFE @ 30 LPM of about 99.8%. It is expected that test materials submitted for VFE testing which have a VFE higher than 99.8% 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 99.8% would be expected to yield a combined uncertainty and an expanded uncertainty greater than the uncertainty values reported here.

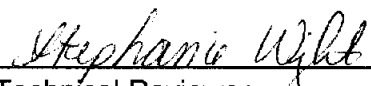
Test samples were not collected by the laboratory and therefore the representative nature of the samples is not included in the uncertainty assessment.

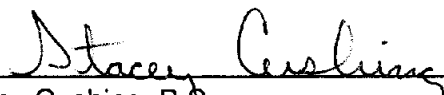
**RESULTS:**

The filtration efficiencies were calculated using the following equation:

$$VFE \% = \frac{\text{Plaques without filter} - \text{Plaques with filter}}{\text{Plaques without filter (Control)}} \times 100$$

The mean particle size (MPS) of the challenge aerosol was determined using a six-stage Andersen sampler. The challenge level, MPS, and filtration efficiencies of the samples are summarized in Table 1.

  
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Technical Reviewer

  
\_\_\_\_\_  
Stacey Cushing, B.S.  
Study Director

24 Mar 2005  
\_\_\_\_\_  
Study Completion Date

TABLE 1. VFE Results  
Sample Identification: Technostat® 200 With 17 g/m<sup>2</sup> Cover Web Both Sides  
Lot #50080-V

SAMPLE IDENTIFICATION	TOTAL PFU RECOVERED	FILTRATION EFFICIENCY
T200/15 PPCW-2 #1	<1*	>99.999969%
T200/15 PPCW-2 #2	9	99.99972%
T200/15 PPCW-2 #3	<1*	>99.999969%

Challenge Level (PFU): 3.2 x 10<sup>6</sup> PFU

Mean Particle Size (MPS): 2.8 μm

\* There were no detected plaques on any of the assay plates for this sample.



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Lab Number 286728

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