NELSON
LABORATORIES

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

| | |
|---|---|
| LABORATORY NUMBER: | 235273 |
| PROCEDURE NUMBER: | SOP/ARO/018E.1 |
| SAMPLE SOURCE: | Pharma Systems AB |
| SAMPLE IDENTIFICATION: | BACT HME/ThermoFlo Filter Code 6000 (A) and 6020 (A) |
| DEVIATIONS: | None |
| DATA ARCHIVE LOCATION: | Sequentially by lab number |
| SAMPLE RECEIVED DATE: | 05 May 2003 |
| LAB PHASE START DATE: | 15 May 2003 |
| LAB PHASE COMPLETION DATE: | 16 May 2003 |
| REPORT ISSUE DATE: | 19 May 2003 |
| TOTAL NUMBER OF PAGES: | 7 |

REFERENCES:

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) 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.

All aerosols were contained so that there were no biosafety problems. The φX174 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 ± 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 PREPARATION:

A 100 mL aliquot of nutrient broth was inoculated with *Escherichia coli*, ATCC #13706, and incubated at 37 ± 2°C for 6-18 hours with rapid shaking. The culture was diluted 1:100, and incubated at 37 ± 2°C. The culture was allowed to grow to a density of 2-4 x $10^8$ CFU/mL. The bacterial culture was inoculated with the φX174 bacteriophage stock culture (ATCC #13706-B1). The culture was incubated for 1-5 hours with rapid shaking. After complete *E. coli* lysis, the φX174 phage culture was centrifuged, then filtered through a 0.2 $\mu$m filter. The stock culture of φX174 was kept at 2-8°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 at a fixed air pressure 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. Test samples were ran by placing them into the sample holder, initiating challenge aerosol, and collection of effluent air into AGIs as with the controls.

The AGI fluid was assayed by placing aliquots of each sample into tubes containing 2.5 mL of top agar and 1-2 drops of *E. coli.* The contents were mixed and poured over the surface of the bottom agar plates. All plates were incubated at 37 ± 2°C for 16 hours.

The filtration efficiencies were calculated using the following equation:

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

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 = 99.94%
Standard Deviation = 0.12% VFE

The combined uncertainty for the VFE test @ 30 LPM is 0.015% VFE and the expanded uncertainty is 0.03% 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 99.9%. It is expected that test materials submitted for VFE testing which have a VFE higher than 99.9% 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 99.9% 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:

The mean particle size (MPS) of the challenge aerosol was determined using a six-stage Andersen sampler and calculated to be 2.8 $\mu$m. The challenge level and filtration efficiencies of the samples are summarized in Table 1.

Stacey Cushing, B.S.
Associate Study Director

Brandy Giles
Brandy Giles, B.S.
Study Director

20 May 2003
Study Completion Date

clc

TABLE 1. VFE Results

| SAMPLE IDENTIFICATION | CHALLENGE LEVEL (PFU) | TOTAL PFU RECOVERED | FILTRATION EFFICIENCY |
|---|---|---|---|
| BACT HME/ThermoFlo Filter Code 6000 (A) and 6020 (A)-1 | $3.6 \times 10^6$ | 99 | 99.997% |
| BACT HME/ThermoFlo Filter Code 6000 (A) and 6020 (A)-2 | $3.6 \times 10^6$ | 45 | 99.999% |
| BACT HME/ThermoFlo Filter Code 6000 (A) and 6020 (A)-3 | $3.6 \times 10^6$ | 54 | 99.999% |
| BACT HME/ThermoFlo Filter Code 6000 (A) and 6020 (A)-4 | $3.6 \times 10^6$ | 117 | 99.997% |