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FINAL REPORT

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

PROCEDURE NO. SOP/ARO/018F.1

LABORATORY NO. 308501.1 AMENDED

PREPARED FOR:

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

LABORATORY NUMBER:	308501.1 Amended
PROCEDURE NUMBER:	SOP/ARO/018F.1
SAMPLE SOURCE:	Medical Research & Development
SAMPLE IDENTIFICATION:	Refer to Table 1
DEVIATIONS:	None
DATA ARCHIVE LOCATION:	Sequentially by lab number
SAMPLE RECEIVED DATE:	26 Oct 2005
LAB PHASE START DATE:	02 Nov 2005
LAB PHASE COMPLETION DATE:	04 Nov 2005
REPORT ISSUE DATE:	07 Nov 2005
STUDY COMPLETION DATE:	11 Nov 2005
AMENDED REPORT ISSUE DATE:	14 Nov 2005

AMENDMENT JUSTIFICATION:

At the request of the sponsor the report was changed from short format to long format.

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 ϕX174 bacteriophage has a diameter of 27 nm (0.027 μm) 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 μm.

The average percent virus filtration efficiency (%VFE) for the reference material must be within the upper and lower control limits established for the VFE test.

CHALLENGE 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 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 (PFU) 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 peptone water (PEPW) to collect the aerosol droplets. The aerosol challenge flow rate was maintained at 30 Liters per minute (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 ± 2°C for 12-24 hours.

STATEMENT OF UNCERTAINTY:

If applicable, the statement of uncertainty is available to sponsors upon request.

RESULTS:

The filtration efficiencies were calculated using the following equation:

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

The 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.

Technical Reviewer

Stacey Cushing, B.S.
Study Director

18 NOV 2005
Amended Report Date

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TABLE 1. VFE Results
Lot #01/2005

SAMPLE IDENTIFICATION	TOTAL PFU RECOVERED	FILTRATION EFFICIENCY
Neumofilt #1	<1*	>99.999909%
Neumofilt #2	<1*	>99.999909%
Neumofilt #3	<1*	>99.999909%
Neumofilt #4	<1*	>99.999909%
Neumofilt #5	<1*	>99.999909%

Challenge Level (PFU): 1.1×10^6 PFU

Mean Particle Size (MPS): 2.9 μm

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

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FINAL REPORT

BACTERIAL FILTRATION EFFICIENCY TEST (BFE) AT AN INCREASED CHALLENGE LEVEL

PROCEDURE NO. SOP/ARO/017E.1

LABORATORY NO. 308500.1 AMENDED

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BACTERIAL FILTRATION EFFICIENCY TEST (BFE) AT AN INCREASED CHALLENGE LEVEL

LABORATORY NUMBER:	308500.1 Amended
PROCEDURE NUMBER:	SOP/ARO/017E.1
SAMPLE SOURCE:	Medical Research & Development
SAMPLE IDENTIFICATION:	Refer to Table 1
DEVIATIONS:	None
DATA ARCHIVE LOCATION:	Sequentially by lab number
SAMPLE RECEIVED DATE:	26 Oct 2005
LAB PHASE START DATE:	01 Nov 2005
LAB PHASE COMPLETION DATE:	04 Nov 2005
REPORT ISSUE DATE:	07 Nov 2005
STUDY COMPLETION DATE:	11 Nov 2005
AMENDED REPORT ISSUE DATE:	14 Nov 2005

AMENDMENT JUSTIFICATION:

At the request of the sponsor the report was changed from short format to long format.

ACCEPTANCE CRITERIA:

The mean particle size of the challenge aerosol must be maintained at 3.0 ± 0.3 μm.

The average percent bacterial filtration efficiency (%BFE) for the reference material must be within the upper and lower control limits established for the bacterial filtration efficiency (BFE) test.

INTRODUCTION:

This report describes the procedure and results of the BFE 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 employed a challenge level of greater than 10^6 colony forming units (CFU) per test sample, providing a higher challenge than would be expected in normal use.

JUSTIFICATION:

This BFE 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.

PROCEDURE:

Approximately 100 mL of soybean casein digest broth (SCDB) was inoculated with *Staphylococcus aureus*, ATCC #6538, and incubated with mild shaking for 24 ± 4 hours at 37 ± 2°C. The culture 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 CFU per test sample.

The droplets were generated in a glass aerosol chamber and drawn through the sample holder and into AGIs in parallel. The AGIs contained 30 mL aliquots of sterile peptone water (PEPW) to collect the aerosol droplets. The aerosol challenge flow rate through the test filter was maintained at 30 Liters per minute (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 assay fluid in the AGIs was assayed using standard plate count or membrane filtration techniques. All plates were incubated at 37 ± 2°C for 48 ± 4 hours prior to counting.

STATEMENT OF UNCERTAINTY:

If applicable, the statement of uncertainty is available to sponsors upon request.

RESULTS:

The filtration efficiencies were calculated using the following equation:

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

Where: C = Average of control values.
T = Count total for test material.

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.

Technical Reviewer

Stacey Cushing, B.S.
Study Director

18 Nov 2005
Amended Report Date

djo

TABLE 1. BFE Results
Lot #01/2005

SAMPLE IDENTIFICATION	TOTAL CFU RECOVERED	FILTRATION EFFICIENCY
Neumofilt #1	<1*	>99.999980%
Neumofilt #2	<1*	>99.999980%
Neumofilt #3	<1*	>99.999980%
Neumofilt #4	<1*	>99.999980%
Neumofilt #5	<1*	>99.999980%

Challenge Level: 5.0×10^6 CFU

Mean Particle Size (MPS): 3.2 μm

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