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FINAL REPORT

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

PROCEDURE NO. STP0010 REV 03

LABORATORY NO. 500235

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

LABORATORY NUMBER:	500235
PROCEDURE NUMBER:	STP0010 REV 03
SAMPLE SOURCE:	Piston Ltd.
SAMPLE IDENTIFICATION:	Refer to Table 1
	P.O. #CsL-PO-046-2-2009
DEVIATIONS:	None
SAMPLE RECEIVED DATE:	02 Nov 2009
LAB PHASE START DATE:	24 Nov 2009
LAB PHASE COMPLETION DATE:	30 Nov 2009
REPORT ISSUE DATE:	01 Dec 2009

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. This method was adapted from ASTM F2101.

ACCEPTANCE CRITERIA:

The mean particle size (MPS) of the challenge aerosol was maintained at 3.0 ± 0.3 µm.

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

The VFE challenge level was ≥ 1×10^6 PFU/test sample when the flow rate is ≥ 30 LPM.

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 25-27 nanometers (nm) (0.025-0.027 µm), therefore, provides a severe challenge to the test sample.

CHALLENGE PROCEDURE:

The stock bacteriophage ΦX174 was prepared by inoculation of ΦX174 into a log phase culture of *E. coli*. 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 at least 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.

RESULTS:

The filtration efficiencies were calculated using the following equation:

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

Where: C = Average of control values.
T = Effluent counts of test material.

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. Testing met the acceptance criteria previously stated in this report.

Piston Ltd.
Lab Number 500235

VFE at an Increased Challenge Level

STATEMENT OF UNCERTAINTY:

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

Adrianne Sandall

Adrianne Sandall, B.S.
Study Director

01 Dec 2009

Study Completion Date

anl

TABLE 1. VFE Results
Sample Identification: Bacterial and Viral Filter, Type: PBF-100

SAMPLE IDENTIFICATION	TOTAL PFU RECOVERED	FILTRATION EFFICIENCY
Lot #20091021/6	<1[a]	>99.999947%
Lot #20091021/7	<1[a]	>99.999947%
Lot #20091021/8	<1[a]	>99.999947%
Lot #20091021/9	9	99.99952%
Lot #20091021/10	2.7×10^1	99.9986%

Challenge Level (PFU): 1.9×10^6 PFU

Mean Particle Size (MPS): 2.8 µm

[a] There were no detected plaques on any of the assay plates for this sample.