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LABORATORIES

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

LABORATORY NUMBER:	235264
PROCEDURE NUMBER:	SOP/ARO/017E.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:	13 May 2003
LAB PHASE COMPLETION DATE:	16 May 2003
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TOTAL NUMBER OF PAGES:	6

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.

ACCEPTANCE CRITERIA:

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

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

INTRODUCTION:

This report describes the procedure and results of the bacterial filtration efficiency (BFE) 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. All aerosols were contained so that there were no biosafety problems.

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 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 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 to collect the aerosol droplets. The aerosol challenge flow rate through the test filter 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 tested by placing them into the sample holder, initiating the challenge aerosol, and collection of effluent air into AGIs as with the controls.

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 49 hours prior to counting. 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.

STATEMENT OF UNCERTAINTY:

Due to the large number of data points available for the standard reference material used in the BFE test, the Type B uncertainty factors have been determined to be incorporated into the Type A uncertainty. The combined uncertainty and expanded uncertainty for the BFE test are calculated as follows:

Statistical analysis of the BFE data resulted in the following:

Mean Bacterial Filtration Efficiency = 99.94%
Standard Deviation = 0.016%

The combined standard uncertainty for the BFE test is 0.003%BFE and the expanded uncertainty at a 95% confidence level is 0.005%BFE.

It should be noted that the statistical analysis was conducted on data from Nelson Laboratories' standard reference material with a mean BFE of 99.94%. It is expected that materials submitted for BFE testing which have a %BFE lower than 99.94 would have a combined uncertainty and an expanded uncertainty greater than the uncertainty values reported here. Conversely, test materials with %BFE values greater than 99.94 would be expected to yield a combined uncertainty and an expanded uncertainty less than the uncertainty values reported here.

RESULTS:

The mean particle size (MPS) of the challenge aerosol was determined using a six-stage Andersen sampler and calculated to be 3.2 μm. The challenge level and filtration efficiencies of the samples are summarized in Table 1.

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20 May 2003
Study Completion Date

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TABLE 1. BFE Results

SAMPLE IDENTIFICATION	CHALLENGE LEVEL (CFU)	TOTAL CFU RECOVERED	FILTRATION EFFICIENCY
BACT HME #1	9.0×10^6	54	99.9994%
BACT HME #2	9.0×10^6	26	99.9997%
BACT HME #3	9.0×10^6	45	99.9995%
BACT HME #4	9.0×10^6	40	99.9996%