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**BIOSAFETY INVESTIGATION UNIT
QUALITY, SAFETY AND SCIENTIFIC RESOURCES DIVISION**

TEST REPORT

DESCRIPTION OF EQUIPMENT: Pharma Breathing Systems Filters **PAGE 1 OF 9**

TEST: Challenge Breathing System Filters against *Mycobacterium tuberculosis* Aerosols

COMMERCIAL IN CONFIDENCE

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MICROBIAL EFFICIENCY TESTING OF BREATHING SYSTEMS FILTERS WITH *MYCOBACTERIUM TUBERCULOSIS*

Summary

Six Thermo-Flo 6000 Breathing system filters (BSF) were challenged with *Mycobacterium tuberculosis*. Each of the filters were challenged with approximately 10^6 colony forming units (cfu) of a clinical isolate of *Myco. tuberculosis*. 90% of the aerosolised bacteria challenging the BSF were found to be less than 2.1 microns. The filter efficiencies varied from 99.996% to 99.998%.

Introduction

Over the last decade, the decrease in the incidence of tuberculosis in the developed world has reversed and an upsurge of cases of tuberculosis has been reported in the US and Europe (1, 2). Many of these cases have been associated with HIV infection and are therefore becoming more prevalent in hospitalised patients. Also multi drug resistant forms of *Mycobacterium tuberculosis* are increasing in frequency and as the treatment of these forms is more difficult, patients tend to remain infectious for longer periods of time. Since large amounts of *Myco. tuberculosis* cells are present in the sputum of untreated patients or patients in the first two weeks of antibiotic therapy there is a potential for infection of respiratory equipment used by tuberculous patients (3).

In this work the efficacy of breathing systems filters, supplied by 3M, to prevent possible contamination of respiratory equipment in hospitals was investigated by challenging these filters with aerosols of a clinical isolate of *Myco. tuberculosis* using a Henderson apparatus with category 3 containment conditions (4).

A system has been developed at the Centre for Applied Microbiology and Research, Porton to test the efficiencies of many types of microbiological filters. An apparatus developed originally by Henderson and Druett (5, 6) to study experimental airborne infection is used where a suspension of micro-organisms in aqueous solution is nebulized by a 3-jet Collison spray (7) forming a fine aerosol containing viable micro-organisms.

The generated aerosols are injected into an air stream flowing into a 77 cm long stainless steel tube of 5 cm diameter. The relative humidity of the air in the spray tube is controlled to a desired value in the air stream. The efficiencies of the filters can be calculated by determining the airborne concentration of viable micro-organisms upstream and downstream of the filter using suitable aerosol sampling techniques and microbiological assay methods.

Materials and Methods

Test micro-organism

A *Mycobacterium tuberculosis* H37RV strain was originally isolated from a clinical specimen and deposited with ATCC in 1948 (ATCC 9360, NCTC 7416). The *Myco. tuberculosis* was prepared by scraping confluent growth from 5 Middlebrook 7H10 agar plates containing supplements into a plastic universal containing 15 ml sterile distilled water. The resultant spray suspension was assayed by microbiological assay (as described below) to determine the colony forming units per ml of distilled water.

Breathing System Filters (BSF).

Six Thermo-Flo 6000 BSF manufactured by Pharma Systems AB Sweden were received individually packed in sealed containers and each filter was labelled with a maroon label. These were tested against a *Myco. tuberculosis* challenge.

Challenging of BSF with aerosols containing *Myco. tuberculosis*

The Henderson apparatus was set up to deliver a challenge of over 10^6 *Myco. tuberculosis* in aerosol at 28 litres per minute. The relative humidity was over 90% at a room temperature of $19^{\circ}\text{C} \pm 4^{\circ}\text{C}$. The apparatus consisted of the following essential parts:-

- Two matching 3-jet Collision sprays (7), one containing 10 ml distilled water and the other 10 ml *Myco tuberculosis* suspension containing over 1×10^7 cfu ml⁻¹ in distilled water. The Collision sprays were arranged so that they could be operated alternately, as required, to nebulize their contents at a pressure of 180 kPa into the air stream in the spray tube.

- Stainless steel spray tube 77 cm length and 5 cm diameter allowed mixing and conditioning of the aerosols generated from the Collision with a supply of clean filtered humidified air at 56 litres per minute.
- A device to determine the relative humidity at the end of the spray tube.
- Suitable clean silicone tubing connectors and tubes to allow insertion of BSF to be tested in the system.
- Two identical 28 l/min Porton all-glass impingers (8) arranged in parallel containing 20 ml sterile distilled water for the collection of bacteria.

The impingers were linked to the vacuum system in the Henderson rig. The total air stream flowing at 56 litres per minute was divided into the two impingers incorporating a critical orifice allowing 28 litres per minute. Two fresh Thermo-Flo 6000 BSF were challenged simultaneously by inserting them in the apparatus upstream of each of the impingers. The air downstream of both the BSFs was sampled for 2 minutes. The efficiency of each the BSFs was determined by microbiological analysis of the collection fluid from each impinger when the BSF was in place compared to the microbiological analysis of the collection fluid with the BSF absent (i.e. the challenge solution). The nebulization and sampling time of 2 minutes was selected so as to challenge each BSF with a total of at least 10^6 bacteria.

Microbiological assay of collection fluids

The colony forming units in the challenge solution were determined by serial dilution of the collection fluid and plating onto Middlebrook 7H10 agar plates and incubated at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for at least 14 days. The collection fluid from the impinger samples with the BSF in place were assayed by serial dilution as described above but also by filtration, of the remaining, solution through $0.2 \mu\text{m}$ cellulose nitrate membrane filters and placing the membranes onto the solid media.

Expression of effectiveness of BSF

The effectiveness of the BSF can be expressed in the following two ways;

(a) The percentage efficiency was calculated using this equation:-

$$\frac{\text{Bacteria collected in impinger with BSF absent} - \text{Bacteria collected in impinger with BSF present}}{\text{Bacteria collected in impinger with BSF absent}} \times 100$$

(b) The titre reduction value was calculated using this equation:-

$$\frac{\text{cfu without BSF in place}}{\text{cfu with BSF in place}}$$

Particle size distribution of aerosols containing *Myco. tuberculosis* determined using the Andersen sampler

This was carried out to demonstrate the distribution of sizes of the particles containing challenge micro-organisms. The pre-sterilised Andersen sampler (9) was assembled with six Middlebrook agar plates in each of the stages. The sampler was linked to the apparatus in place of one of the impingers without a BSF present. The challenge suspension in the Collision was prepared by diluting the bacterial spray suspension (prepared as described above) by 10^3 . The Andersen sampler was operated in parallel with an all-glass impinger operating at the same flow rate (28 l min^{-1}) for 20 seconds, nebulization occurred for the total 20 seconds. The particle size analysis was determined by counting the colonies on the agar plates, placed in the different stages, after incubation at $37^\circ\text{C} \pm 2^\circ\text{C}$ for at least 14 days. A correction factor was determined to account for the possibility of more than one organism going through the holes in the Andersen sampler stages.

Containment

All tests involving *Myco. tuberculosis* were carried out inside a contained Henderson apparatus in a Category 3 biohazard facility. After each run the cabinet enclosing the Henderson apparatus was ventilated for at least 10 minutes before the samples were removed for dilution and plating in an adjacent Class III microbiological safety cabinet.

Results

The distribution of aerosol particle size using the Andersen sampler showed that over 80% of the challenge particle containing *Myco. tuberculosis* were less than 2.1 microns (Figure 1).

The efficiencies of the 6 fresh Thermo-Flo 6000 BSF against aerosolised *Myco. tuberculosis* were above 99.98% these results are shown in Table 1. Meaningful results were obtained with 4 BSF and the efficiencies of all of these were found to be close, varying from 99.996% to 99.998%. This is equivalent to a titre reduction value of between 4.7×10^4 to 2.4×10^4 .

FIGURE 1. Particle Size Distribution of *Mycobacterium tuberculosis* Aerosols in Henderson Apparatus.

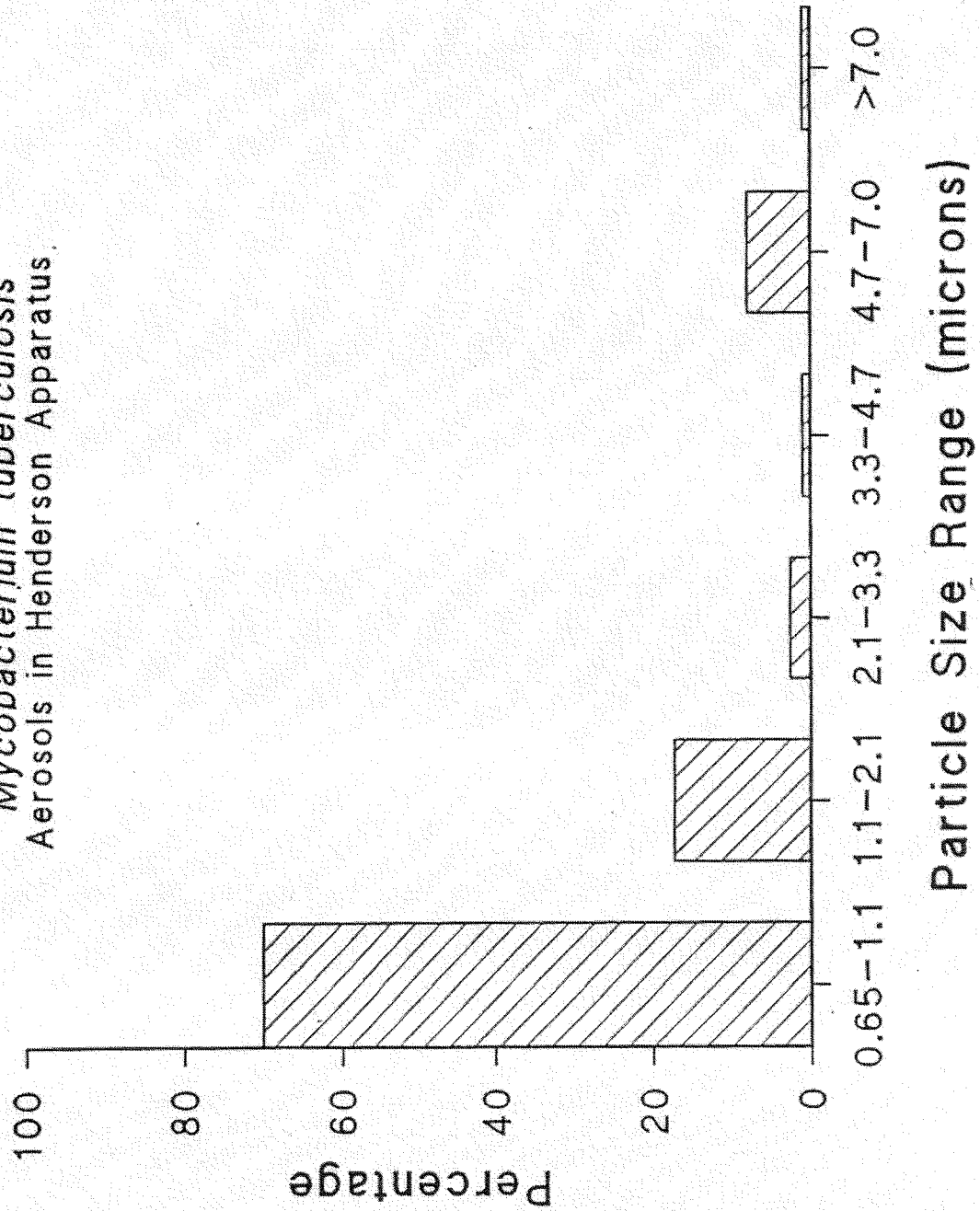


Table 1. Filter Integrity Tests Using Micro-Organisms

Date	17/04/96	Apparatus	Contained Henderson
Operators	A. M. Bennett S. E. Speight	Challenge Micro-Organisms	<i>Mycobacterium tuberculosis</i>
Batch N ^o	-	Suspension Fluid	Bacteria Suspension
Spray	3-jet Collison	Concentration	6.9×10^7 cfu/ml

Temperature: $19^{\circ}\text{C} \pm 4^{\circ}\text{C}$:- RH >90%

Filters Tested: Fresh Thermo-Flo 6000

Sampling Time min at litres/min Sampler

Filter Type	Colony forming units (cfu/)	Volume collected (ml)	Total (cfu) collected	% Efficiency	Titre reduction
No Filter	33/ 0.1ml 10^2 dilution	20	6.6×10^5	-	-
1	0/ 0.1ml neat ; 19 on membrane	20	19	99.997	3.5×10^4
2	0/ 0.1ml neat; memb' contaminated	20	-	>99.99	$>6.6 \times 10^3$
3	0/ 0.1ml neat; memb' contaminated	20	-	>99.99	$>6.6 \times 10^3$
4	0/ 0.1ml neat; 14 on membrane	20	14	99.998	4.7×10^4
5	0/ 0.1ml neat; 26 on membrane	20	26	99.996	2.5×10^4
6	0/ 0.1ml neat; 27 on membrane	20	27	99.996	2.4×10^4

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