A Test Protocol and Evaluation of the Bacterial Filtration Efficiency of the Intersurgical Clear-Therm 3 HMEF, code 1541

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Introduction

The Biosafety Investigation Unit at the Health Protection Agency (HPA) Porton Down specialise in aerosol and airborne infection studies, and have developed a system to test the efficiencies of many types of microbiological breathing filters. A suspension of micro-organisms in aqueous solution is nebulised by a 3-jet Collison spray forming a fine monodispersed aerosol containing viable micro-organisms. The generated aerosols are injected into an air stream flowing into a stainless steel tube. The relative humidity of the air in the spray tube is controlled to a desired value and maintained using wet and dry bulb thermometers in the air stream. The efficiencies of these filters are calculated by determining the airborne concentration of viable micro-organisms upstream and downstream of the filters using suitable aerosol sampling techniques and microbial assay methods.

Test Organism

The bacterial strain chosen to challenge the test filters was based on a non-pathogenic model providing the highest possible viable challenge allowing a full quantitative assessment of the filters. Spores of Bacillus subtilis var niger (0.96µm to 1.25µm long and from 0.55µm to 0.67µm wide) were selected as the bacterial model. Their robust nature enables survival during the aerosolization process.

Technical Protocol
Henderson Rig

The Henderson Rig was designed to deliver a challenge of over 10^7 Bacillus subtilis spores in aerosols of a relative humidity of 96% or above at a specified flow rate. The Henderson Rig is illustrated in Figure 1.

The apparatus consisted of the following essential parts:

Two 3-jet Collison sprays: one containing 10ml distilled water and the other 10ml Bacillus subtilis suspension containing 3.7 x 10^9 cfu per ml in distilled water.

The Collison sprays were arranged so that they could be operated alternatively to nebulise their contents at a pressure of 180Kpa into the air stream in the spray tube.

Stainless steel tube: 77cm length and 5cm diameter allowed mixing and conditioning of the aerosols generated from the Collison with a supply of clean filtered humidified air at 60L/min.

Wet and Dry thermometers: downstream of the spray tube, to determine the relative humidity of the aerosol.

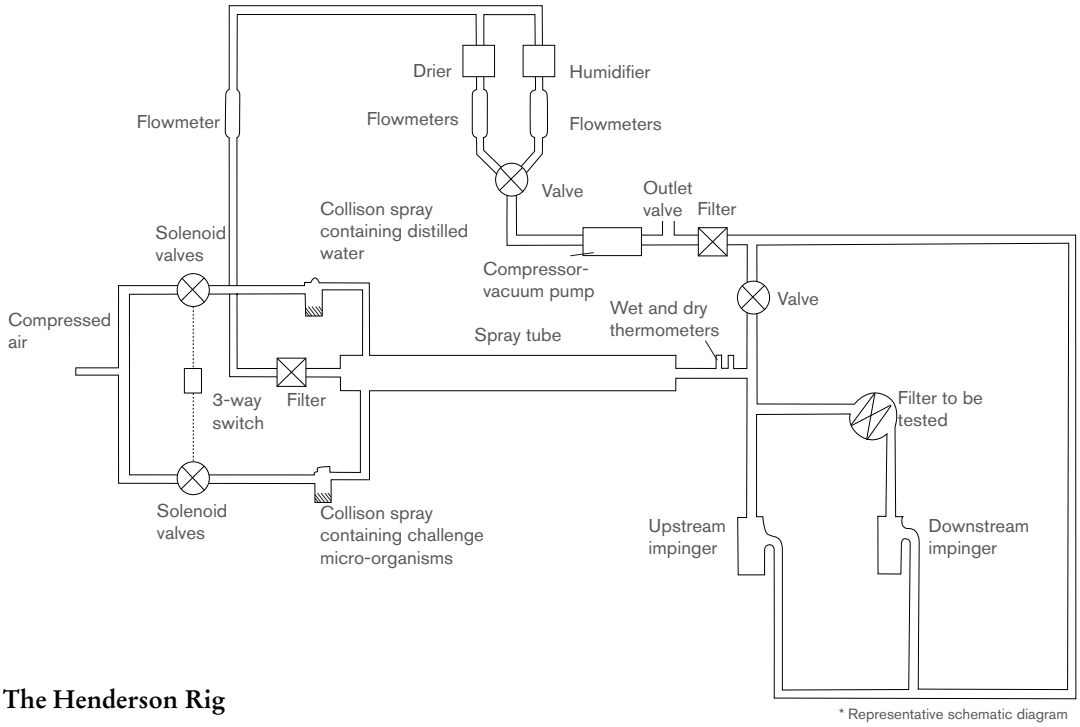

Figure 1 - The Henderson Rig

* Representative schematic diagram

Sterile tubing, connectors and tapers: to allow insertion of the filter to be tested in the system.

Porton All Glass Impingers: incorporating critical orifices to control the flow of sampled air. Each impinger contained 10ml buffer solution (phosphate buffer containing mannitol and antifoam PBMA) and were connected to a vacuum pump.

The impingers were operated in parallel at 30 litres per minute with one placed downstream of the filter to be tested.

Sampling Technique

Each filter was inserted in turn into the Henderson Rig, and the Collison spray containing the Bacillus subtilis spore suspension was activated. The air was then sampled at the specified flow rate for one minute by the impingers. Collecting fluid was then removed from the impingers and assayed for spores as described below.

Assay of Bacillus subtilis in collecting fluids

The collecting fluid from the impinger linked to the spray tube was suitably diluted in PBMA. The number of spores was determined in a 10^4 fold diluted fluid, by spreading 0.1ml on duplicate Tryptone Soya Broth agar (TSBA) plates. The TSBA plates were incubated at 37ºC for 18 hours and any orange colonies were counted.

Undiluted suspensions (0.1ml) of the collecting fluid from each impinger placed behind the filter, were spread on duplicate TSBA plates. The volume of the remainder of the collecting fluid, was measured and filtered through a 0.2 µm pore 47 mm diameter polycarbonate membrane filter (Whatman International, Maidstone, Kent No 7060-4202), placed on the sintered surface of a sterile filter holder. The filter membrane was placed on a TSBA plate. The TSBA plates were incubated at 37ºC for 18 hours and any distinctive orange colonies were counted.

Determination of 'Filtration Efficiency'

- Filtration Efficiency is defined as expressed below

$$\frac{\text{cfu collected without filter in place} - \text{cfu with filter in place}}{\text{cfu collected without filter in place}} \times 100$$

- Microbial Penetration Value (MPV) this is defined as the number of colony forming units passing through the filter per 10^7 challenge micro-organisms

Results

Sample	Challenge (CFU)	Collected after Filter (CFU)	Filtration Efficiency %	MPV
1541	3.10×10^7	3.42×10^2	99.9989	110
1541	3.60×10^7	7.30×10^2	99.9980	203
1541	3.85×10^7	5.80×10^2	99.9985	151

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