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A Test Protocol and Evaluation of the Bacterial Filtration Efficiency of the Intersurgical Pulmo-Protect™ lung function filter, code 1691050

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Introduction

The Biosafety Investigation Unit at Public Health England (PHE) 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.

Bacterial 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 107 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 Collision sprays: one containing 10ml distilled water and the other 10ml Bacillus subtilis suspension containing 3.7 x 109 cfu per ml in distilled water. The Collision 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 Collision 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.

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

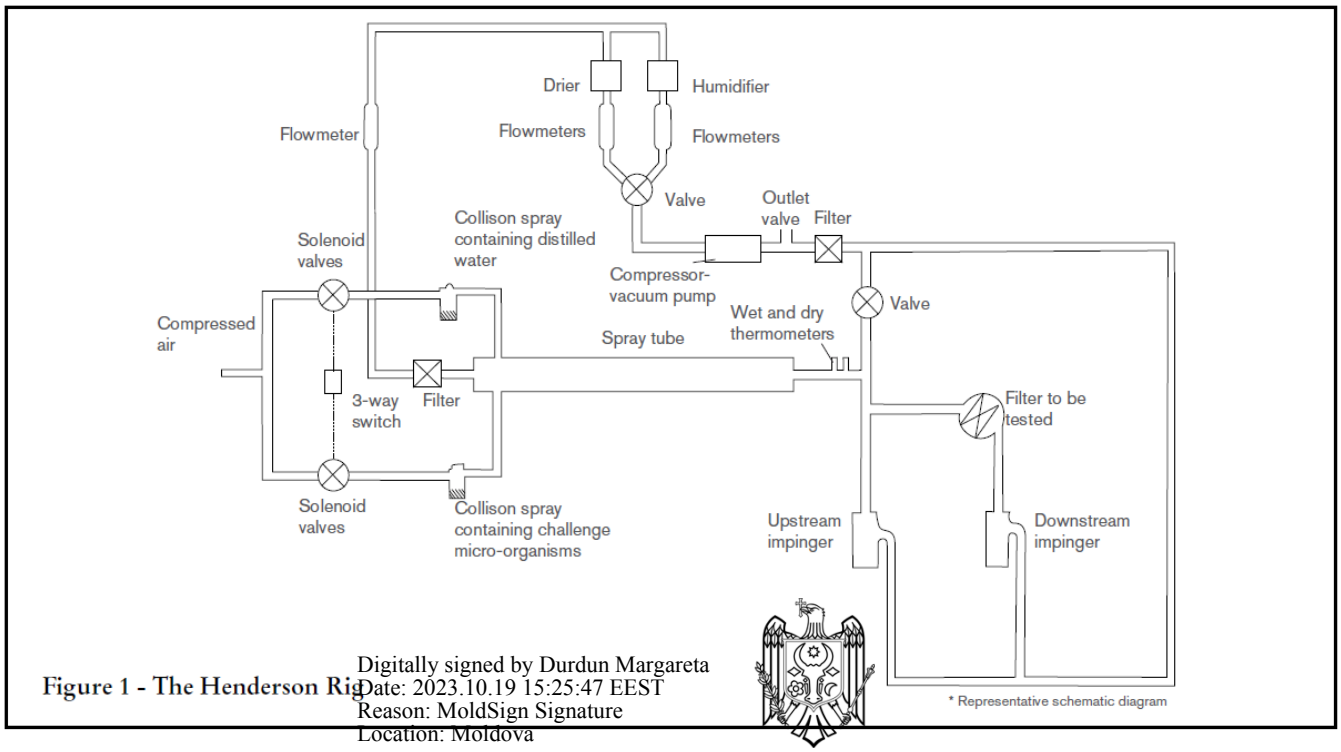

Figure 1 - The Henderson Rig

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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 750 litres per minute with one placed downstream of the filter to be tested.

Bacterial Sampling Technique
Each filter was inserted in turn into the Henderson Rig, and the Collision 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 104 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 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 'Bacterial Filtration Efficiency'
Filtration Efficiency is defined as expressed below:

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

Microbial Penetration Value (MPV) this is defined as the number of colony forming units passing through the filter per 107 challenge micro-organisms.

Results

Filter	Bacterial Filtration Efficiency (%)
1691050	>99.8[10]

Table 1: Bacterial filtration efficiency of 1691050 filter.

Conclusion

This protocol shows the Intersurgical Pulmo-Protect™ lung function filter (1691050) to be >99.8% efficient when tested against a Bacterial Aerosol Challenge.

References:

1. *PHILLIP, I. and SPENCER, G. (1965). Pseudomonas aeruginosa cross-infection due to contaminated respiratory apparatus. Lancet ii, 1365- 1367.*
2. *HENDERSON, D. W. (1952) An apparatus for the study of airborne infections. J.Hyg. Camb. 50, 53-67.*
3. *DRUETT, H. A. (1969) A mobile form of the Henderson apparatus. J. Hyg. Camb. 67, 437-448.*
4. *HINDS, W. C. (1982) Properties, behaviour and measurement of airborne particles. In "Aerosol Technology". Published by John Wiley & Sons, New York.*
5. *ANDERSEN, A. A. (1958) New sampler for the collection, Sizing and enumeration of viable particles. J. Bacteriol. 76, 471-484.*
6. *SHARP, R J. SCAWEN, M. D. and ATKINSON, A. (1989) Fermentation and downstream processing of Bacillus. In 'Bacillus'. Edited by C. R Harwood, Plenum Publishing Corporation.*
7. *COX, C. S. (1987). In "Aerobiological Pathway of Microorganisms". Published by John Wiley & Sons, Chichester, New York, Brisbane, Toronto, Singapore.*
8. *MAY, K R. (1973) The Collison nebulizer. Description, performance and application. Aerosol Sci. 4, 235-243.*
9. *MAY, K. R and HARPER, G. J. (1957). The efficiency of various liquid impinger samplers in bacterial aerosols. Brit. J. Ind. Med. 14, 287-297*
10. *Report No.19-029*