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A Test Protocol and Evaluation of the Viral 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.

Viral Test Organism

Due to potential health hazards human viruses are not often used to evaluate respiratory filters. RNA phages are similar in size to the smallest human viruses, therefore filtration efficiency can be comparatively gauged by measuring the penetration of aerosolised RNA phages.

MS-2 phage is an unenveloped single stranded RNA coliphage, 23nm in diameter with a molecular weight of 3.6 x 106 Daltons. It has been established that MS-2 coliphage sprayed from the supernatant of centrifuged spent bacterial lysate are known to remain infectious. By spraying this suspension from a Collision nebuliser, the airborne coliphage are carried in droplets, which are much larger than the infected particles, consisting mostly of bacterial lysate and media constituents.

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.

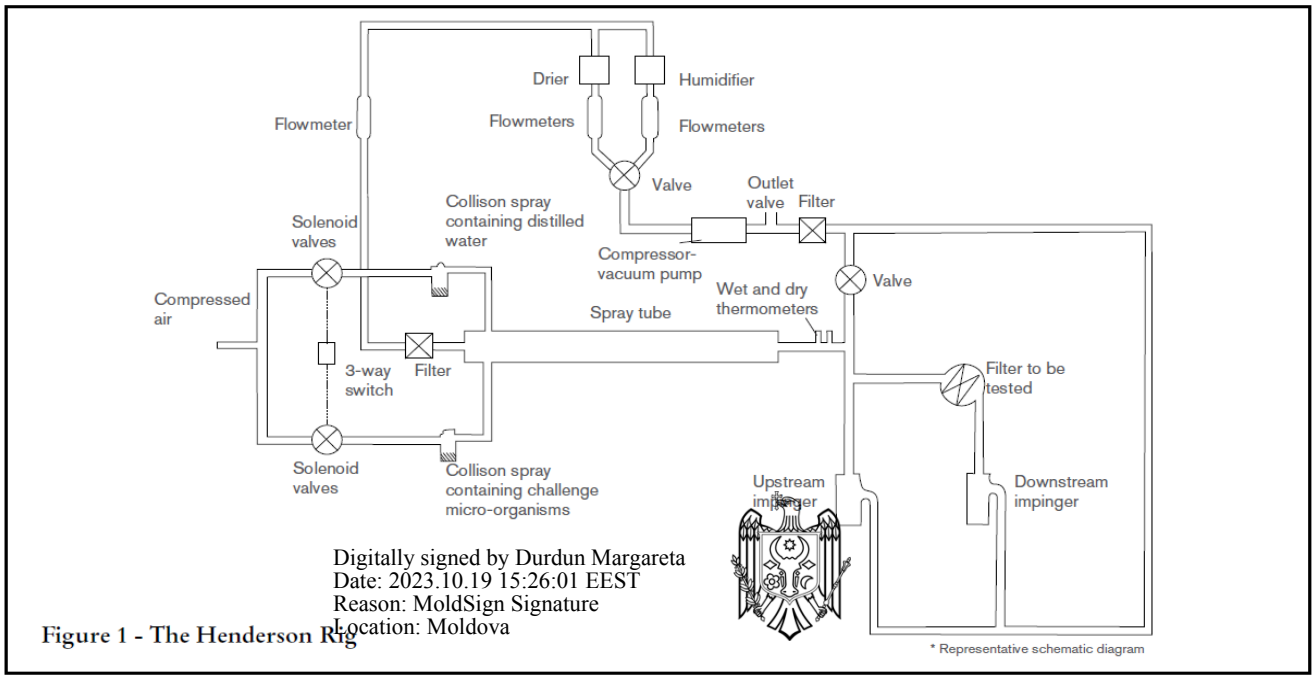

Figure 1 - The Henderson Rig

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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.

Challenging filters with MS-2 phage
A high–titre suspension of MS-2 for challenging the filters was prepared as follows:

The E. coli 9481 host was inoculated on a fresh TSBA plate, which was incubated at 37± 2°C for 19-29hr. The E coli was sub cultured from this plate by a 10µl loop to 0ml sterile Trytone Soya broth (TSB) in a 500ml flask. After mixing thoroughly the flask was placed in a shaking incubator (120 rpm) for 150 mins at 37 ± 2°C.

The suspension of coliphage was then prepared by inoculating a total of 4 x 1011 plaque forming unit (pfu) coliphage suspension into the 500ml flask containing the 60ml TSB. The suspension was then aerated by shaking 37 ± 2°C for a further 3 hours. The suspension was centrifuged twice at 2,000g for 20 mins each to remove the cell debris and the supernatant was transferred to a fresh flask.

The concentration of phage was determined as follows:

The filters were challenged on the Henderson rig by spraying for 1 minute MS-2 suspension containing over 3.65 x 1011 pfu per ml in 50% nutrient broth in the system described above. A total of at least 1 x 109 pfu was used to challenge each filter. The concentration of MS-2 collected in the impingers placed before and after the filter, was determined in suitably diluted samples in PBMA as described in the next paragraph.

Assay of MS-2 coliphage in various suspensions and collecting fluids
A fresh TSBA plate was inoculated with Escherichia coli NCIMB 9481 from a stock plate previously stored at 4 ± 2°C. The plate was incubated at 37 ± 2°C for 19-20 hrs. The E.coli 9481 was subcultured by transferring a 10 µl loopful from the plate to 10ml sterile nutrient broth in a glass universal bottle. After mixing, the universal bottle was incubated at 37 ± 2°C for 260mins before use. Meanwhile, stoppered bottles containing 3ml volumes of soft phage agar were heated for at least 90 minutes at 90-100°C then stored at 60 ± 20°C until required. These bottles were then cooled to 45°C before use. The suitably diluted MS-2 suspension in PBMA (100 µl) was added to the soft agar followed immediately by three drops of the E.coli 9481 suspension using a 50 D (20 µl per drop) Pasteur pipette. After mixing, it was poured immediately on a TSBA plate. Duplicate samples were carried out (the dilution should give 30-100 plaque forming units per plate) the plates were incubated at 37 ± 2°C overnight, and after incubation the clear plaques were counted. The MS-2 suspension was stored at 4 ± 2°C for up to 7 days and re-assayed if required. The MS-2 has been shown to be stable under these conditions.

Determination of 'Viral Filtration Efficiency'
Filtration Efficiency is defined as expressed below:

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

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	Viral Filtration Efficiency (%)
1691050	>99.5[10]

Table 1: Viral filtration efficiency of 1691050 filter.

Conclusion
This protocol shows the Intersurgical Pulmo-Protect™ lung function filter, code 1691050 to be >99.5% efficient when tested against a Viral Aerosol Challenge.

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