Public Health England

An Evaluation of Filtration Efficiencies Against Bacterial and Viral Aerosol Challenges

Report No. 18/040

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About Public Health England

We work with national and local government, industry and the NHS to protect and improve the nation's health and support healthier choices. We address inequalities by focusing on removing barriers to good health.

We were established on 1 April 2013 to bring together public health specialists from more than 70 organisations into a single public health service.

About Biosafety Investigation Unit

The Biosafety Investigation Unit at Porton Down has been carrying out independent evaluations of infection control interventions in laboratories, health care, containment, workplace and domestic settings for over twenty years. Our expertise is in air and water microbiology applied to nosocomial, pharmaceutical and containment situations. We have developed and offer standard techniques for the determination of the efficacy of filters and air disinfection units, the performance of safety cabinets, sealed centrifuges rotors and air samplers. We are also able to assess liquid and gaseous disinfectants and the microbial air quality of healthcare facilities, workplaces and other environments.

The Biosafety Investigation Unit provides specialist bespoke research, testing and evaluation services for commercial customers that delivers independent analysis and reports. However as a public sector body we are not able to endorse any particular products or recommend them for use by the NHS or others.

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Executive summary

The efficiencies of six mouthpiece filters, supplied by Bedfont Scientific Ltd., were determined against aerosols containing micro-organisms. Three filters were tested with aerosols containing bacterial spores (*Bacillus atrophaeus* NCTC 10073) and three were tested with aerosols containing viral particles (MS-2 coliphage ATCC 15597-B1). The challenges were conducted against fresh filters at 6 litres min^{-1} at a relative humidity of approximately 95%.

The results are summarised as follows:-

Filter Number	Test Organism	% Efficiency
1	*Bacillus atrophaeus*	99.13
2	*Bacillus atrophaeus*	99.27
3	*Bacillus atrophaeus*	96.84
1	MS-2 coliphage	95.42
2	MS-2 coliphage	96.47
3	MS-2 coliphage	98.00

Introduction

Contamination of respiratory apparatus during mechanical ventilation has been recognised since 1965 as a source of noscomial infections (1). Disposable filters placed between the patient and the ventilation system are designed to prevent such contamination. There is a need for a standard method to test the effectiveness of these filters against bacteria and viruses. A system has been developed at Public Health England, Porton Down (PHE) to test the efficiencies of many types of microbiological filters. An apparatus, developed originally by Henderson and Druett (2, 3) to study experimental airborne infection, is used where a suspension of micro-organisms in aqueous solution is nebulised by a 3-jet Collison spray 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 internal diameter. The relative humidity of the air in the spray tube is controlled to a desired value and monitored using wet and dry bulb thermometers in the air stream. The efficiencies of the filters are calculated by determining the airborne concentration of viable micro-organisms upstream and downstream of the filter using suitable aerosol sampling techniques and microbial assay methods.

The choice of bacterial strains to challenge and test these filters is based on a non-pathogenic model providing the highest possible challenge concentration of viable micro-organisms to allow a fully quantitative assessment of the filters to be made. To do this, spores of *Bacillus atrophaeus* were used as the bacterial model because they are known to survive the stresses caused by aerosolisation. The spores were washed thoroughly and finally suspended in distilled water before nebulisation. During nebulisation the water is rapidly evaporated from the droplets formed (even at high relative humidities) so that monodispersed aerosols of viable spores actually challenge the filter in this system (4).

Because of the health hazards involved, it is unrealistic to evaluate these filters using human viruses. Fortunately, RNA-phages are of a similar size as the smallest human viruses and the efficiencies of the filters for removing human viruses form air streams can be gauged by measuring the penetration of aerosolized coliphage through the filter. MS-2 phage is an unenveloped single stranded RNA coliphage, 23 nm in diameter with a

molecular weight of 3.6 x 10^6 Daltons. MS-2 coliphage sprayed from the supernatant of centrifuged spent bacterial lysate are known to remain infectious at the conditions tested here (5). By spraying this suspension from a Collison nebuliser, the airborne coliphage are carried in droplets, which are much larger than the infectious particles, consisting mostly of bacterial lysate and media constituents.

Materials and Method

Test organisms

Bacillus atrophaeus (NCTC 10073)

The *B. atrophaeus* spores (2.28 x 10^9 colony forming units (cfu) per ml) which had been thoroughly washed in distilled water were suspended in distilled water. The suspension was prepared from batches previously prepared by the PHE Production Division (6).

MS-2 coliphage (ATCC 15597-B1)

A vial of MS-2 phage (ATCC 15597-B1) was obtained from LGC Standards, Teddington, Middlesex. A stock suspension of coliphage was prepared by inoculating 0.1 ml of a 10^{11} plaque forming unit (pfu) per ml coliphage suspension into 500 ml nutrient broth containing 1 x 10^8 *Escherichia coli* (ATCC 15597) during the logarithmic growth phase. The suspension was aerated by shaking at 37°C. This was used as the stock suspension of MS-2. The suspension was centrifuged twice at 2,000g for 20 minutes each to remove the cell debris and filtered through a 0.2µm filter.

A high-titre suspension of MS-2 for challenging the filtered mouthpieces was prepared as follows:-

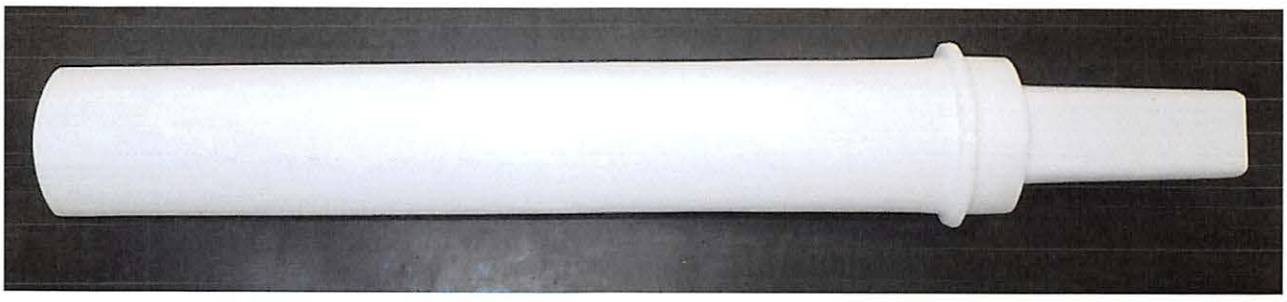
The *E. coli* host was inoculated on a fresh TSA plate, which was incubated at 37±2°C for 19 - 20 hr. The *E. coli* was sub-cultured from this plate by a 10 µl loop to 100ml sterile Tryptone Soya broth (TSB) in a 500 ml flask. After mixing thoroughly the flask was placed in a shaking incubator (120 rpm) for 150 mins at 37 ±2°C. The suspension of coliphage was then prepared by inoculating a total of 4 x 10^{11} plaque forming unit (pfu) coliphage suspension into the 500 ml flask containing the 100 ml TSB. The suspension was then aerated by shaking at 37 ±2°C for a further 3 hours. The suspension was centrifuged twice at 2,000g for 20 minutes each to remove the cell debris. The supernatant was transferred to a fresh flask. The concentration of phage was 1.90 x 10^{12} pfu/ml. This was diluted to

1.90 x 10^{11}pfu/ml for use as the challenge test suspension. The phage assay is described later.

Filters

The efficiencies of six mouthpiece filters, supplied by Bedfont Scientific Ltd., were determined against aerosols containing micro-organisms. Three filters were tested with aerosols containing bacterial spores (*Bacillus atrophaeus* NCTC 10073) and three were tested with aerosols containing viral particles (MS-2 coliphage ATCC 15597-B1). The challenges were conducted against fresh filters at 6 litres min^{-1} at a relative humidity of approximately 95%.

Figure 1. Mouthpiece filter supplied by Bedfont Scientific Ltd.



Challenging filters with bacterial aerosols

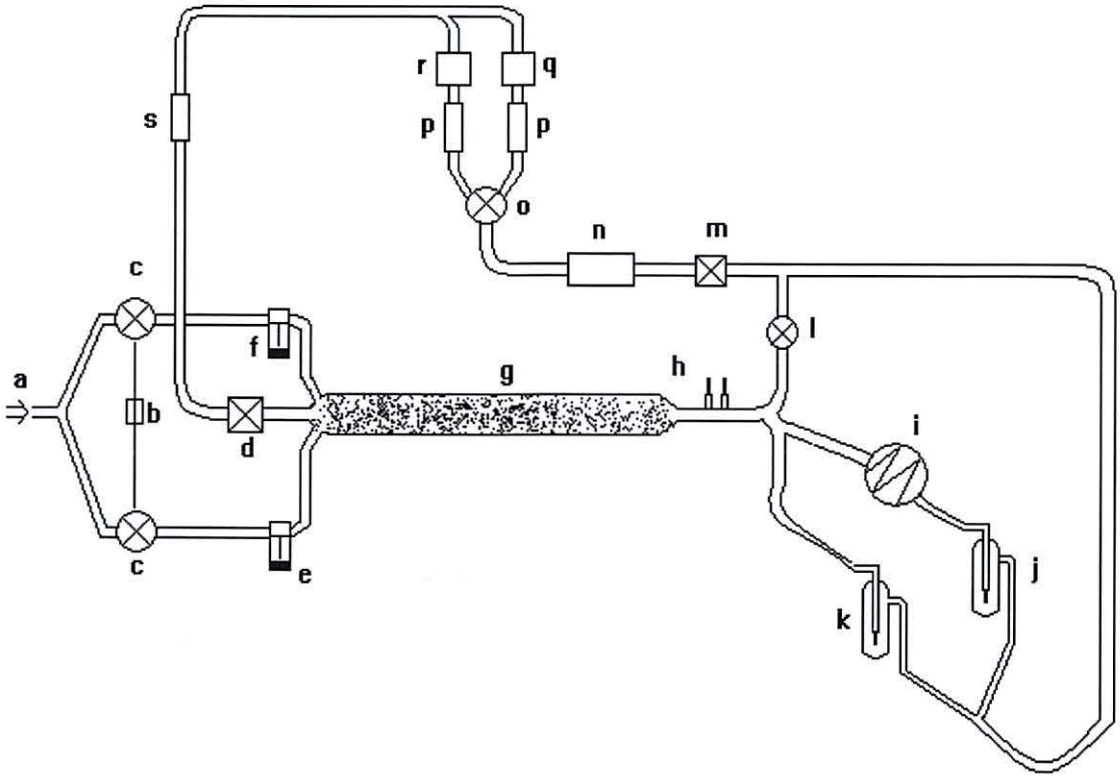
The Henderson apparatus (Figure 2.) was designed to deliver a high challenge level of micro-organisms in aerosols at a relative humidity of approximately 95% or above (measured by a wet and dry thermometer) (7) at 6 litres per minute.

The apparatus consisted of the following essential parts:-

- Two 3-jet Collison sprays (8), one containing 10 ml distilled water and the other the microbial suspension. The Collison sprays were arranged so that they could be operated alternatively to nebulise 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 to allow mixing and conditioning of the aerosols generated from the Collison with a supply of clean filtered humidified air at 60 litres per minute.
- Wet and dry thermometers downwind of the spray tube to determine the relative humidity.
- Suitable sterile silicone tubing connectors and tapers to allow insertion of the filter to be tested in the system.
- Two 6 l/min Porton All-Glass impingers (9) incorporating critical orifices to control the flow of sampled air. Each impinger contained 10 ml buffer solution (Phosphate buffer containing manucol and antifoam [PBMA]) and was connected to a vacuum pump. The impingers were operated in parallel.

The filters were inserted in the apparatus and the Collison spray containing the microbial suspension was activated. The air was sampled for five minutes by the impingers. The collecting fluid was removed from the impingers and assayed for microbes as described below.

Figure 2. Henderson apparatus for challenging breathing system filters with microbial aerosols



Key:

a	Compressed air	k	Upstream Impinger
b	3-Way Switch	l	Valve
c	Solenoid Valves	m	Filter
d	Filter	n	Compressor-Vacuum Pump
e	Collison Spray Containing Challenge Micro-organisms	o	Valve
f	Collison Spray Containing Distilled Water	p	Flowmeters
g	Spray Tube	q	Humidifier
h	Wet and Dry Thermometers	r	Drier
i	Filter to be Tested	s	Flowmeter
j	Downstream Impinger		

Assay of *B. atrophaeus* in collecting fluids

The collecting fluid from the impinger linked to the spray tube (i.e. without filter) was suitably diluted in PBMA. The number of spores was determined in a 10^3 fold diluted fluid by spreading 0.1 ml on duplicate Tryptone Soya agar (TSA) plates. The TSA plates were incubated at 37°C for 24 hours and any orange colonies were counted. The neat, 10^{-1}, 10^{-2} and 10^{-3} dilution of the collecting fluid from the impinger placed behind the filter was also spread (0.1 ml) on duplicate TSA plates. Volumes of 1ml, 5ml and the remainder of the collecting fluid were filtered through a 0.2 μm pore 47 mm diameter polycarbonate membrane filter (Whatman International, Maidstone, Kent. Cat. N°. 7060-4202) placed on the sintered surface of a sterile filter holder. The filter membranes were placed on TSA plates. These TSA plates were incubated at 37°C for 24 hours and any distinctive orange colonies were counted.

Assay of MS-2 coliphage in collecting fluids

A fresh TSA plate was inoculated with *Escherichia coli* ATCC 15597 from a stock plate previously stored at 4 ± 2°C. This plate was incubated at 37 ± 2°C for 19 - 20 hrs. The *E. coli* 15597 was subcultured by transferring a 10 μl loopful from the plate to 10 ml sterile nutrient broth in a glass universal bottle. After mixing, the universal bottle was incubated at 37 ± 2°C for 260 minutes before use. Meanwhile, stoppered bottles containing 3 ml volumes of soft phage agar were heated for at least 90 minutes at 90 to 100°C and then stored at 60 ± 2°C until required. These bottles were then cooled to 45°C before use. The suitably diluted MS-2 suspension (100 μl) was added to the soft agar followed immediately by 3 drops of the *E. coli* 15597 suspension using a 50 D (20 μl per drop) Pasteur pipette. After mixing, it was poured immediately on a Tryptone Soya Agar (TSA) plate. Duplicate samples were carried out (the dilution selected should give 20 to 200 plaque forming units (pfu) per plate). The plates were incubated at 37 ± 2°C overnight. The clear plaques were counted.

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Determination of effectiveness of the filter

The effectiveness of the filter is expressed in the following ways:-

- Percentage efficiency. This is defined as follows:-

$$\frac{\textit{cfu collected without filter in place - cfu with filter in place}}{\textit{cfu collected without filter in place}} \; X \; 100$$

Results

Test Conditions

Date	14th January 2019	Challenge Micro-organisms	*Bacillus atrophaeus* NCTC 10073
Operators	Anna Moy & Leyla Musleh		
Apparatus	Henderson	Suspension Fluid	Sterile Distilled Water
Spray	3 Jet Collison	Concentration	2.28×10^9 cfu/ml

Relative Humidity (RH):	97%	Temperature:	22 ± 3°C

Samplers	All glass Impingers	Sampling Time	5	min at	6	Litres/min

Collecting Fluid	PBMA	Volume	10ml

Filter	3 x Nobreath mouthpiece filters supplied by Bedfont Scientific Ltd

Results

Filter Number	Total Challenge (cfu#)	Total Collected after filter (cfu)	% Efficiency
1	9.35×10^6	8.15×10^4	99.13
2	9.35×10^6	6.80×10^4	99.27
3	9.35×10^6	2.95×10^5	96.84

#cfu - colony forming units

Test Conditions

Date	23rd January 2019	Challenge Micro-organisms	MS-2 Coliphage ATCC 15597-B1
Operators	Anna Moy & Leyla Musleh		
Apparatus	Henderson	Suspension Fluid	50% Nutrient Broth
Spray	3 Jet Collison	Concentration	1.90×10^{11} pfu/ml

Relative Humidity (RH):	96%	Temperature:	22 ± 3°C

Samplers	All glass Impingers	Sampling Time	5	min at	6	Litres/min

Collecting Fluid	PBMA	Volume	10ml

Filter	3 x Nobreath mouthpiece filters supplied by Bedfont Scientific Ltd

Results

Filter Number	Total Challenge (pfu#)	Total Collected (pfu)	% Efficiency
1	9.50×10^8	4.35×10^7	95.42
2	9.50×10^8	3.35×10^7	96.47
3	9.50×10^8	1.90×10^7	98.00

#pfu - plaque forming units

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