An evaluation of filtration efficiencies against bacterial and viral aerosol challenges

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Report Prepared For	Mr. Marco D'Alò of MADA
Operator	Miss A. Moy
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Report Written By	**Report Authorised By**
Name Miss Anna Moy **Name Title** Biosafety Scientist	**Name** Mrs Sara Speight **Title** Senior Biosafety Scientist

SUMMARY

The efficiencies of 4 MADA pulmonary function testing filters (Spirometry filter 82), supplied by MADA, were determined against aerosols containing micro-organisms. The filters were challenged with bacterial spores of *Bacillus atrophaeus* (previously *Bacillus subtilis* var niger) and viral aerosols of MS-2 coliphage NCIMB 10108, fresh from the packaging. The filters were challenged at 30 and 750 litres min^{-1}.

The results are summarised as follows:-

Filter N°.	Aerosol challenge	Flow Rate (l/min)	% Efficiency
1	*B. atrophaeus*	30	99.9988
2	*B. atrophaeus*	750	99.68
3	MS-2	30	99.94
4	MS-2	750	99.89

INTRODUCTION

Contamination of respiratory apparatus during expiration has been recognised since 1965 as a source of noscomial infections (1). Disposable filters placed between the patient and the apparatus 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 the Health Protection Agency, Porton Down (HPA) to test the efficiencies of many types of microbiological filters including breathing system 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. This system is used to test the filters at 30 litres/minute.

The Health Protection Agency, Porton Down (HPA) also has the ability to test the efficiencies of many types of microbiological filters using our small test rig facility. This rig is based on the apparatus developed originally by Henderson and Druett to study experimental airborne infection, where a suspension of micro-organisms in aqueous solution is nebulized 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 long stainless steel tube. 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. Filters can be challenged with micro-organisms on the small test rig up to flow rates of 2000 litres per minute. This system is used to test the filters at 750 litres/minute.

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* (electron micrographic examination show that the spores used are from 0.96 µm to 1.25 µm long and from 0.55 µm to 0.67 µm wide) were used as the bacterial model because they are known to survive the stresses caused by aerosolization. The spores were washed thoroughly and finally suspended in distilled water before nebulization. During nebulization the water is rapidly evaporated form the droplets formed (even at high relative humidities) so that monodispersed aerosols of viable spores (of the above size) actually challenge the filter in this system (4). Analysis of the distribution of the aerosol particle size by an Andersen sampler (5) has previously showed that over 80 % of the particles containing *B. atrophaeus* spores challenging the filters were less than 2.1 microns in size.

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 from air streams can be gauged by measuring the penetration of aerosolised 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 (6). By spraying this suspension from a Collison nebulizer, 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 METHODS

Test organisms

Bacillus atrophaeus spores (NCTC 10073)

The *B. atrophaeus* spores (>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 HPA Production Division (7).

MS-2 phage (NCIMB 10108)

A vial of MS-2 phage (NCIMB 10108) was obtained from the National Collection of Industrial and Bacteria, Torry Research Station, Aberdeen. 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* (NCIMB 9481) during the logarithmic growth phase. The suspension was aerated by shaking at 37°C. The bacterial cells lysed within 30 minutes and the

suspension was centrifuged to remove the cell debris. The supernatant was transferred to a fresh flask and 10 drops of chloroform were added to kill any contaminating bacteria. This was used as the stock suspension of MS-2. The concentration of phage was determined as described later.

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 - 20 hr. The *E. coli* was sub-cultured from this plate by a 10 µl loop to 60 ml 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 60 ml TSB. The suspension was then aerated by shaking at 37 ±2°C for a further 3 hours. The suspension was centrifuged twice at 2,000 g for 20 minutes each to remove the cell debris. The supernatant was transferred to a fresh flask. The concentration of phage was determined as described below.

Filters

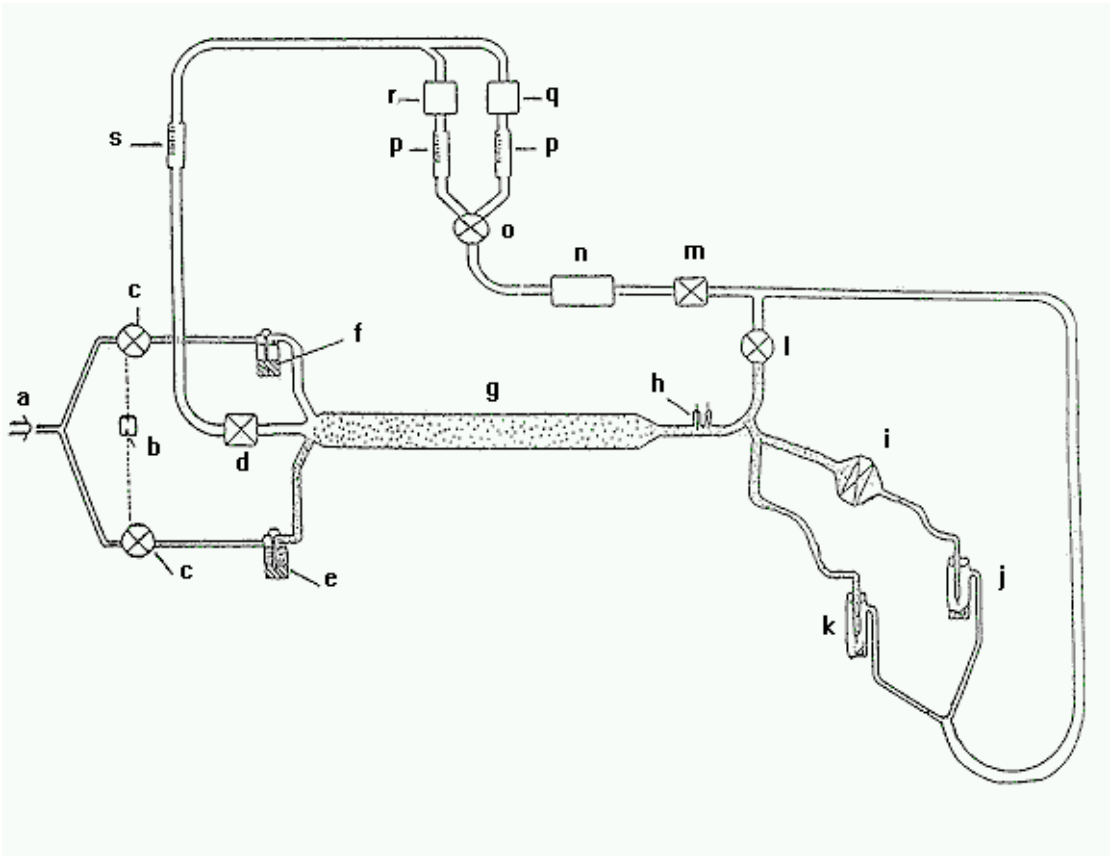
Four MADA pulmonary function testing filters (Spirometry filter 82) were provided for testing by MADA. Their efficiencies were determined against bacterial and viral aerosols containing *B. atrophaeus* spores and MS-2 coliphage, respectively, at 30 and 750 litres/minute.

Challenging filters at 30 l/min using the Henderson Apparatus

The Henderson apparatus (Figure 1) was designed to deliver a high titre challenge of *B. atrophaeus* spores or MS-2 coliphage in aerosols at a relative humidity of 95% or above (measured by a wet and dry thermometer) (8) at 30 litres per minute.

The apparatus consisted of the following essential parts:-

FIGURE 1. HENDERSON APPARATUS FOR CHALLENGING 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		

- Two 3-jet Collison sprays (9), one containing 20 ml distilled water and the other either 30 ml *B. atrophaeus* suspension (containing >10^9 cfu per ml in distilled water) or 30 ml of MS-2 coliphage (containing 3.55 x 10^{11} pfu per ml in 50% ($^{v}/_{v}$) nutrient broth). 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.

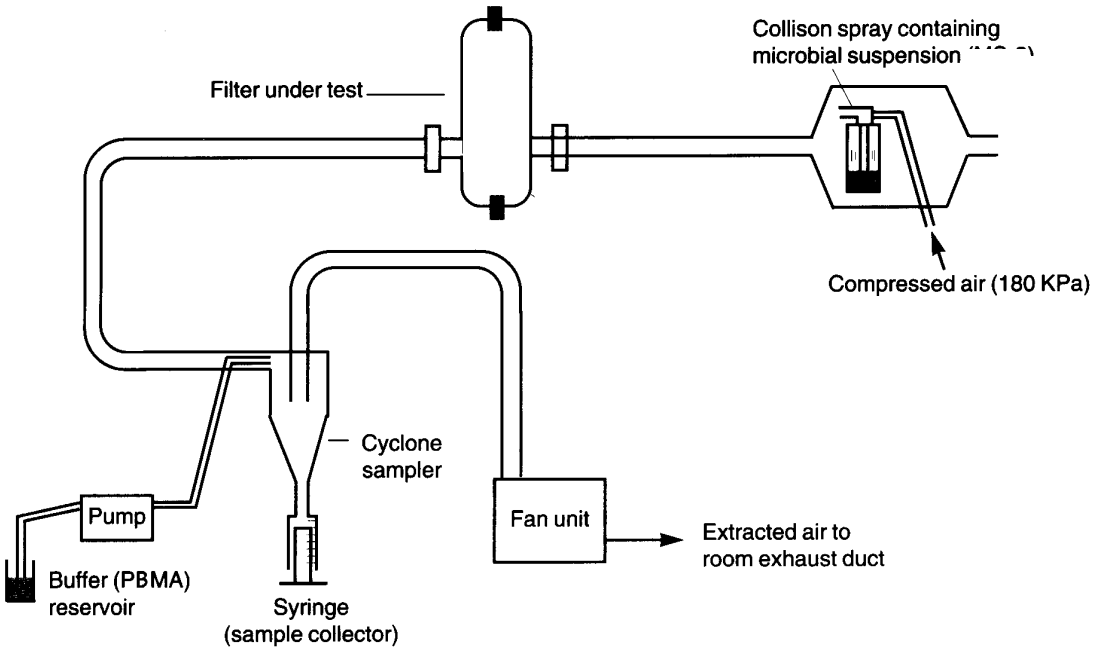
Two 30 l/min Porton All-Glass impingers (10) incorporating critical orifices to control the flow of sampled air. Each impinger contained 10 ml buffer solution (Phosphate buffer containing manucol and antifoam [PBMA]) and was connected to a vacuum pump. The impingers were operated in parallel at 30 litres per minute for the filter.

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

<u>Challenging filters on the small test rig at 750 litres/minute with aerosols containing bacterial and viral particles</u>

The small test rig (Figure 2) was designed to deliver a high titre challenge of *B. atrophaeus* spores and MS-2 coliphage in aerosols at 750 litres per minute.

FIGURE 2. SMALL TEST RIG FOR TESTING FILTERS WITH MICROBIAL AEROSOLS



The apparatus consisted of the following essential parts:-

- One pre-weighed 3-jet Collison spray, containing either 30 ml *B. atrophaeus* suspension (containing >10^9 cfu per ml in distilled water) or 30 ml of MS-2 coliphage (containing 3.55 x 10^{11} pfu per ml in 50% ($^{v}/_{v}$) nutrient broth). The Collison spray was operated to nebulise its contents at a pressure of 180 KPa into the air stream in the spray tube.

- Stainless steel spray tube 90 cm length and 5 cm diameter to allow mixing and conditioning of the aerosols generated from the Collison.

- Suitable sterile tubing connectors and tapers to allow insertion of the filter to be tested in the system.

- One Cyclone sampler (11) (manufactured by The Hampshire Glass Company, Southampton) operates the flow of sampled air via a vacuum pump. The air

containing the microbial aerosols is drawn through the system at a flow rate of 750 litres per minute. Sterile phosphate buffer containing manucol and antifoam (PBMA) was used as the collection fluid and was fed into the cyclone inlet at a rate of approximately 1ml per minute by a peristaltic pump. The particles in the air stream were deposited by centrifugal force on the cyclone wall and were collected by the swirling liquid, which was withdrawn by a syringe at the end of the challenge period. The volume of collection fluid collected by the cyclone was measured for each filter.

Each filter was inserted in turn in the apparatus and the pre-weighed Collison spray was activated. The air was sampled for 5 minutes by the Cyclone. The collecting fluid was removed from the samplers and assayed for spores or MS-2 as described below. The Collison spray was weighed after each test to determine the weight loss. The challenge concentration was determined by operating the system with the filters removed.

Assay of *B. atrophaeus* in collecting fluids

The collecting fluids from the samplers linked to the spray tube (i.e. without filter) were suitably diluted in PBMA and plated (0.1 ml) on duplicate Tryptone Soya agar (TSA) plates. The TSA plates were incubated at 37°C for 18 hours and any orange colonies were counted. Suitably diluted suspensions (0.1 ml) of the collecting fluid from each sampler placed behind the filter was also spread on duplicate TSA plates and these TSA plates were incubated at 37°C for 18 hours and any distinctive orange colonies were counted.

Assay of MS-2 coliphage in various suspensions and collecting fluids

A fresh TSA plate was inoculated with *Escherichia coli* NCIMB 9481 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* 9481 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 in PBMA (100 µl) was added to the soft agar followed immediately by 3 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 (Tryptone Soya Broth agar) plate. Duplicate samples were carried out (the dilution selected should give 30 to 100 plaque forming units (pfu) per plate). The plates were incubated at 37 ± 2°C overnight. The clear plaques were counted.

Determination of effectiveness of the filter

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

- Percentage efficiency. This is defined as follows:-

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

RESULTS

The results of challenging the filters with aerosolised bacterial and viral particles at 30 litres/minute and 750 litres/minute are shown in Tables 1, 2, 3 and 4.

TABLE 1 FILTER INTEGRITY TESTS CHALLENGING WITH *B. ATROPHAEUS* AT 30 LITRES / MINUTE

Date	June 2010	Challenge Micro-organisms	*Bacillus atrophaeus*
Operator	A. Moy	Suspension Fluid	Distilled Water
Apparatus	Henderson	Concentration	3.53×10^9 cfu/ml
Spray	3-Jet Collison		

Relative Humidity (RH):	95%
Temperature:	22 ± 3°C

Filters Tested: MADA Spirometry filter 82

Sampling Time 1 min at 30 litres/min Sampler All glass impinger

Collecting Fluid PBMA Volume 10 ml

Filter	Challenge (cfu)	Collected After Filter (cfu)	% Efficiency
Spirometry filter 82	1.13×10^8	1.33×10^3	99.9988

cfu – colony forming units

TABLE 2 FILTER INTEGRITY TESTS CHALLENGING WITH *B. ATROPHAEUS* AT 750 LITRES / MINUTE

Date	July 2010	Challenge Micro-organisms	*Bacillus atrophaeus*
Operator	A. Moy	Suspension Fluid	Distilled Water
Apparatus	Small rig	Concentration	5.00×10^9 cfu/ml
Spray	3-Jet Collison		

Filters Tested: MADA Spirometry filter 82

Sampling Time 5 min at 750 litres/min Sampler Cyclone

Collecting Fluid PBMA Volume Variable ml

Filter	Challenge (cfu)	Collected After Filter (cfu)	% Efficiency
Spirometry filter 82	1.30×10^9	4.14×10^6	99.68

cfu – colony forming units

TABLE 3 FILTER INTEGRITY TESTS USING MS-2 COLIPHAGE AT 30 LITRES/ MINUTE

Date	June 2010	Challenge Micro-organisms	MS-2 coliphage
Operator	A. Moy	Suspension Fluid	50% Nutrient Broth
Apparatus	Henderson	Concentration	3.55×10^{11} pfu/ml
Spray	3-Jet Collison		

Relative Humidity (RH):	95%
Temperature:	22 ± 3°C

Filters Tested: MADA Spirometry filter 82

Sampling Time 1 min at 30 litres/min Sampler All glass impinger

Collecting Fluid PBMA Volume 10 ml

Filter	Challenge (pfu)	Collected After Filter (pfu)	% Efficiency
Spirometry filter 82	5.55×10^{9}	3.15×10^{6}	99.94

pfu – plaque forming unit

TABLE 4 FILTER INTEGRITY TESTS USING MS-2 COLIPHAGE AT 750 LITRES/ MINUTE

Date	July 2010	Challenge Micro-organisms	MS-2 coliphage
Operator	A. Moy	Suspension Fluid	50% Nutrient Broth
Apparatus	Small rig	Concentration	3.55×10^{11} pfu/ml
Spray	3-Jet Collison		

Filters Tested: MADA Spirometry filter 82

Sampling Time 5 min at 750 litres/min Sampler Cyclone

Collecting Fluid PBMA Volume Variable ml

Filter	Challenge (pfu)	Collected After Filter (pfu)	% Efficiency
Spirometry filter 82	1.88×10^{9}	2.09×10^{6}	99.89

pfu – plaque forming unit

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