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Efficacy Testing of the Airsteril MP20 Air Purification Device to Reduce Aerosol and Surface Microbial Contamination

Report 37/09

Commercial in Confidence

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SUMMARY

Two studies were carried out with the Airsteril MP20 Air Purification Device to evaluate if it could reduce both aerosol and surface microbial contamination.

1. The aerosol study involved challenging the Airsteril MP20 Air Purification Device separately with MS-2 coliphage NCIMB 10108 (an unenveloped single stranded RNA coliphage) and *Staphylococcus epidermidis* NCIMB 12721 (a Gram positive cocci)

2. The surface decontamination study involved exposing stainless steel discs contaminated with know concentrations of MS-2 coliphage NCIMB 10108 and Methicillin Resistant *Staphylococcus aureus* NCIMB 13162 (MRSA) (Gram positive cocci)

The results of the two studies are shown in Tables 1 and 2, respectively.

Table 1. Results of Aerosol study

Micro-organism	Percentage Efficiency		
	Run 1	Run 2	Run 3
MS-2 coliphage	87.18%	92.17%	89.04%
Staph. epidermidis	98.11%	97.69%	97.94%

Table 2. Results of Surface decontamination study

Test Number	Micro-organism			
	MS-2 coliphage		MRSA	
	% Efficiency	Log Reduction	% Efficiency	Log Reduction
Test Disc 1	39.69	0.22	32.10	0.17
Test Disc 2	59.47	0.39	45.24	0.26
Test Disc 3	21.46	0.10	51.81	0.32
Test Disc 4	15.85	0.07	44.14	0.25
Test Disc 5	no effect	No effect	1.42	0.006

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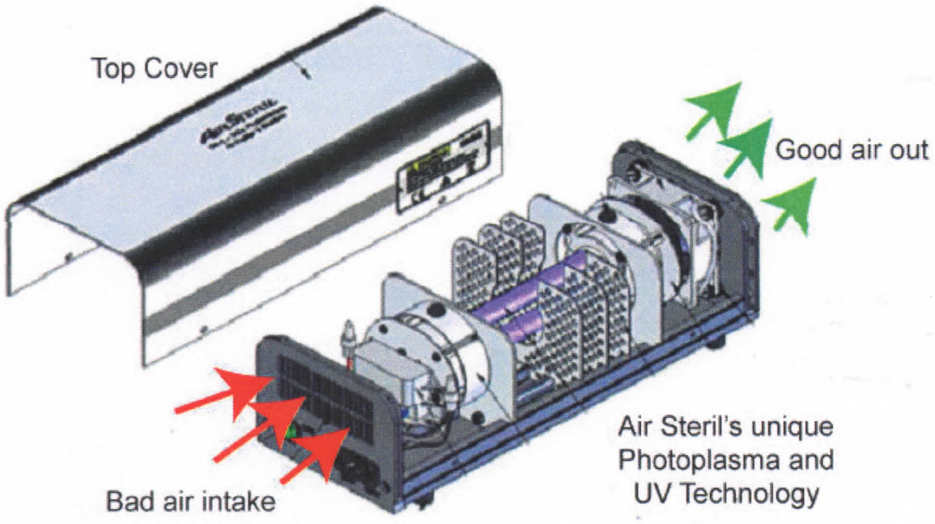
INTRODUCTION

Airborne micro-organisms can cause health risks in a number of different environments particularly in healthcare facilities. Airsteril (UK) Ltd has an air purification device (Figure 1, MP20) designed for removing particles and odour from indoor environments. The device operates by drawing air at 300 litres per minute through a chamber which combines ultraviolet UVC and Photocatalytic Oxidation PCO technology then from here it is returned to the room it came from. The MP20 Air purification device was supplied to HPA CEPR by Airsteril (UK) Ltd to test its ability to reduce both aerosol and surface microbial contamination. The aerosol studies were carried out using *Staphylococcus epidermidis* NCIMB 12721 (a Gram positive, cocci) and MS2 coliphage NCIMB 10108 (an unenveloped single stranded RNA coliphage). The surface decontamination studies were carried out using Methicillin Resistant *Staphylococcus aureus* MRSA NCIMB 13162 (Gram positive, cocci) and MS2 coliphage NCIMB 10108.

Figure 1



MATERIALS AND METHODS

1. Methodology for aerosol study

The Airsteril MP20 device was placed in a HEPA filtered chamber to provide a controlled environment. High-pressure airlines and electrical supplies were supplied in the chamber to control the nebuliser and samplers, respectively. The test micro-organisms were aerosolised using a 3-jet Collison nebuliser. The airborne micro-organisms were introduced directly into the inflow aperture of the Airsteril MP20 device for 5 minutes. Air samples were taken simultaneously from the exit vent located on the opposite side of the device using a glass cyclone sampler operating at 620 litres per minute for 5 minutes. This procedure was carried out for each of the two micro-organisms separately. To determine the concentration of micro-organisms challenging the device it was necessary to carry out a test run (for each of the two micro-organisms) with the ultraviolet UVC and Photocatalytic Oxidation PCO technology disconnected. Before the test experiments were started the ultraviolet UVC and Photocatalytic Oxidation PCO technology was reconnected. The test experiments were carried out in triplicate and the challenge sample was carried out once.

This methodology was carried out using two different micro-organisms:

- *Staphylococcus epidermidis* NCIMB 12721 (Gram positive, cocci, ca0.6 microns diameter)

- MS2 bacteriophage NCIMB 10108 (MS-2, model virus, 23nm diameter)

2. Surface decontamination experiment

A separate experiment was carried out exposing stainless steel discs contaminated with known quantities of micro-organisms to the operating MP20 air purification device over a 1 hour period.

Eighteen individual stainless steel discs were prepared by inoculating 10µl of MRSA (9 discs) and MS-2 coliphage (9 discs) onto the surface of the discs and drying at 37°C for 1 hour and then storing at room temperature until used. Ten of the discs (5 MRSA and 5 MS-2) were place in the chamber and the Airsteril MP20 device was operated for a 1 hour period. At the end of the test time the discs were placed into individual universal containers containing 2 ml of Phosphate Buffered Saline (PBS) and removed from the cabinet. The samples were mixed using a vortex mixer for a minimum of 1 minute then assayed using the culturing techniques described below, to determine the concentration of viable micro-organisms recovered from the discs. The remaining two discs for each micro-organism (positive controls) were placed in the chamber without the air purification device operating for 1 hour. These discs were processed using the same method as the test discs.

This methodology was carried out using two different micro-organisms:

- Methicillin Resistant *Staphylococcus aureus* MRSA NCIMB 13162 (Gram positive, cocci)

- MS2 bacteriophage NCIMB 10108 (MS-2, model virus, 23nm diameter)

3. Preparation and Assay of Micro-organisms

MS-2 bacteriophage NCIMB 10108

A spray suspension of MS-2 for challenging the unit was prepared using the following method:-

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 (140 rpm) for 150 mins at 37 ±2°C. The suspension of E. *coli* was then inoculated with 0.5 ml of a MS-2 coliphage suspension, giving a total inoculum of 4 x 10^{11} plaque forming unit (pfu). The

resultant 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 resultant concentration of phage was 2.5x 10^{11} plaque forming units per ml (pfu/ml).

Microbial Assay of MS-2 Bacteriophage

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 spray suspension and collection fluids from the cyclone were suitably diluted in sterile distilled water and 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 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 and any clear plaques were counted.

Staphylococcus epidermidis NCIMB 12721

A spray suspension of *Staph. epidermidis* was prepared by inoculating one flask containing 100 ml each of Tryptone Soya Broth. A full (generous) 10 µl loop of *Staph. epidermidis* was taken from a stock plate previously stored at 4°C ± 2°C and added to the flask. The culture suspension was mixed thoroughly by shaking and placed in a 37°C ± 2°C shaking water bath for 24 hours. The resultant suspension was 4.3 x 10^9 colony forming units per ml (cfu/ml).

Microbial Assay of *Staph. epidermidis in collecting fluid*

The spray suspension and collection fluids from the cyclone were assayed by plating out 0.1 ml of a ten fold serial dilution in duplicate onto Tryptone Soya agar (TSA) plates. The TSA plates were incubated at 37 ± 2 °C for 24 hours and any colonies were counted.

Methicillin Resistant *Staphylococcus aureus* MRSA NCIMB 13162

A suspension of MRSA was prepared by inoculating one flask containing 100 ml each of Tryptone Soya Broth. A full (generous) 10 µl loop of MRSA was taken from a stock plate previously stored at 4°C ± 2°C and added to the flask. The culture suspension was mixed thoroughly by shaking and placed in a 37°C ± 2°C shaking water bath for 24 hours. The resultant suspension was 9.85 x 10^8 cfu/ml.

Microbial Assay of MRSA in collecting fluid

The suspension and collection fluids from the cyclone were assayed by plating out 0.1 ml of a ten fold serial dilution in duplicate onto Tryptone Soya agar (TSA) plates. The TSA plates were incubated at 37 ± 2 °C for 24 hours and any colonies were counted.

4. Determination of effectiveness of the Airsteril MP20 Air Purification Device

The effectiveness of the device is expressed as either:-

Percentage efficiency =

$$\frac{\text{cfu/pfu collected with device off (challenge)} - \text{cfu/pfu with device on (test)}}{\text{cfu/pfu with device off (challenge)}} \times 100$$

Or

Log Reduction = Log (Ave total cfu/pfu of 4 control disc ÷ Total cfu/pfu test disc)

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RESULTS

TABLE 1. Aerosol challenge results for MS-2 coliphage NCIMB 10108

Date	August 2009	Challenge Micro-organisms	MS-2 coliphage NCIMB 10108
Operator	H. Mittal	Suspension Fluid	50% nutrient broth
Spray	3-Jet Collison	Concentration	2.50×10^{11} pfu/ml

Sampling time 5 min at 620 litres/min sampler Glass Cyclone

Collecting fluid PBMA Volume (See table below) ml

Test Number	Dilution	Sample Volume (ml)	Average plaque forming units from duplicate plates (100µl)	Total plaque forming
Test 1	10^{-6}	3.87	107	4.14×10^{9}
Test 2	10^{-6}	3.52	72	2.53×10^{9}
Test 3	10^{-6}	3.50	101	3.54×10^{9}
Challenge	10^{-7}	3.36	96	3.23×10^{10}

Test N°.	Challenge (pfu)	Collected After Filter (pfu)	% Efficiency
1	3.23×10^{10}	4.14×10^{9}	87.18
2	3.23×10^{10}	2.53×10^{9}	92.17
3	3.23×10^{10}	3.54×10^{9}	89.04

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TABLE 2. Aerosol challenge results for *Staphylococcus epidermidis* NCIMB 12721

Date	August 2009	Challenge Micro-organisms	*Staphylococcus epidermidis* NCIMB 12721
Operator	H. Mittal	Suspension Fluid	Nutrient broth
Spray	3-Jet Collison	Concentration	4.30×10^9 cfu/ml

Sampling time 5 min at 620 litres/min sampler Glass Cyclone

Collecting fluid PBMA Volume (See table below) ml

Test Number	Dilution	Sample Volume (ml)	Average colony forming units from duplicate plates (100µl)	Total plaque forming
Test 1	10^{-3}	4.50	300	1.35×10^7
Test 2	10^{-3}	4.67	353	1.65×10^7
Test 3	10^{-3}	4.46	329	1.47×10^7
Challenge	10^{-5}	5.22	136.5	7.13×10^8

Test N°.	Challenge (pfu)	Collected After Filter (pfu)	% Efficiency
1	7.13×10^8	1.35×10^7	98.11
2	7.13×10^8	1.65×10^7	97.69
3	7.13×10^8	1.47×10^7	97.94

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TABLE 3. Surface Decontamination results for MS-2 coliphage NCIMB 10108

Date	August 2009	Challenge Micro-organisms	MS-2 coliphage NCIMB 10108
Operator	H. Mittal	Suspension Fluid	50% nutrient broth
		Concentration	2.50×10^{11} pfu/ml

Exposure time 60 min

Sample fluid PBMA volume 2 ml

Test Number	Dilution	Sample Volume (ml)	Average colony forming units from duplicate plates (100µl)	Total plaque forming
Test Disc 1	10^{-6}	2	22, 21	4.30×10^{8}
Test Disc 2	10^{-6}	2	15, 14	2.89×10^{8}
Test Disc 3	10^{-6}	2	25, 31	5.60×10^{8}
Test Disc 4	10^{-6}	2	31, 29	6.00×10^{8}
Test Disc 5	10^{-6}	2	30, 50	8.00×10^{8}
Control Disc 1	10^{-6}	2	36, 32	6.80×10^{8}
Control Disc 2	10^{-6}	2	33, 39	7.20×10^{8}
Control Disc 3	10^{-6}	2	32, 29	6.10×10^{8}
Control Disc 4	10^{-6}	2	41, 43	8.40×10^{8}
Ave of 4 controls used in calculation	-	-	-	7.13×10^{8}

Test N°.	% Efficiency	Log Reduction
Test Disc 1	39.69	0.22
Test Disc 2	59.47	0.39
Test Disc 3	21.46	0.10
Test Disc 4	15.85	0.07
Test Disc 5	no effect	No effect

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TABLE 4. Surface Decontamination results for MRSA NCIMB 13162

Date	August 2009	Challenge Micro-organisms	Methicillin Resistant *Staphylococcus aureus* MRSA
Operator	H. Mittal	Suspension Fluid	Nutrient Broth
		Concentration	9.85 x 10^8 cfu/ml

Exposure time: 60 min

Sample fluid: PBMA Volume: 2 ml

Test Number	Dilution	Sample Volume (ml)	Average colony forming units from duplicate plates (100µl)	Total plaque forming
Test Disc 1	10^{-4}	2	25, 37	6.20 x 10^6
Test Disc 2	10^{-4}	2	22, 28	5.00 x 10^6
Test Disc 3	10^{-4}	2	22, 22	4.40 x 10^6
Test Disc 4	10^{-4}	2	25, 26	5.10 x 10^6
Test Disc 5	10^{-4}	2	47, 43	9.00 x 10^6
Control Disc 1	10^{-4}	2	50, 40	9.00 x 10^6
Control Disc 2	10^{-4}	2	43, 48	9.10 x 10^6
Control Disc 3	10^{-4}	2	52, 43	9.50 x 10^6
Control Disc 4	10^{-4}	2	40, 49	8.90 x 10^6
Ave of 4 controls used in calculation	-	-	-	9.13 x 10^6

Test N°.	% Efficiency	Log Reduction
Test Disc 1	32.10	0.17
Test Disc 2	45.24	0.26
Test Disc 3	51.81	0.32
Test Disc 4	44.14	0.25
Test Disc 5	1.42	0.006