Efficacy Testing of the Air Revolution Unit to Reduce Aerosol Microbial Contamination

Report No. 128-11

Commercial In Confidence

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SUMMARY

The effectiveness of an novel air disinfection device and a control unit, provided by Air Revolution Technologies (Pty) Ltd, to remove airborne bacteria and viruses were determined by challenging the devices directly with aerosols of four different microorganisms:-

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

• *Brevundimonas diminuta* NCIMB 11091 (Gram negative, rod, 0.3 x 0.8 microns)

• *Staphylococcus epidermidis* NCTC 13360 (Gram positive, cocci, ca 0.6 microns diameter)

• *Bacillus atrophaeus* NCTC 10073 (Gram positive, spore, 1.1 x 0.6 microns)

The two devices tested were Unit A – fan only (used to determine the challenge concentration of the microbial aerosol), and Unit B – Fan, UV and TiO_2 material. The results of the tests are summarised below as percentage efficiency of the units and log reduction in the units.

	Test	MS-2 Bacteriophage		Brevundimonas diminuta		Staphylococcus epidermidis		Bacillus atrophaeus	
		% Efficiency	Log Red^n	% Efficiency	Log Red^n	% Efficiency	Log Red^n	% Efficiency	Log Red^n
Unit A Fan Only	1	colony forming units detected from Unit A were used to calculate the challenge concentration							
	2								
	3								
Unit B Fan, UV + TiO_2	1	90.526	1.02	98.695	1.88	99.764	2.63	82.686	0.76
	2	88.21.	0.93	98.339	1.78	99.099	2.05	78.467	0.67
	3	86.552	0.87	97.399	1.58	99.511	2.31	78.906	0.68
Average		88.539	0.94	98.144	1.75	99.458	2.33	80.020	0.70

MATERIALS AND METHODS

Methodology for aerosol study

The Air Revolution Units (Figure 1) were each placed in turn in a HEPA filtered chamber which provided a controlled environment. High-pressure airlines and electrical supplies were supplied in the chamber to control the nebuliser and sampler, respectively. Each unit was positioned horizontally in the test chamber for the test procedure. The test micro-organisms were aerosolised using a Collison nebuliser.

The volume of microbial suspension aerosolised for each test was determined by weight loss from the Collison nebuliser (1). The airborne micro-organisms were introduced directly into the inflow aperture of the Air Revolution Unit 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 (2) operating at 650 ± 50 litres per minute for 5 minutes. The sampler was carefully positioned to ensure that it did not affect the airflows within the disinfection device.

The cyclone captured any airborne micro-organisms exiting the unit into collecting fluid (Phosphate buffer plus manucol and antifoam) (PBMA) which was injected into the inlet of the cyclone at a rate of about 1-2 ml per minute. Any micro-organisms present in the sampled air were deposited by centrifugal forces on the cyclone wall and washed off by the swirling collection fluid. The fluid was withdrawn from the base of the cyclone by a sterile syringe at the end of the sampling period. The volume of sample fluid collected was measured and assayed for the appropriated micro-organism, as described below.

This procedure was carried out for both units with each of the four micro-organisms, separately. The concentration of micro-organisms challenging the unit was calculated from the results achieved by the unit with a fan only. The test experiments for each unit were carried out in triplicate.

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

- MS-2 Bacteriophage NCIMB 10108 (MS2, model virus, 23nm diameter)
- *Brevundimonas diminuta* NCIMB 11091 (Gram negative, rod, 0.3 x 0.8 microns)
- *Staphylococcus epidermidis* NCTC 13360 (Gram positive, cocci, ca 0.6 microns diameter)
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Test Micro-organisms

Bacillus atrophaeus (NCTC10073)

Bacillus atrophaeus NCTC 10073 is a non-pathogenic, aerostable spore which is resistant to the stresses of aerosolisation. A *B. atrophaeus* spore suspension (3.57 x 109 colony forming units (cfu) per ml) was used as the spray suspension. This suspension had been thoroughly washed and suspended in distilled water and was prepared from batches previously prepared by the HPA Production Division. The *B. atrophaeus* collected from the air was assayed as follows. The collecting fluid from the cyclones was suitably diluted in phosphate buffer containing manucol and antifoam (PBMA). The number of spores in the air samples was determined by spreading 0.1ml of the appropriate dilution on duplicate Tryptone Soya agar (TSA) plates. The TSA plates were incubated at 37oC for 24 hours and any orange colonies were counted.

MS-2 coliphage (NCIMB 10108)

MS-2 coliphage NCIMB 10108 is an aerostable unenveloped single stranded RNA bacteriophage which is known to remain infectious under test conditions. Because of the health hazards involved, it is unrealistic to evaluate these air disinfection systems using human viruses. Fortunately, RNA-phages are of a similar size as the smallest human viruses and the efficiencies of these systems for removing human viruses from air streams can be gauged by measuring the penetration of aerosolized coliphage through the system. A vial of MS-2 coliphage (NCIMB 10108) was obtained from the National Collection of Industrial and Marine Bacteria, Torry Station, Aberdeen.

The spray suspension was produced by adding 0.2ml of high titre stock suspension, individually, to three 500ml flasks, each containing 60ml of a 150 minute culture of the host strain *Escherichia coli* NCIMB 9481 in a shaking water bath at 37$_{o}$C±2$_{o}$C. After 240 minutes the culture was centrifuged at 2000g for 20 minutes. The supernatant was collected and centrifuged again, then stored at 4$_{o}$C±2$_{o}$C prior to use. The suspension was assayed using the phage assay described below.

The resultant suspension was 5.55 x 10_{11} plaque forming unit per ml (pfu/ml). This suspension was used neat as the spray suspension. The spray suspension and all the air samples were assayed for MS-2 as follows:- A fresh Tryptone Soya Broth agar (TSBA) plate

was inoculated with *E. coli* 9481 and incubated at 37$_o$C±2$_o$C for 18-20 hours. A 10Fl loopful was transferred aseptically to 10ml sterile nutrient broth in a universal bottle. After mixing thoroughly the broth was incubated at 37$_o$C±2$_o$C for 260 minutes. In the meantime small bottles containing 3 - 4 ml soft phage agar were heated at 90$_o$C – 100$_o$C for 90 minutes and then cooled and maintained at 60$_o$C until required.

Immediately before use the molten agar was cooled to 45$_o$C. A bottle containing the molten soft agar was inoculated with 100µl of suitably diluted MS-2 suspension and immediately three drops of the *E. coli* suspension were added by a Pasteur pipette. After replacing the lid of the bottle the contents were rapidly mixed before being poured onto a TSBA plate ensuring that the surface was evenly covered. The plates were incubated immediately at 37oC±2oC and the plaques formed were counted after 24 hrs.

Brevundimonas diminuta (NCIMB 11091, ATCC 19146)

Brevundimonas diminuta NCIMB 19146 is a non-pathogenic, vegetative bacterium. *Brev. diminuta* is commonly used as a bacterial model for work testing the efficiency of disinfection systems because it is the smallest known free living micro-organism. The *Brev. diminuta* used was nebulised from distilled water.

Brev. diminuta was prepared by inoculating four 500ml flasks each containing 100ml of Tryptone Soya Broth. A full (generous) 10Fl loop of *Brev. diminuta* was taken from a stock plate previously stored at 4°C ± 2°C and added to each of the flasks. The culture suspension was mixed thoroughly by shaking and placed in a 30°C ± 2°C shaking incubator for 24 hours. The resultant suspension was centrifuged at approximately 2,000 g for 30 minutes and the pellet formed was washed by resuspending in sterile distilled water and centrifuging as above. The pellet was resuspended in 100ml of sterile distilled water and stored at 4°C ± 2°C until used.

The suspension was assayed by plating out 0.1 ml of a ten fold serial dilution in duplicate onto Tryptone Soya Agar (TSA) plates and incubating the plates at 30°C ± 2°C for 48 hours. The colonies were counted after incubation to determine the concentration of the bacteria (colony forming units (cfu) per millilitre of suspension. The resultant suspension was 2.55 x 1010 cfu/ml. This suspension was used neat as the spray suspension.

Staphylococcus epidermidis (NCTC 13360)

Staphylococcus epidermidis (NCTC 13360) is a non-pathogenic, vegetative bacterium. *Staph. epidermidis* is used as a bacterial model for work testing the efficiency of disinfection systems because it is non-pathogenic *Staphylococcus* species commonly used a surrogate for *Staph. aureus* or MRSA. The *Staph. epidermidis* used was nebulised from the spent nutrient media it was grown in. *Staph. epidermidis* was prepared by inoculating a 500ml flask containing 100 ml of Tryptone Soya Broth.

A full (generous) 10 Fl loop of *Staph. epidermidis* was taken from a stock plate previously stored at 4°C ± 2°C and added to each of the flasks. The culture suspension was mixed thoroughly by shaking and placed in a 37°C ± 2°C shaking incubator for 20 hours. The resultant suspension was stored at 4°C ± 2°C until used.

The suspension was assayed by plating out 0.1 ml of a ten fold serial dilution in duplicate onto Tryptone Soya Agar (TSA) plates and incubating the plates at 37°C ± 2°C for 24 hours. The colonies were counted after incubation to determine the concentration of the bacteria (colony forming units (cfu) per millilitre of suspension. The resultant suspension was 2.20 x 109 cfu/ml. This suspension was used neat as the spray suspension.