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**Microbial Aerosol Challenge
Testing of the IQ Air Cleanroom
H13 Unit**

Report 850/03

Commercial in Confidence

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Report Prepared For	Mr. Jonathon Wells
Operator	Mr. Graham Pitt and Mr. Simon Parks
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Report Written By  Name Mrs. Sara Speight Title Research Scientist	Authorised By  Name Mr Allan Bennett Title Head of Biosafety Investigation Unit
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SUMMARY

The IQ Air Cleanroom H13 Unit was challenged with four different micro-organisms, *Brevundimonas diminuta* NCIMB 11091, MS-2 coliphage NCIMB 10108, *Bacillus subtilis* var niger NCTC 10073 and *Staphylococcus epidermidis* NCIMB 12721. The percentage efficiencies of the IQ Air Cleanroom H13 Unit against these four airborne micro-organisms are as follows:-

Micro-organism	Percentage Efficiency		
	Run 1	Run 2	Run 3
<i>Brev. diminuta</i>	99.923	99.846	99.870
MS-2 coliphage	99.665	99.604	99.524
<i>B. subtilis</i> var niger	99.687	99.690	99.697
<i>Staph. epidermidis</i>	99.879	99.886	99.870

INTRODUCTION

Airborne micro-organisms can cause a health risk in a number of different environments particularly in healthcare facilities. Air Science Ltd. has designed air cleaning systems for airborne infection control and particulate contamination challenges for indoor environments. The IQAir systems filter the air by recirculation or by creating positive and negative pressure environments with special ducting adaptors. The IQAir Cleanroom H13 Unit was supplied to HPA Porton down by Air Science Ltd. to test its percentage efficiency with four different micro-organisms.

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

Bacillus subtilis var niger NCTC 10073 (Gram positive, spore, 1.1 x 0.6 microns)

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

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



MATERIALS AND METHODS

The IQAir Cleanroom H13 Unit was placed in an environmental room (dimension 3m x 3m x 2m high). The room is fitted with a filtered extract and supply ventilation system and remotely controlled high-pressure airlines and electrical supplies which were used to control the nebuliser and samplers, respectively. The IQAir Cleanroom H13 Unit was tested using InFlow and OutFlow ducting adaptors supplied by Air Science Ltd. Using a 3-jet Collison nebuliser the airborne micro-organism was introduced directly into the InFlow aperture of the unit for 5 minutes. Air samples were taken simultaneously from the unit using a glass cyclone sampler operating at 770 litres per minute. The downstream filter sample was taken from the OutFlow duct and the challenge sample was taken from directly before the filter via a sample port added to the InFlow duct. This procedure was carried out for each of the four micro-organisms. The downstream sample was carried out in triplicate and the challenge sample was carried out once.

Preparation of Test Micro-Organisms

Bacillus subtilis var niger NCTC 10073

A suspension of *Bacillus subtilis* var niger NCTC 10073 spores was prepared by diluting, in sterile distilled water, stock batches previously prepared by the CAMR Production Division. The concentration of the suspension was determined in 10⁹ fold dilution by spreading 0.1 ml on duplicate TSBA plates. The TSBA plates were incubated at 37 ± 2°C for 18 hours and the colonies were counted. The concentration was 3.46 x 10⁹ cfu per ml.

MS2 bacteriophage NCIMB 10108

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



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The *E. coli* 9481 host was inoculated on a fresh TSBA plate, which was incubated at $37 \pm 2^\circ\text{C}$ 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 $37 \pm 2^\circ\text{C}$. The suspension of coliphage was then prepared by inoculating a total of 4×10^{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 $37 \pm 2^\circ\text{C}$ 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 4.30×10^{11} pfu per ml this was determined as described below.

A fresh TSBA plate was inoculated with *Escherichia coli* NCIMB 9481 from a stock plate previously stored at $4 \pm 2^\circ\text{C}$. This plate was incubated at $37 \pm 2^\circ\text{C}$ 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 $37 \pm 2^\circ\text{C}$ 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 $60 \pm 2^\circ\text{C}$ 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 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 $37 \pm 2^\circ\text{C}$ overnight. The clear plaques were counted.

Brevundimonas diminuta NCIMB 11091

Brev. diminuta was prepared by inoculating two flasks containing 50 ml each of Tryptone Soya Broth. A full (generous) 10 μl loop of *Brev. diminuta* was taken from a stock plate previously stored at $4^\circ\text{C} \pm 2^\circ\text{C}$ and added to each of the flasks. The culture suspension was mixed thoroughly by shaking and placed in a $30^\circ\text{C} \pm 2^\circ\text{C}$ shaking water bath for 24 hours. The resultant suspension was centrifuged at



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approximately 2,000 g for 15-20 minutes and the pellet formed was washed by resuspending in sterile distilled water and centrifuging as above. The pellet was resuspended in 100 ml of sterile distilled water and stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ until used.

The suspension was assayed by plating out 0.1 ml of a ten fold serial dilution in duplicate onto Tryptone Soya Broth Agar (TSBA) plates and incubating the plates at $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ 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 2.00×10^{10} cfu/ml. This suspension was diluted 1 in 10 for use as the spray suspension (2.00×10^9 cfu/ml).

Staphylococcus epidermidis NCIMB 12721

Staph. epidermidis was prepared by inoculating two flasks containing 50 ml each of Tryptone Soya Broth. A full (generous) 10 μl loop of *Staph. epidermidis* was taken from a stock plate previously stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and added to each of the flasks. The culture suspension was mixed thoroughly by shaking and placed in a $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ shaking water bath for 24 hours.

The suspension was assayed by plating out 0.1 ml of a ten fold serial dilution in duplicate onto Tryptone Soya Broth Agar (TSBA) plates and incubating the plates at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ 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 4.25×10^9 cfu/ml. This suspension was used as the spray suspension.

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RESULTS

TABLE 1 Results of *Brevundimonas diminuta* NCIMB 11091

Date	Sept 2003	Challenge Micro-organisms	<i>Brevundimonas diminuta</i> NCIMB 11091
Operator	G. Pitt	Suspension Fluid	Sterile distilled water
Spray	3-Jet Collison	Concentration	2.00×10^9 cfu/ml

Sampling time min at litres/min sampler

collecting fluid volume ml

Test N°.	Challenge (cfu)	Collected After Filter (cfu)	% Efficiency
1	8.325×10^5	645	99.923
2	8.325×10^5	1280	99.846
3	8.325×10^5	1075	99.871

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TABLE 2 Results of MS-2 Coliphage NCIMB 10108

Date	Sept 2003	Challenge Micro-organisms	MS-2 coliphage NCIMB 10108
Operator	G. Pitt	Suspension Fluid	50% nutrient broth
Spray	3-Jet Collision	Concentration	4.30×10^{11} pfu/ml

Sampling time min at litres/min sampler
 collecting fluid volume ml

Test N°	Challenge (pfu)	Collected After Filter (pfu)	% Efficiency
1	2.73×10^8	9.15×10^5	99.665
2	2.73×10^8	1.08×10^6	99.604
3	2.73×10^8	1.30×10^6	99.524

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TABLE 3 Results of *Bacillus subtilis* var niger NCTC 10073

Date	Sept 2003	Challenge Micro-organisms	<i>Bacillus subtilis</i> var niger NCTC 10073
Operator	G. Pitt	Suspension Fluid	Sterile distilled water
Spray	3-Jet Collision	Concentration	3.46×10^9 cfu/ml

Sampling time min at litres/min sampler

collecting fluid volume ml

Test N°.	Challenge (cfu)	Collected After Filter (cfu)	% Efficiency
1	8.61×10^6	26975	99.687
2	8.61×10^6	26700	99.690
3	8.61×10^6	26100	99.697

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TABLE 4 Results of *Staphylococcus epidermidis* NCIMB 12721

Date	Sept 2003	Challenge Micro-organisms	<i>Staphylococcus epidermidis</i> NCIMB 12721
Operator	G. Pitt	Suspension Fluid	Sterile distilled water
Spray	3-Jet Collison	Concentration	4.25×10^9 cfu/ml

Sampling time min at litres/min sampler

collecting fluid volume ml

Test N°.	Challenge (cfu)	Collected After Filter (cfu)	% Efficiency
1	2.70×10^7	32750	99.879
2	2.70×10^7	30750	99.886
3	2.70×10^7	35000	99.870