Evaluation of the performance of the INOV8 device.

A report prepared by

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Objectives of the study
The objectives of the study were to evaluate the performance of the device in terms of its ability to reduce the concentration of airborne *Staphylococcus aureus, Mycobacterium parafortuitum* and *Clostridium difficile* in the test chamber.

The Device
The performance of an air treatment device of this type is highly dependent upon its location. Therefore it is important during the evaluation process to locate the device in an area in which the concentration of microorganisms in the air is high, thereby giving the device the best chance of performing well. In the test chamber, previous CFD modeling has shown that the best location for a device is between the point of aerosol dispersion and the back wall of the chamber (Figure 1). This is an area where there is likely to be a high concentration of airborne microorganisms. Due to the fact that the device could not be operated remotely the testing period was split and the test chamber purged between taking the control samples and switching on the device to allow the operator to enter the room safely.

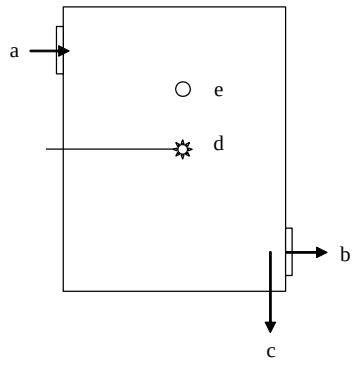

Figure 1: The experimental layout of the test chamber
(a, air inlet; b, air outlet; c, sampling point; d, the point at which the bacterial/fungal aerosol is introduced, e, the location of the device)

The Test Microorganisms
The experiments were performed using three different bacterial species which were considered to be important nosocomial pathogens or surrogates for those species deemed to hazardous to work with.

- *Clostridium difficile* is an anaerobic spore-forming gram positive bacterium that causes a whole range of clinical conditions and is the most commonly diagnosed bacterial cause of infectious hospital acquired diarrhoea in developed countries.

- *Mycobacterium parafortuitum* (ATCC 19687) is an acid-fast gram positive bacterium which is commonly used as a surrogate for *M. tuberculosis*. It is considered to be a better substitute than *M. bovis* BCG as it is faster growing and is generally thought to be less hazardous.

- *Staphylococcus aureus* (ATCC 13709) is a gram positive bacterium and is a major cause of hospital acquired infections. Hospital strains are usually resistant to a variety of antibiotics (e.g. MRSA) and many are resistant to antiseptics and disinfectants which aids it survival in the hospital environment.

Experimental Methodology

The experiments described in this report were carried out in the aerobiological test chamber at the University of Leeds (see Figure 1), which consists of a 32.25m^3 hermetically sealed negatively pressurised room in which the air flow rate, temperature and relative humidity can be constantly controlled and monitored. The experiments were carried out with the ventilation system set at 3 AC/hr at ambient temperature (approx 20°C) and relative humidity (approx 50%).

During the microbiological experiments the bacterial aerosols were generated using a 6-jet Collison nebuliser operating at a flow rate of 12 l/min and at a pressure of 20 psi. This was connected to the room via a 25 mm diameter pipe which terminated in a plastic sphere containing twenty four 3mm diameter holes through which the aerosol was dispersed. Air samples were collected through a plastic pipe located close to the room air outlet grille. This pipe was connected to a six stage Andersen sampler loaded with sterile agar plates. During the sampling process air passed through the sampler and the bacteria were deposited onto the agar plates. Each stage of the sampler represents a particular size range and this allows the size distribution of the aerosol to be determined. All the experiments were performed using only stages 5 and 6 (the smallest two stages) since previous experiments have shown that the majority of the aerosol is captured in these two stages. The sampling time was varied depending upon the concentration of the bacterial culture with the aim of collecting between 200 and 300 colony forming units on the agar plates.

The test room was set up as shown in Figure 1 prior to the start of each experimental run and the chamber door closed and locked and both the sampling port (c) and the nebuliser port (d) sealed. The air fans were then switched on and operated at maximum speed (approx 12AC/hr) for 30 minutes in order to ensure the chamber was sterile. During this purging period the test device remained switched off. During the initial purging period the pre-sterilised nebuliser was prepared and filled with 100ml of bacterial suspension at a concentration of approximately 10^5 organisms/ml of sterile distilled water. The nebuliser was then connected to the inlet tube ready for the start of the experiment.

After the initial purging period the ventilation rate was reduced to 3 AC/hr and nebulisation of the bacterial culture then began and the concentration in the test chamber was allowed to stabilize for 30 minutes. A total of ten samples were then taken at 3 minute intervals during which time the device remained switched off. Once all ten samples had been taken the nebuliser was switched off and the room purged for 1 hour to remove any bacterial contamination from the air. The device was then switched on and the ventilation rate returned to 3 AC/hr and the device operated without nebulisation for 2 hours. After 2 hours the nebuliser was then switched on and the concentration in the test chamber was allowed to stabilize for 30 minutes. A further ten samples were then taken at 3 minute intervals.

The agar plates were incubated at 37°C for 24 hours after which the number of colonies on each plate were counted. All the counts were then subjected to positive hole correction in order to account for multiple impaction (Macher 1989). The corrected counts for each set of plates (stages 5 and 6) were added together to give a total count and multiplied to give a count per m^3 of test chamber air. Each set of samples represents ten replicates taken during steady state, the first ten being the concentration without the device and the second with the device. The mean was taken of the ten replicate samples to give a mean concentration with and without the device. This allowed the mean reduction in concentration to be calculated used to give an indication as to the efficacy of the device

In order to determine the statistical significance of the results a t-test was carried out on the two data sets (before and after). The purpose of the test is to determine whether the

means of the two data sets are statistically different from each other. The test yields a p-value and the smaller the p-value the less likely the difference between the two data sets is the result of chance.

Results

Figure 2 shows the concentration of airborne *S. aureus* during the trial and it can be seen that there is a dramatic drop in the concentration of airborne *S. aureus* when the device was switched on. The concentration during the control period ranged from 8871 to 17900 cfu/m^3 with an average concentration of 12158 cfu/m^3. When the device was in operation the concentration ranged from zero to 654 cfu/m^3 with an average concentration of only 348 cfu/m^3 which represents a kill of 97.1% was recorded. A t-test carried out on the control and test data sets showed the difference between the two data sets to be highly significant (P< 0.01)

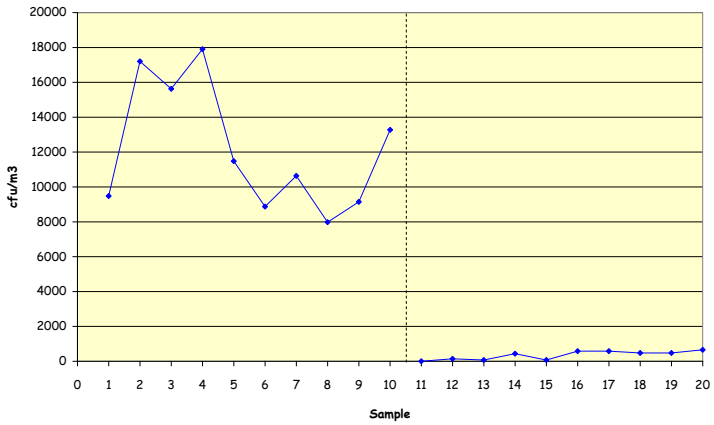

Figure 2 Effect of the device on the concentration of airborne *S. aureus*

Figure 3 shows the result from the final test in which the device was used against aerosols of *Clostridium difficile*. It can be seen that there was a dramatic drop in the concentration of the bioaerosol when the device was switched on. The mean concentration during the control period was 1637 cfu/m^3 (range 789-2436 cfu/m^3) compared to a mean of 166 cfu/m^3 (range 50-361 cfu/m^3) after the device was switched on, a drop of 89.91%.

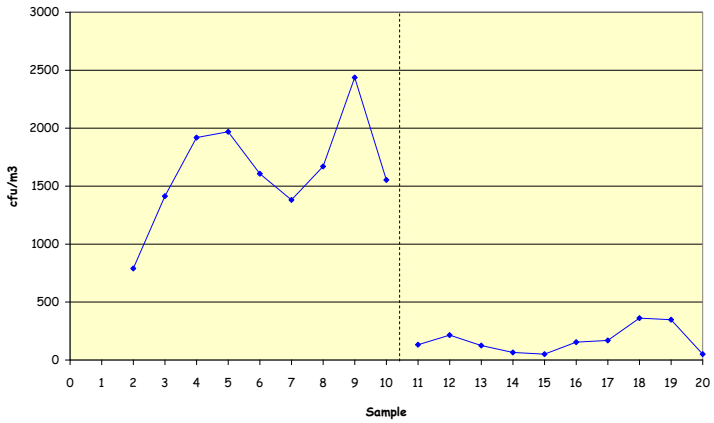

Figure 3 Effect of the device on the concentration of airborne *Clostridium difficile*

Figure 4 shows the effect of the device on the concentration of airborne *M. parafortuitum*. The concentration during the control period ranged from 4191 to 8873 cfu/m^3 with an average concentration of 5705 cfu/m^3 It can be seen that after the device was switched on there was a dramatic drop in the concentration of airborne *M. parafortuitum*. This was evident in the first four samples and was followed by a steady increase in the following six samples up to a level in excess of that measured during the control period. Taking the data from the first four samples the average concentration was 3016cfu/m^3 and this would represent a drop of 47%. However taking the ten replicates the average concentration with the device switched on was 6469cfu/m^3 which represents an increase in concentration of 13%. A t-test carried out on the control and test data sets showed the difference between the two data sets was not significant (P> 0.01)

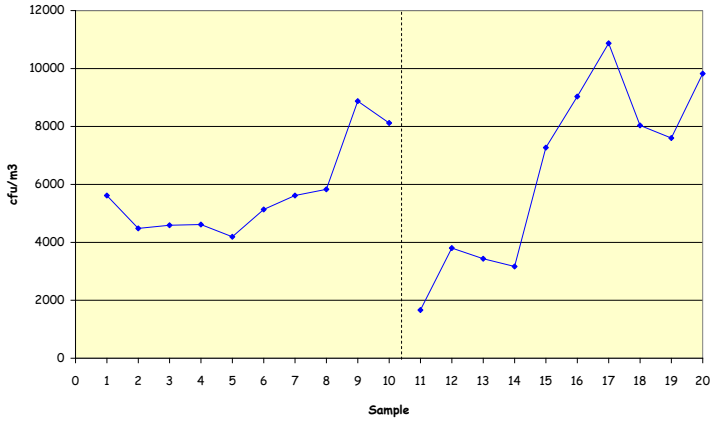

Figure 4 Effect of the device on the concentration of airborne *M. parafortuitum*

Figure 5 shows the concentration of airborne *M. parafortuitum* in the test chamber during this test and includes data obtained previously for *M. parafortuitum* that has not been homogenized prior to use. It can be seen that in this study there is a drop in the concentration of airborne M. parafortuitum which is maintained throughout the 10 replicate samples. With the non-homogenised culture the initial drop in the first four samples and the concentration then increased in the following six samples up to a level in excess of that measured during the control period.

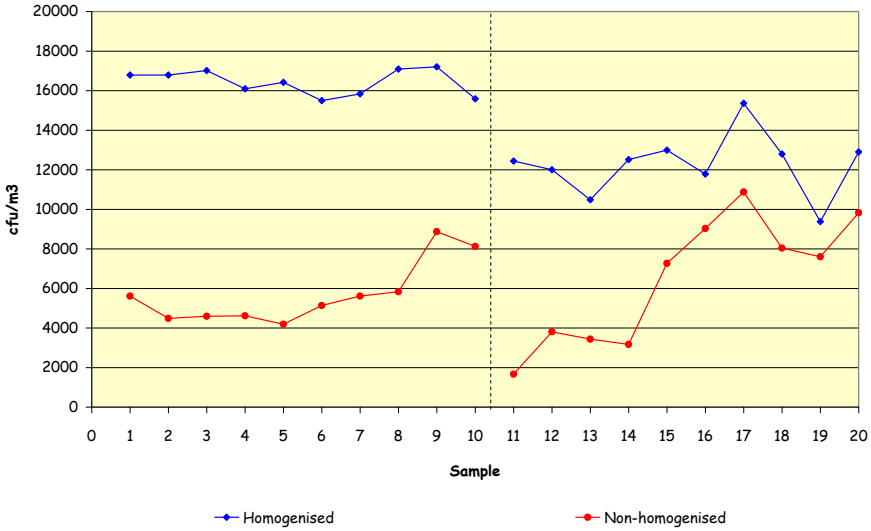

Figure 5 Effect of the device on the concentration of airborne *M. parafortuitum*

The concentration during the control period ranged from 15496 to 17203 cfu/m^3 with an average concentration of 16429 cfu/m^3. When the device was in operation the concentration ranged from 9378 to 15360 cfu/m^3 with an average concentration of 12263 cfu/m^3 which represents a kill of 25.4%. A t-test carried out on the control and test data sets showed the difference between the two data sets to be highly significant ($P< 0.01$)

Discussion

It was evident from the results that the performance of the device was extremely species specific with variable success dependent upon the bioaerosol used. The device was very effective at reducing the concentration of airborne *Staphylococcus aureus* (97%) and *Clostridium difficile* (89%), which are at present the two most important nosocomial pathogens particularly within the UK.

The initial test carried out with aerosols of *Mycobacterium parafortuitum* proved rather disappointing with an overall increase in the average concentration of the bacterium after the device was switched on. One of the reasons put forward to explain this result is the fact that when grown in liquid culture *M. parafortuitum* has a tendency to grow in clumps rather than as individual bacteria. When aerosolised these clumps together with any individual bacteria will be dispersed into the air and will be exposed to the disinfection properties of the device. When the device is activated any individual bacteria in the air will be killed and this would account for the apparent decrease in the concentration seen in the first few samples. However over time the action of the device upon the clumps of bacteria may result in the clump initially breaking up leading to an apparent increase in the concentration of bacteria as was seen in the later samples.

Homogenisation of the Mycobacterium parafortuitum culture prior to nebulisation appears to have had a significant impact on the ability of the INOV8 device to reduce the airborne concentration. This may provide some evidence in support of the hypothesis that due to the fact that M. parafortuitum grows in clumps the initial effect of a disinfection device is to break up the clumps which may in turn lead to an apparent increase in the airborne concentration.

Having homogenised the culture prior to use in this case we can be confident that the bacteria being introduced into the test chamber were more likely to be individuals. If this is the case then one would expect to see a decrease in the airborne concentration as a result of the disinfection capabilities of the device and this is exactly what happened.

Conclusions

The overall conclusions from the experiments carried out can be summarized as follows:

- The device is capable of significantly reducing the concentration of airborne *Staphylococcus aureus* and C*lostridium difficile*.
- Homogenisation of the *Mycobacterium parafortuitum* culture had a significant impact on the disinfection capacity of the INOV8 device.
- Under test conditions the INOV8 device achieved a reduction in the concentration of airborne *Mycobacterium parafortuiitum*.