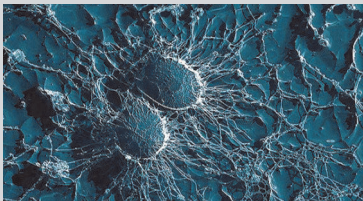


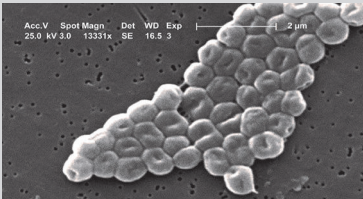
Inov8 Air Disinfection Combats Antibiotic Resistant Bacteria

Inov8 Air Disinfection, Effective Against:

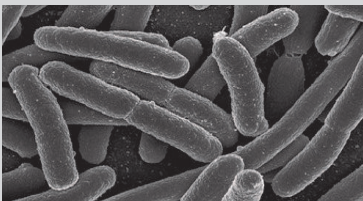
Staphylococcus aureus (i.e MRSA).



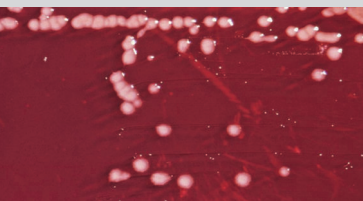
Acinetobacter spp.



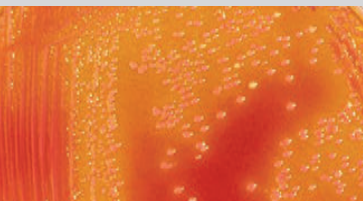
Escherichia coli



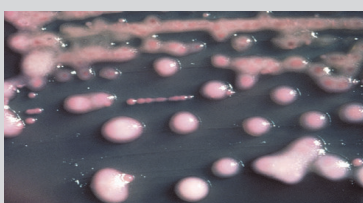
Pseudomonas spp.



Serratia spp.



Klebsiella spp.



Hydroxyl Radicals Provide a Solution to Increasing Antibiotic Resistant Microorganisms

Pathogenic organisms that do not form part of the commensal flora have numerous strategies that enable them to move from a source or reservoir to a host in a viable state. For example, *Gram positive* organisms such as *Staphylococcus aureus* are very well adapted to surviving on skin surfaces and this is an important route of transmission within hospitals.

Gram negative organisms tend to survive in fluids such as contaminated water and are usually responsible for infections such as *Vibrio cholera*. The spores produced by some fungi and bacteria such as *Mycobacterium tuberculosis* exhibit a resistance to the effect of drying in the environment. Bacteria such as *Klebsiella* can pose a major risk of infection in hospitals due to contamination of fluids used in medicine. Other bacteria, such as *Salmonella* and *Shigella*, thrive under conditions of poor hygiene and are transmitted by faecal-oral spread.

In addition to these survival strategies, some of these microorganisms have also developed antimicrobial resistance, which is the ability to resist the action of antimicrobial drugs. This feature can make the treatment of infections more difficult and increase hospital costs. Disease-causing microorganisms that have become resistant to antibiotic drug therapy are an increasing public health problem. Wound infections, gonorrhoea, tuberculosis, pneumonia, septicemia and childhood ear infections are just a few of the diseases that have become hard to treat with antibiotics. One part of the problem is that bacteria and other microbes that cause infections are remarkably resilient and have developed several ways to resist antibiotics and other antimicrobial drugs.

Example of antibiotic resistant bacteria include *Staphylococcus aureus* (i.e, MRSA), *Escherichia coli*, *Klebsiella* spp., *Serratia* spp., *Pseudomonas* spp., *Acinetobacter* spp. The resistance often arises as a result of changes in the microorganism's genes. In some cases, the genes causing resistance can be transferred between different strains of microorganism, and when this happens the recipient organisms will also become resistant.

Some of the mechanisms of resistance to antibiotics, shown in Figure 1, include reduced uptake into cells (Chloramphenicol), active efflux from the cell (Tetracycline), reduced binding of antibiotic to cell target (β -lactams, Erythromycin, Lincomycin), enzymatic cleavage or modification to inactivate antibiotic molecule (β -lactams, Aminoglycosides, Chloramphenicol), metabolic bypass of inhibited reaction (Sulfonamides, Trimethoprim), overproduction of antibiotic target or titration (Sulfonamides, Trimethoprim).

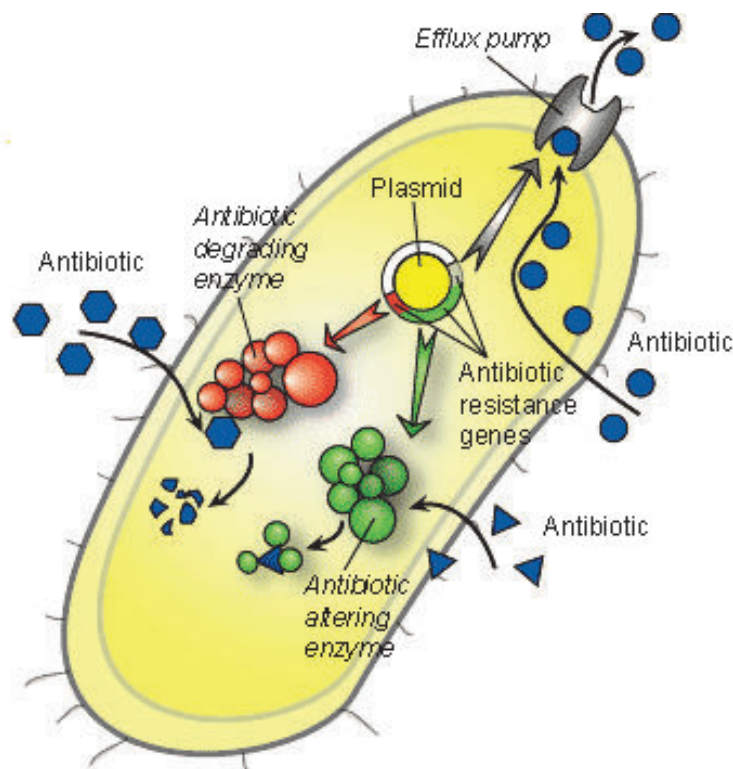


Figure 1. Mechanisms of resistance to antibiotics.

Hydroxyl radical reactions are considered very important in the pathogenesis of many diseases. Inov8 AD air disinfection technology generates hydroxyl radicals at levels that are comparable to those found in outdoor air. The reactions between these hydroxyl radicals and bacteria are non-strain specific, therefore resistant and non-resistant bacterial strains will be equally susceptible to hydroxyl radicals attack.

The attack process essentially leads to a cascade of reactions that may induce damage to nucleic acids, structural and functional changes in proteins, as well as oxidation of lipids. For example, lipids in a bacterial cell wall react with hydroxyl radicals by losing hydrogen and forming a lipid radical. Oxidation of proteins (Garrison WM (1987), Singh J & Thornton J M (1992)) are far more complicated where a protein radical will lead many possible reactions, one of which resulting in a non-protein radical that can be a further hydroxyl radical. In a microbial cell, due to interactions between proteins and lipids, the oxidative reactions can transfer from lipids to proteins and so on, causing destruction to main cellular structures which will render the bacteria non viable.



1. Laboratory Results

Inov8 has conducted extensive experimental validation against antibiotic resistant microorganisms. The tests were performed at two important laboratories in the UK, the HPA and Leeds University. The objectives of these studies were to evaluate the performance of the Inov8 device in terms of its ability to reduce the concentration of viable airborne and surface microorganisms.

1.1 Reduction of Continuously Introduced Microorganisms (Steady State)

A 32.25m³ hermetically sealed negatively pressurized room in which the air flow rate, temperature and relative humidity can be constantly controlled and monitored was used. 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%). A total of ten samples were taken at 3 minute intervals during which time the device was switched off. The device was then switched on 2 hours and a further ten samples were then taken at 3 minute intervals. There was no significant change in negative ion concentrations between control period and when the device was switched on (1000 ions/cm³ average). (More details on the experimental methodology is available on request)

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/m³ with an average concentration of 12158 cfu/m³. When the device was in operation the concentration ranged from zero to 654 cfu/m³ with an average concentration of only 348 cfu/m³ which represents a kill of 97.1%. A t-test carried out on the control and test data sets showed the difference between the two data sets to be highly significant ($P < 0.01$).

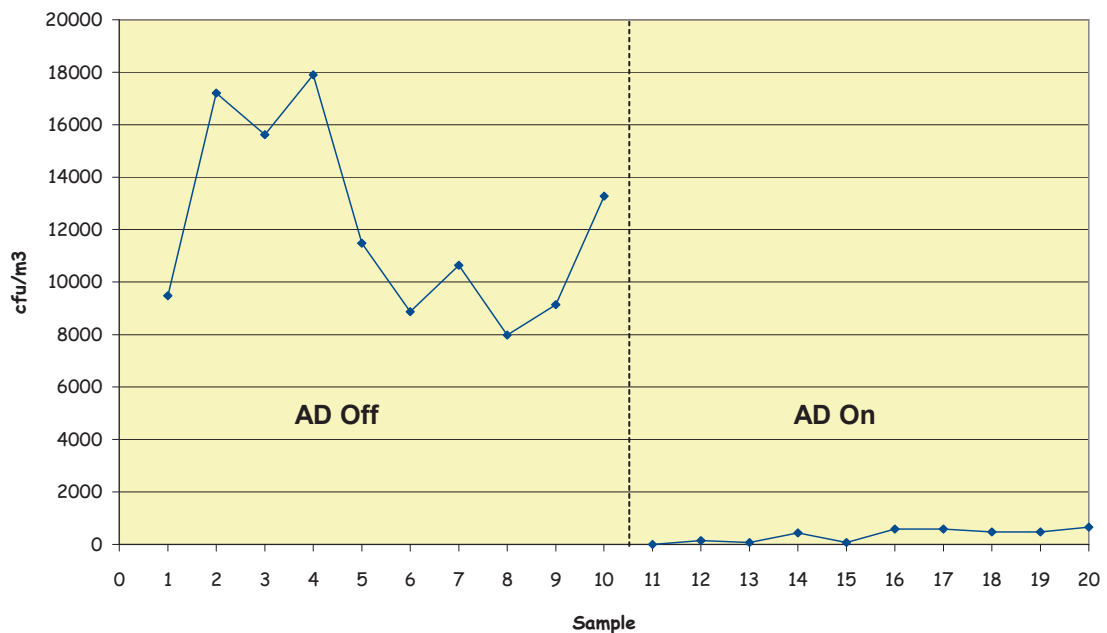


Figure 2. Effect of the device on the concentration of airborne *S. aureus*.



Figure 3 shows the concentration of airborne *P. aeruginosa* and it is clear that there is a drop in the concentration of airborne *P. aeruginosa* when the device was switched on. The concentration during the control period ranged from 8435 to 11128 cfu/m³ with an average concentration of 9773 cfu/m³. When the device was in operation the concentration ranged from 4314 to 8771 cfu/m³ with an average concentration of 6312 cfu/m³ which represents a kill of 35.4%. It can be seen that the first four samples taken with the device in operation showed a dramatic decrease in concentration and that in the subsequent six samples the concentrations steadily increased. A t-test carried out on the control and test data sets showed the difference between the two data sets to be highly significant ($P < 0.01$).

This may provide some evidence in support of the hypothesis that due to the fact that *P. aeruginosa* 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.

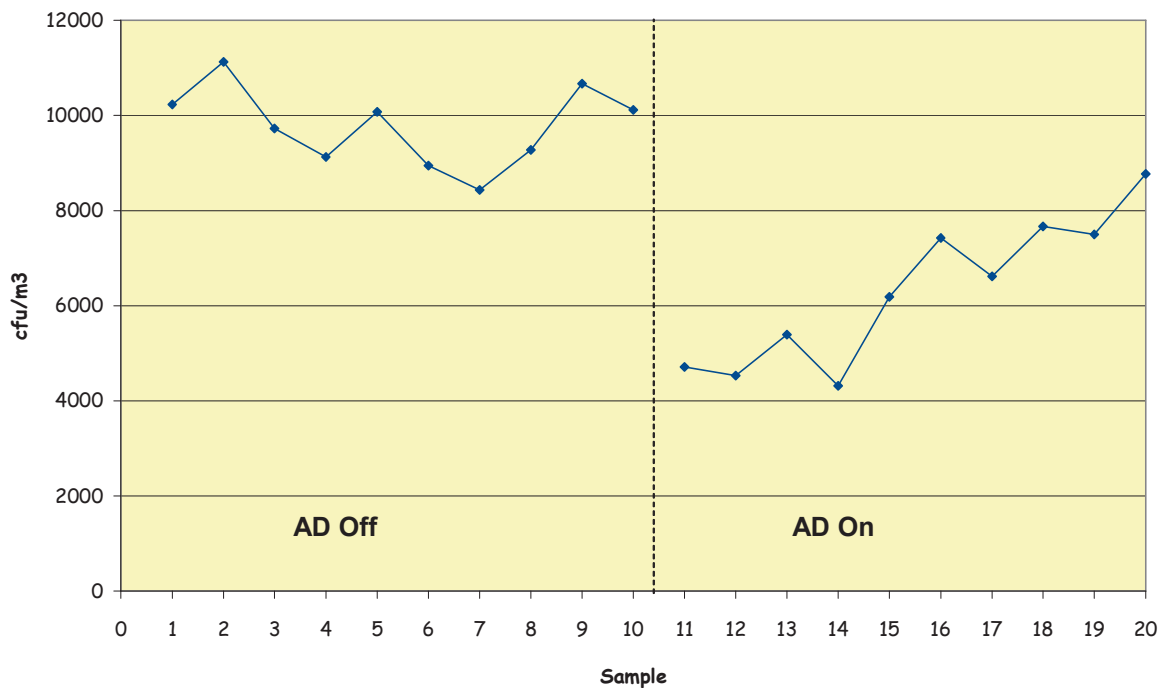


Figure 3. Effect of the device on the concentration of airborne *P. aeruginosa*



Figure 4 shows the effect of the device on the concentration of airborne *S. marcescens*. The concentration during the control period ranged from 6521 to 8989 cfu/m³ with an average concentration of 7784 cfu/m³. It can be seen that after the device was switched on there was a relatively large drop in the concentration of airborne *S. marcescens*. The concentration with the device operating ranged from 3135 to 6189 cfu/m³ with an average of 4222 cfu/m³. This represents a reduction in the concentration of 45.8% and a t-test carried out on the control and test data sets showed the difference between the two data sets was highly significant ($P < 0.01$).

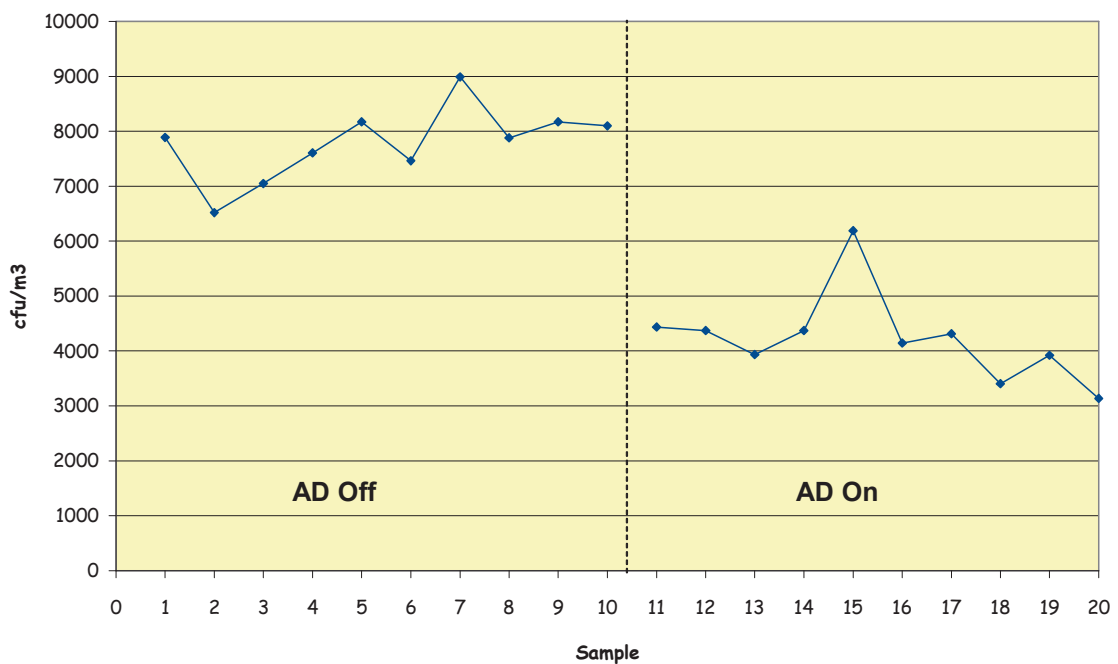


Figure 4. Effect of the device on the concentration of airborne *S. marcescens*



Figure 5 shows the result from the final test in which the device was used against aerosols of *B. cepacia*. It can be seen that there was a drop in the concentration of the bioaerosol when the device was switched on. The concentration during the control period ranged from 8039 to 10407 cfu/m³ with an average of 9182 cfu/m³. When the device was in operation the concentration dropped to an average of 5213 cfu/m³ (range 4064-6007 cfu/m³ representing a drop of 43.2%.

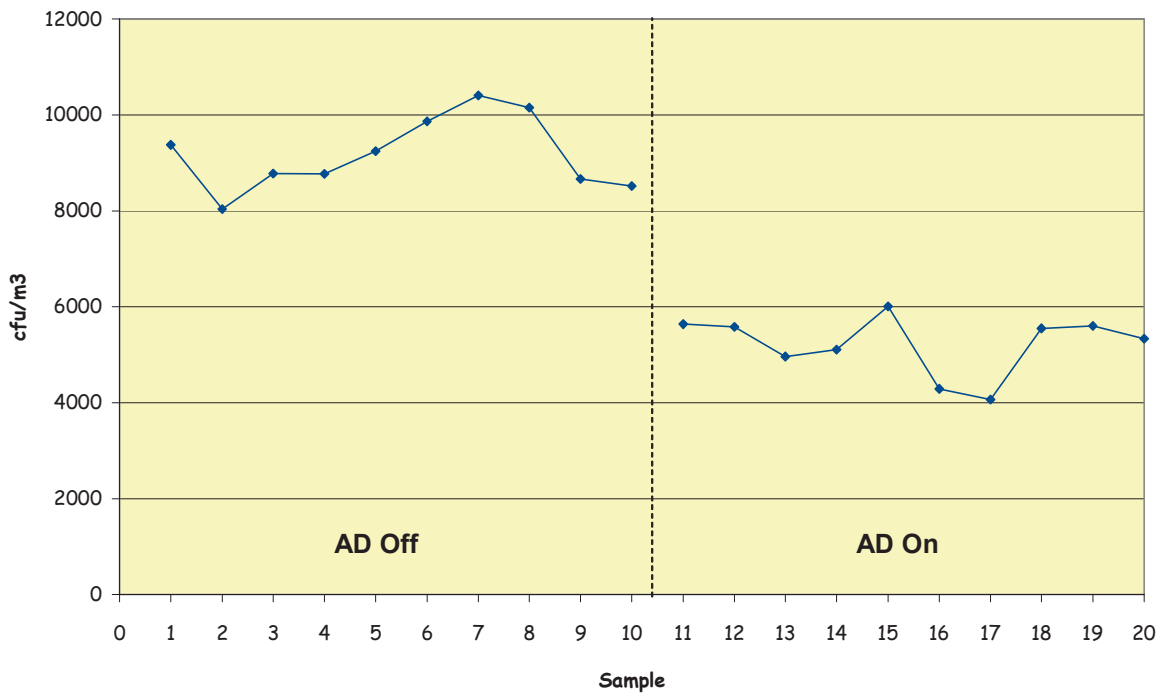


Figure 5 Effect of the device on the concentration of airborne *B. cepacia*.

1.2 Reduction of Aerosolised E-coli.

In this test, conducted at the HPA laboratories, the effectiveness of the Inov8 AD device was measured against aerosols of *Escherichia coli* ATCC 8739 a Gram negative rod, usually 0.5 to 3.0 microns diameter. The aerosol concentration of the test micro-organism in an environmental chamber was measured with the device operating and with the device switched off. Testing was carried out in triplicate. The device was shown to be able to significantly reduce the concentration of *E-coli* by as much as five logs with excellent repeatability.

A 3m x 3m x 2m environmental room was used for the studies. *Escherichia coli* was prepared by inoculating two flasks containing 50 ml each of Tryptone Soya Broth. 10-20mls of the microbial suspension was added to a 3-jet Collision nebulizer. A fan was situated below the nebuliser. In each experiment the Collision was operated to nebulise its contents at a pressure of 180 KPa for two minutes with the fan running during the same time period. The microbial aerosol was sampled using 6 all glass impingers, operating at 30l/min and containing 20mls of PBMA (phosphate buffer with manucol and antifoam). Each sampler was programmed to sample for two minutes at the times stated in the test design. In all three *E. coli* experiments with the black lid device, a four log reduction in the microbial concentration was obtained within 20 minutes of aerosolisation. The *E. coli* results showed very good reproducibility.

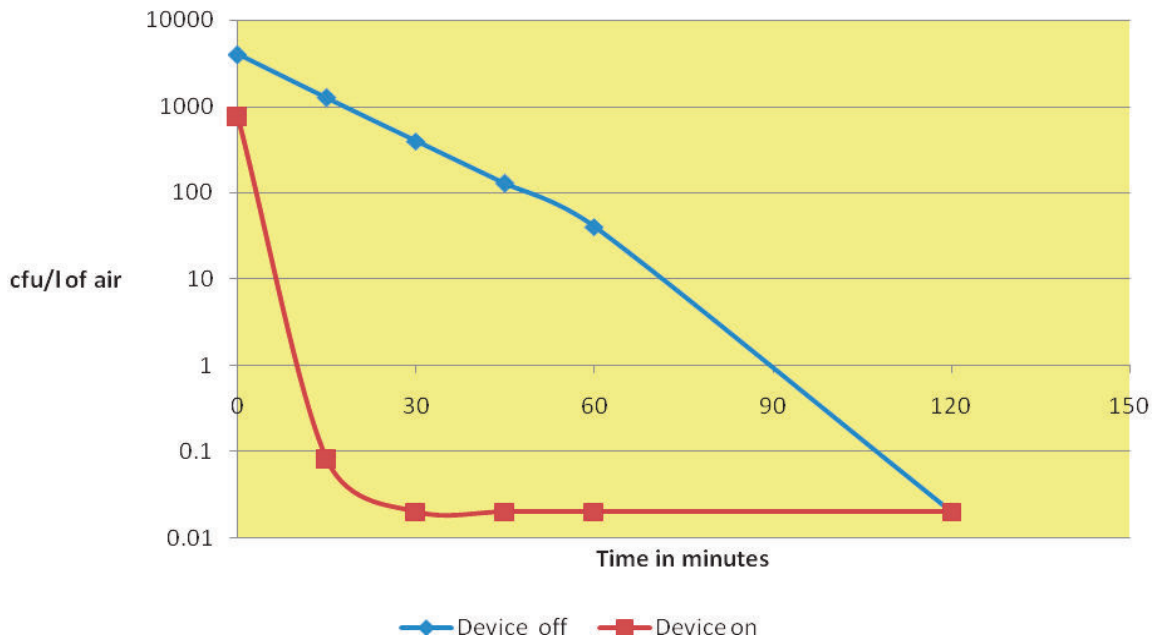


Figure 6 Reduction of E-Coli challenge concentration (Colony forming Units/litre of air) vs time in minutes.

1.3 Reduction of Surface Microbial Contamination

In this study, the aim was to reduce the surface contamination levels of *Staphylococcus aureus*, *Burkholderia cepacia*, *Mycobacterium parafortuitum* and *Acinetobacter baumannii* on cotton cloths. The experimental methodology was carried out as follows:

A total of twenty four (24) identical cloths were sterilized by autoclaving at 121°C for 15 minutes. After autoclaving the cloths were inoculated with 0.1ml of concentrated culture, six for each species. The cloths were then be hung up and allowed to dry.

Three of each set of six remained in the laboratory as controls and the others were placed into the test chamber and exposed to the Inov8 device. After the tests were completed each of the cloths were placed into a sterile stomacher bag along with 100ml of sterile Ringer's solution and stomached for 2 minutes. After stomaching 0.1ml of the suspension was plated out onto a sterile tryptone soya agar plate and incubated for 24 hours at 37°C. The number of colonies were then counted and multiplied up to give a total number of recovered colony forming units (cfu's). The mean recovered counts were used to calculate the reduction after exposure as a percentage reduction.

Figure 7 shows the concentration of recoverable microorganisms from the series of inoculated cotton cloths before and after exposure to the Inov8 device. It can be seen that there is a great deal of variation in the level of reduction from one species to another. For example the highest reduction was obtained with *Acinetobacter baumannii* from an initial concentration of 433 cfu/cloth down to 9 cfu/cloth, a reduction of 97.9% (p<0.01) The least effective was against *Burkholderia cepacia* with a reduction from 411 cfu/cloth to 297 cfu/cloth a reduction of only 27.9% (p>0.01) The reductions for *Staphylococcus aureus* and *Mycobacterium parafortuitum* were 65.4% (p<0.01) and 48.4% (p<0.01) respectively.

Overall the device achieved a statistically significant reduction in contamination levels *A. baumannii*, *S. aureus* and a smaller yet significant reduction for *M. parafortuitum*. The device was less effective against surface *B. cepacia*. It is concluded that the surface disinfection performance of the device is species dependent.

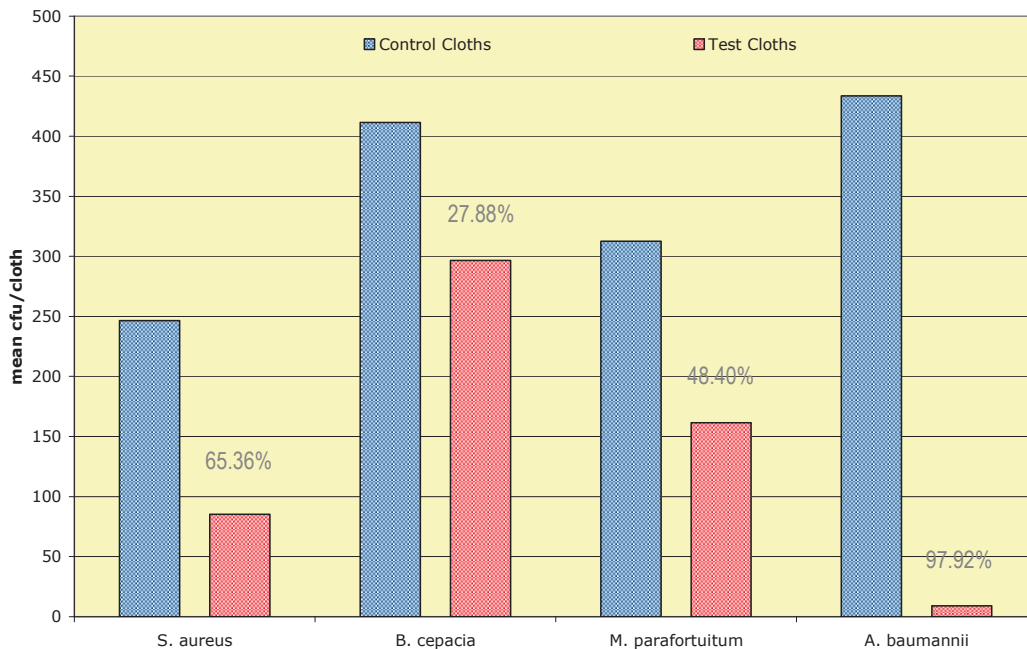


Figure 7. Effect of the device on the level of surface contamination of cotton cloths.



***Air Disinfection Solutions:
Effective Against
Antibiotic Resistant Microorganisms***

References

Pollack A., "Rising Threat of Infections Unfazed by Antibiotics", 2010, New York Times (26th February 2010).

Garrison W. M., "Reaction Mechanisms in the Radiolysis of Peptides, Polypeptides, and Proteins", 1987, Chem. Rev. 87:381-398 -9920

Singh J. & Thornton J. M., "Atlas of Protein Side-Chain Interactions", 1992, Vols. I & II, IRL press, Oxford.



Inov8 AD Applications in Healthcare



Inov8 AD units in ambulances.

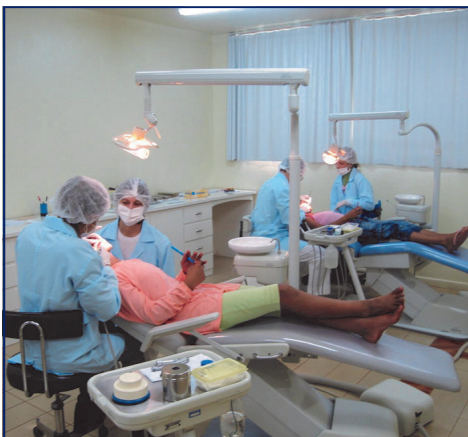
“Environmental colony counts were significantly higher in rooms with patients in them. The Inov8 (AD) system significantly reduced environmental colony counts in occupied and unoccupied rooms. Introduction of the Inov8 (AD) system into an occupied room consequently significantly reduced colony count.”

“In the absence of any opportunity to prevent Norovirus outbreaks in hospitals, the AD units have become the only means by which we can minimise the threat.”

*Gill Hill,
Matron in Infection Prevention and Control,
Hereford Hospitals NHS Trust 2009.*



Inov8 AD units in hospital theatres.



Inov8 AD units in dental clinics.



Features



New Technology

Attacks viruses, bacteria and fungi

Effective against Norovirus, S. Aureus, C. Diff, H1N1 and flu variants

99.999 % effective against MS2 coliphage

Compact (42cm high), quiet and easy to use

Low power consumption

Anti-tamper features

WM - AD Air Disinfection

Inov8's new wall mounted Air Disinfection unit is a device designed for use in enclosed indoor spaces. The unit replicates a process that occurs in nature through the degradation of Ozone by naturally occurring olefins. A bi-product of this reaction is the Hydroxyl Radical, a reactive Oxygen Species which is highly unstable and reacts with bacteria, viruses and fungi by attacking most known species.

The AD has been laboratory tested against:

- The MS2 coliphage, a safe surrogate for Norovirus, H1N1 and flu viruses.
- Vegetative and spore forms of *Clostridium difficile*.
- *Staphylococcus aureus (MRSA)*, *Mycobacterium parafortuitum*, *Aspergillus fumigatus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus subtilis*, *Burkholderia cepacia*, *Staphylococcus epidermidis* and *Acinetobacter baumannii*.

The unit has also been evaluated extensively in several UK hospitals with exceptional disinfecting results and proven reduction in reported cross infection cases.

The AD produces rapid disinfection in enclosed spaces and is also very effective at removing odours. It does all this without the need for air circulation or filtration and is quiet, compact and easy to operate.

In contrast with some competitive products, it is not necessary to evacuate the room during sanitisation; the AD unit is designed to operate in busy lived-in environments.



**Air Disinfection Solutions:
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www.inov8.com**

Technical Specifications

Electrical supply (SELV) ¹	12V AC 1.5A @ 50/60 Hz (SELV) ¹
External Ozone level	≤ 0.2 ppm
Olefin consumption	1g per day (approx.) (or 1-2ml)
Weight	3.5 Kg (approx.)
Consumable bottle capacity	180 ml
Minimum life of consumable	90 days
Operating controls	Single Person
Casing grade	Extruded aluminium
External power adapter supply	230V AC +/- 10 % 50 Hz (EU) Class II - Double insulated 110V AC +/- 10 % 60 Hz (USA) Double Insulated
Dimensions	Height 480 (mm) x Diametre 145 (mm)
Positioning	Wall Mounted or Floor Standing
Treatment area	30 m ³ to 300 m ³
Approvals	CE (EU)
Operating noise level	38 db

⁽¹⁾ SELV: Safe Electrical Low Voltage

Product Configurator	Part Number
Wall Mounted UK Spec.	DH-WM-01-UK
Wall Mounted EU Spec.	DH-WM-01-EU
Cradle Accessory (Required for floor standing)	CR-O1

Sales Contact

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United Kingdom.**