ASSESSMENT OF UVD ROBOT AGAINST REDUCTION OF MULTI DRUG RESISTANT KLEBSIELLA PNEUMONIAE, ACINETOBACTER BAUMANII AND CLOSTRIDIUM DIFFICILE ON SURFACES.

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ASSESSMENT OF ORGANISM REDUCTION BY UVC

Summary of the project:
The UVD robotic device was placed in a room containing two boards to create shadowed areas. The robot moved in front, between and behind the boards covering all areas of shadowing. Coupons containing a preset number of organisms were placed in container in two chosen positions with differing levels of shadowing and exposed for different time periods. The numbers of organisms remaining after the test period were counted and compared to the control and log reduction calculated.

The UVD robot effected a greater than four log reduction for all organisms tested within three minutes. *Klebsiella pneumoniae* and *Acinetobacter baumanni* showed a greater than 6 and 7 log reduction (respectively) within three minutes and *C difficile* spores showed a 4 log reduction within 3 minutes.

1.0.Introduction

The electromagnetic spectrum is divided into seven regions ordered by decreasing wavelengths and increasing energy and frequency. These are radio waves, microwaves, infrared (IR) visible light, ultra violet (UV), X rays and gamma rays. Ultra violet light falls in the range between visible light and X rays. It can be subdivided into three sub-bands namely UVA (315nm-400nm), UVB (280-315nm) and UVC (180-280nm).

UV radiation (photons) has sufficient energies to break chemical bonds so can be useful in chemical processing but it can also cause severe damage to materials and cellular tissues.

Most natural UV energy comes from the sun with 10% of sunlight being attributed to UV. 95% is UVA and 5% UVB. There is no measurable UVC energies from solar radiation on the earths' surface because ozone, oxygen and water vapour absorbs it.

UVC can be produced artificially using lamps (usually vaporised mercury or other gas) that emit this radiation at a particular wavelength (254nm). Cells absorb this UV radiation through photons which cause ionisation of cellular substances including DNA. UVC breaks molecular bonds within the DNA resulting in thymine dimers, which disrupts the structure and results in cell death for the microorganism.

UV has been used for many years within industrial, laboratory and medical settings as a surface disinfectant. This means UVC has a huge potential as a disinfection process, especially in the health care environment as it kills the microorganisms on surfaces and in the air through DNA disruption and it does this very rapidly. Using UVC as a final disinfection process post cleaning will reduce the risk of acquiring healthcare associated infections and hopefully reduce the need for antibiotics.

Globally there is a huge impending threat to society in the form of multiple antimicrobial resistant (AMR) microorganisms and a number of these organisms are listed on the WHO global priority list of antibiotic resistant bacteria (1). When these organisms are present in a healthcare setting on high touch areas such as chair arms, taps, hospital equipment etc there is a high infection control risk that they could be transmitted to a susceptible patient, either through touch or by airborne transmission. Although cleaning should reduce numbers of organisms to prevent transmission it is known that they often persist and current practice in many hospitals uses hydrogen peroxide vapour to eliminate persistent organisms (2). However, this method is expensive to use and can be time consuming. If UVC can reduce numbers of these organisms as efficiently as is claimed then every time a room, a hospital ward, an operating theatre is vacated then UVC should be used to help keep the environment as biologically clean as possible and to prevent risk of infection and reduce inappropriate overuse of antibiotics.

UVD robots have 8 UVC emitting lamps placed vertically on top of a robotic platform which moves either autonomously or can be controlled either by a phone or tablet from outside the area being disinfected. This can help reduce shadowing as the device can move to an area adjacent to the item creating the shadowing and in this way can reduce or eliminate shadowing which is a limitation of this disinfection method.

When the UVD Robot operates autonomously, the operator maps the room once. The robot remembers the map and uses the information in all subsequent disinfection processes. An unlimited number of hotspots can also be added in close proximity of high touch, high risk positions to the map. This information can all be retained for future process. The speed of the robot in disinfection mode is 10cm per second, the 360-degree coverage of the UVC lamps means there is always a minimum of 3 lamps radiating UVC on any given point on a surface. The ability of the UVC emitting device to move autonomously or remotely allows minimisation of shadowing (which is a known limitation of UV disinfection). The UVD robotic system can be controlled remotely from a tablet device, computer or smartphone from outside the room.

Typically, a UVD robot will take approximately 10-15 minutes to disinfect a single occupancy patient room. Following disinfection, the robot will return to its' station until it is called upon again.

Details of the UVC emitter device.

The UVD-robotic whole-room disinfection system uses one tower containing 8 UV-continuous emitting lamps (254nm) to access a 360° field of irradiation placed on a movable robot (UVDR Model B manufactured in Denmark by UVD Robots ApS).

Methods

2.1 Organisms used in the study

Klebsiella pneumoniae beta lactamase producer NCTC 13443

Acinetobacter baumanii (NCTC 12156)

Spores of *Clostridium difficile* NCTC 11209.

2.2 Preparation of inoculum (with light soil ~ 0.3g/l bovine serum albumin [BSA]), for all organisms

Inocula were prepared in-Maximum Recovery Diluent (MRD) (Lab M Ltd, LAB103) using log phase cultures of *Klebsiella pneumoniae/ Acinetobacter baumanii* (approximately 10^8 cfu/ml). As per EN 14561, 1ml of interfering substance (BSA - 3g/l)) and 9ml of test organism was mixed to ensure even dispersion.

C. difficile was prepared using a frozen spore suspension (approximately 10^6 cfu/ml) and prepared as above using EN14561.

Accurate viable counts were undertaken using a standard dilution method. The original inoculum for each organism was:

Klebsiella pneumoniae = 1.73×10^7 cfu/ml
Acinetobacter baumannii = 2.58×10^8 cfu/ml
C. difficile= 5.77×10^6 cfu/ml

2.3 Preparation of the coupons

Stainless steel coupons (of the type and grade specified in BS EN 13697 – namely 1.5mm depth /20mm diameter stainless steel, manufacturer code 304 2B) were inoculated with 50µl of the inoculum and dried for approximately 40minutes at 36°C. The method was validated and showed no loss of viability of *C. difficile* over the time period tested and a 1-2 log reduction of activity of *K.* pneumoniae and *A. baumanii* during the test period. This was due to organisms dying on the coupons due to dessication. Therefore all log reductions were calculated against their own controls rather than to the original inoculum.

2.4 Effect of UVD robot on the viability of organisms.

Dried suspensions of each organism on stainless steel coupons (held at a 45 degree angle) were placed in two positions within a designated area of $32m^2$. Test coupons (exposed to UVC) (n=3) and unexposed control coupons (n=3) were placed at the same locations. The unexposed control was prepared by wrapping with 3 layers of aluminium foil (to shield from UV- irradiation) around the petri dish containing the coupons. There was a separate petri dish for each organism under test (with an identical un-exposed control).

The robot moved into position 1 and the coupons were exposed for the required contact time. The robot then moved into position 2 and the coupons were exposed for the required amount of time. The robot moved autonomously between positions without any human interaction. The mapping was carried out prior to the testing by a member of the UVD Robot installation team. Melbec Microbiology Ltd prepared and processed the discs. See figure 1A.

An indicator was placed in the same area as the test coupons for comparison to the log reduction of the microorganism under test. The indicators turned bright pink indicating the UVC robot was delivering over 1000 Joules per sq. metre within the first run at 3 minutes.

This was repeated on three separate occasions at 3minutes, 5minutes and 10minutes exposure. 15minutes exposure was also undertaken for *Clostridium difficile*

2.5 Processing the coupons

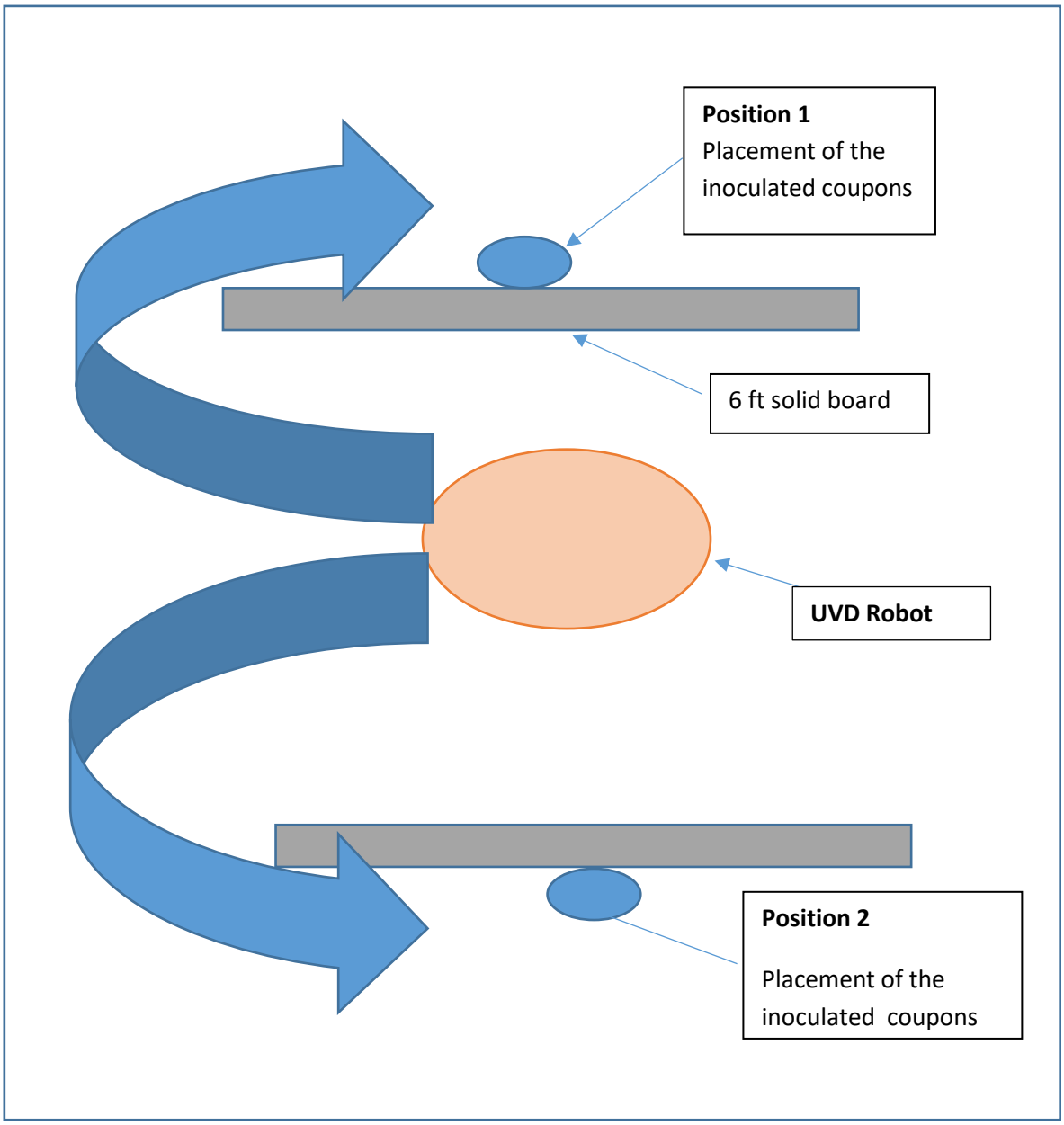
On completion of each UV-disinfection cycle, the coupons were processed within the laboratory. Each coupon was aseptically transferred to 2ml sterile Maximum Recovery Diluent (MRD) in a sterile bottle containing sterile glass beads. The MRD was vortexed mixed at full power for 30 seconds.

1ml was transferred to 9ml MRD, mixed and a ten-fold dilution series undertaken to determine an accurate viable count. 1ml of each dilution was added to 12ml molten Tryptone Soy Agar (Lab M Ltd, LAB011) as a pour plate and allowed to set. All plates were transferred to the relevant incubation conditions (37°C in air for 48hrs for *K. pneumoniae* and *Acinetobacter baumanii,* and 37°C in anaerobic conditions for 48hrs for the *C. difficile).*

Following 48hrs incubation, all colonies were counted and recorded.

All testing was done in triplicate and the log reductions were calculated by comparison of the test mean log viable count and the control mean log viable count.

Figure 1A Diagram of the room used



3.0 Results

3.1 Antimicrobial effect of UVC

There was a log reduction of organism numbers observed for all bacteria tested. There was high log reduction (> log 6 - > log7) for the two Gram negative organisms, *K. pneumoniae* and *A. baumannii)* (Table 2) at 3 minutes.

The *C difficile* was tested at 5 minutes and this showed a log >4 reduction.

Unfortunately, there was a loss of organism numbers through natural desiccation which reduced the limit of detection at 5minutes and 10 minutes for the Gram negative bacteria. This was not observed with *C. difficile* because it is a spore and survives desiccation. Where the log reduction was reported as > (greater than) this is the limit of detection for that organism.

Table 2; Log reduction in all organisms in two positions at different time intervals.

	3 minutes Log reduction	**5 minutes Log reduction**	**10 minutes Log reduction**	**15 minutes Log reduction**
Klebsiella pneumoniae				
Position1	>6.24*	>4.3*	>4.76*	N/T
Position2	5.81	>4.3*	>4.76*	N/T
Acinetobacter baumanii				
Position 1	7.11	>5.96*	>6.3*	N/T
Position2	>7.41*	>5.74*	>6.3*	N/T
Clostridium difficile				
Position 1	N/T	4.1	4.27	4.78
Position2	N/T	4.05	4.42	3.52

Key: * limit of detection, N/T: not tested

Discussion

The log reductions seen with the three different organisms tested did vary depending upon the organism type. In the presence of low soil, the log reduction was greater at 3 minutes exposure in *A. baumanii* & *K. pneumoniae* showing a mean log reduction of log 7.26 and >log 6. There was a 4 log reduction in the number of viable *C difficile* spores at 5 minutes increasing slightly at 15 minutes.

Unfortunately, due to the effect of desiccation, in *K. pneumoniae* and *A. baumanii* the log reduction seen at 5 minutes and 10 minutes appeared less than at 3 minutes. This meant that the starting numbers of organisms on the control coupons were less than at 3 minutes, therefore giving the appearance of a smaller log reduction at 5 and 10 mins. This was not the case, in real terms, the limit of detection was smaller at 5 and 10mins than at 3 mins. In future experiments, the coupons should be prepared just prior to the actual operation of the UVD robot rather than preparing them all in advance. Although they were all used within a 5 hr period, there was still a 1-2 log reduction of numbers seen on the control coupons.

4.0 Conclusion

This was a snap shot study undertaken to determine whether an effective log reduction could be attained at a low time period. Three different bacteria strains were chosen: two Gram negative multi-antibiotic resistant strains, *K. pneumoniae* and *Acinetobacter baumanii* and a spore forming organism (*Clostridium difficile),* all known to be causing problems in the UK in health care settings.

All three bacteria were drastically reduced within a five minute exposure to UVC via the UVD robot. Shading was not an issue because the robot could move between positions and position itself so that sufficient UVC was emitted in these areas.

References

1. WHO Global list of antibiotic resistant bacteria- https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1
2. Literature Review and Practice Recommendations: Existing and emerging technologies used for decontamination of the healthcare environment. Airborne Hydrogen Peroxide. https://hpspubsrepo.blob.core.windows.net/hps-website/nss/1810/documents/1_hpv-lr-v1.1.pdf

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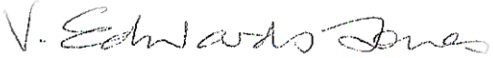
Signed V Edwards-Jones



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