ASSESSMENT OF ORGANISM REDUCTION AND DOSIMETER COLOUR CHANGE DUE TO UVC OUTPUT.

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ASSESSMENT OF ORGANISM REDUCTION BY UVC

1.0 Introduction

1.1 Details of the UVC emitter device.

Surfacide UV whole-room disinfection system uses three towers containing 3 UV-emitting (254nm) bulbs to access a 360° field of irradiation. The operation of the system requires the lamps to be placed in a "triangular" arrangement to cover all/as many surfaces. The towers have a built-in laser mapping system that scans the size of the room and calculates the duration of the disinfection.

The system may be set as "low", "medium" and "high", depending on the operator requirements for the level of disinfection desired. The towers are controlled remotely from a tablet device placed outside the room.

On day 1, the UVC emitter system was placed in a single occupancy room and the dosimeter colour change indicator/device tested against *Clostridium difficile* with no interfering substance (no soil) (Figure 1A). On day 2, the system was placed in one area of a four bedded bay as described in Figure 1B and privacy curtains were used to segregate the area. During UV-disinfection, all personnel vacated the room and a warning sign placed to alert public, patient and staff of the disinfection underway.

1.2 Assessment of usability and operation of the UV system

The time taken to complete UV disinfections of rooms was measured on each occasion. On all occasions for day 1 the system operated for 38minutes and on day 2 for 28mins.

The UVC emitted during the operation was estimated using a Surfacide® Helios™ UV-360 UV disinfection device.

1.3 Organisms used in the study

Klebsiella pneumoniae beta lactamase producer NCTC 13443

Staphylococcus aureus MRSA NCTC 13142

Spores of *Clostridium difficile* NCTC 11209.

1.4 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/ MRSA* (approximately 10^8 cfu/ml). This was mixed in equal volumes with BSA. This was vortex mixed to ensure even dispersion.

C. difficile was prepared using a frozen spore suspension (approximately 10^6 cfu/ml) and mixing in equal parts with BSA. This was vortex mixed to ensure even dispersion.

Accurate viable counts were undertaken using a standard dilution method.

The original inoculum for each organism was:

Klebsiella pneumoniae = 2.2 x 10^8 cfu/ml
MRSA = 1.73 x 10^8 cfu/ml
C. difficile= 3.8 x 10^6 cfu/ml

Note: the suspension used on day 1 for *C. difficile* on day one did not contain interfering substance.

1.5 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 appropriate BSA-inoculum and dried for approximately 40mins at 37^{o}C before being placed in a sterile petri dish. All plates and coupons were added to a moist chamber at room temperature and transported to the facility for testing with UVC. The method was validated and showed minimal loss of viability of the organisms under test.

1.6 Effect of UV-C (high power) on viability of organisms (day 1)

Spore suspensions on stainless steel coupons were placed in four positions within a single occupancy room. The area was 27. 9m^2. Test coupons (exposed to UVC) (n=2) and unexposed control coupons (n=2) 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).

A dosimeter indicator was placed in the same petri dish as the test coupons for comparison to the log reduction of the microorganism under test. A UVC radiometer was operated at the same time to determine the amount of UVC emitted over the exposure period.

The locations used were:

Position 1) table (full exposure) high touch.
Position 2 behind stand (shade) low touch.
Position 3) patient sink (partly shaded) high touch.
Position 4) equipment stand (full exposure) high touch.

The Surfacide® Helios™ UV-360 UV disinfection device was operated at high setting for **38 minutes** following scan of the room.

This was repeated on three separate occasions.

1.7 Affect of UV-C (high power) on viability of organisms (day2)

Spore and bacterial suspensions on stainless steel coupons were placed in four positions within a single area of a four bed bay. The area was 13. 5 m^2 and the area was cordoned off using privacy curtains. Test coupons (n=2) and unexposed control coupons (n=2) 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).

A dosimeter indicator was placed in the same petri dish for comparison to the log reduction of the microorganism under test. A UVC radiometer was operated at the same time to determine the amount of UVC emitted over the exposure period.

The locations used were:
Position 1) underneath the patient's mattress (semi- shadowed-area), low touch.
Position 2) patient sink (partly shaded) high touch.
Position 3) equipment stand (full exposure) high touch.
Position 4) Floor behind locker (shadowed area) low touch.

The Surfacide® Helios™ UV-360 UV disinfection device was operated at high setting for **28minutes** following scan of the room.

This was repeated on three separate occasions.

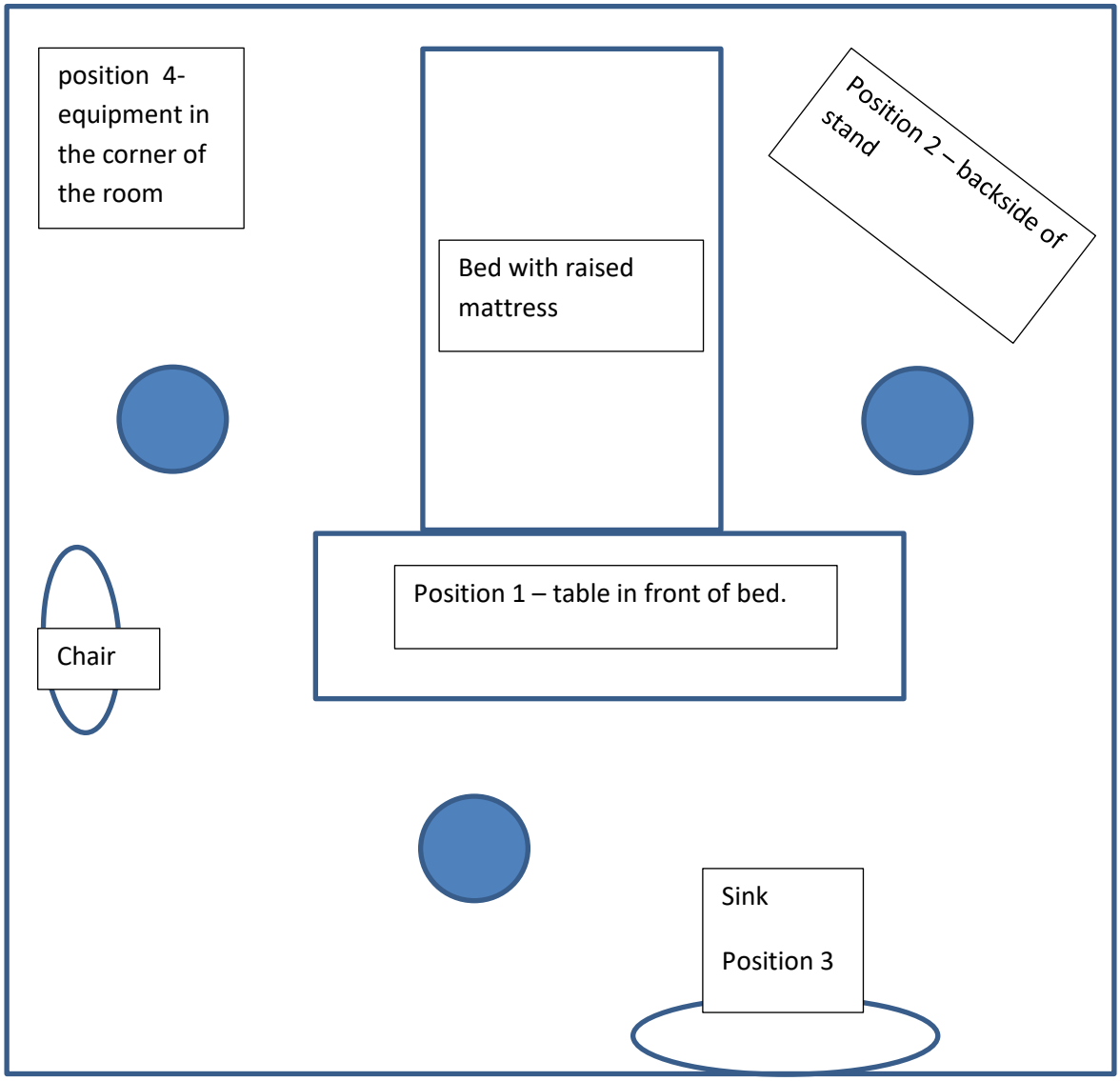
1.8 Processing the coupons:

On completion of each UV-disinfection cycle, the coupons were returned to the laboratory for processing and plating. The coupons were aseptically transferred to 2ml sterile MRD in a sterile bottle containing sterile glass beads. The MRD was vortexed at full power for 30 seconds.

One ml was transferred to 9ml MRD, mixed and a ten-fold dilution series undertaken to determine an accurate viable count. One ml 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^{o}C in air for 48hrs for *K. pneumoniae* and MRSA and 37^{o}C in anaerobic conditions for 48hrs for the *C. difficile).*

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

Figure 1A Diagram of the room used on Day 1



Key : blue circles (UV-C emitters)

Position 1 Table in front of bed (No shade)

Position 2 Behind stand (Shade)

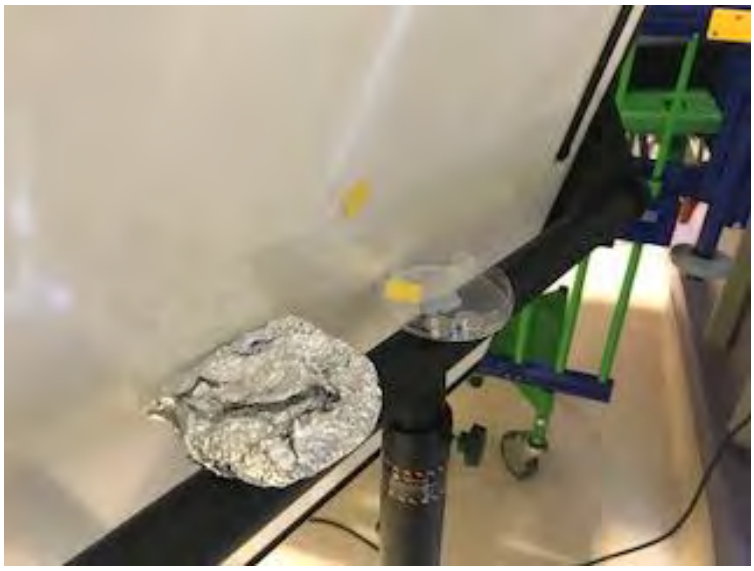
Position 3 Sink (Partly shade)

Position 4 Equipment in room Corner (No shade)

Photographs of Day 1

Photograph of the room

Position 1 –the table



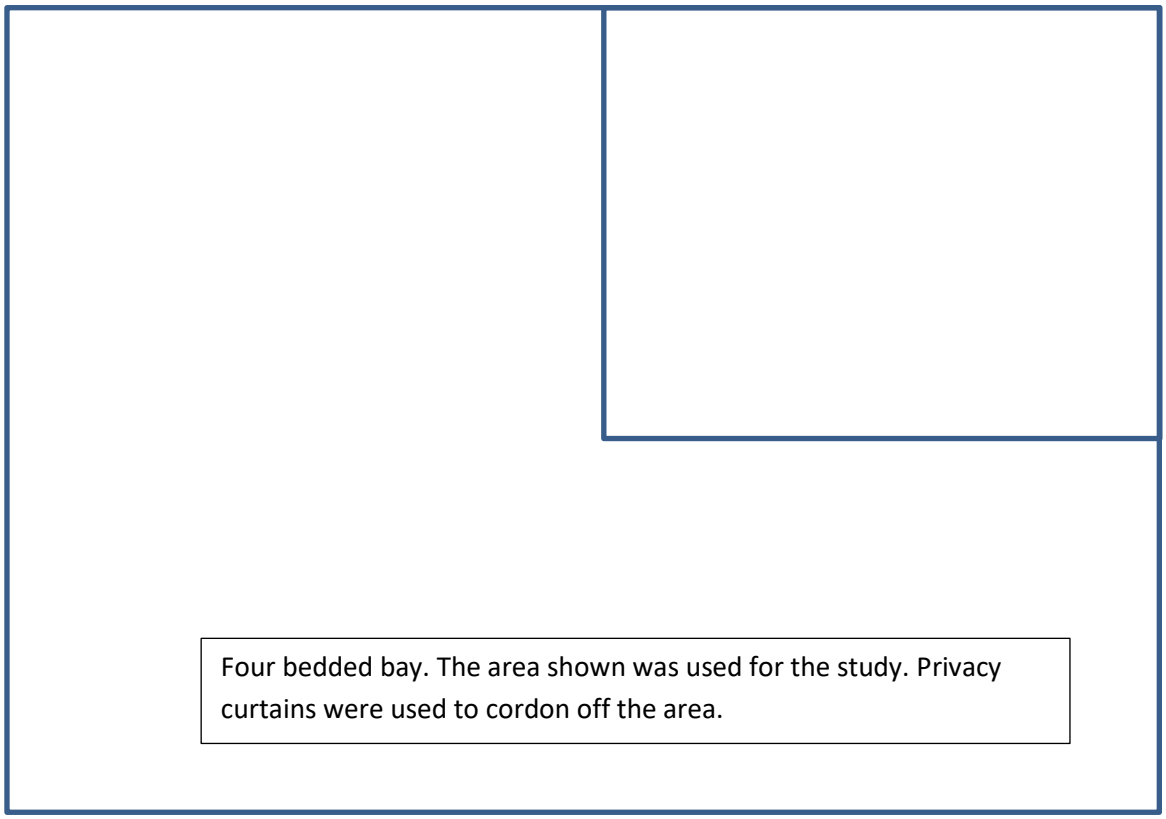
Position 2 – behind the stand

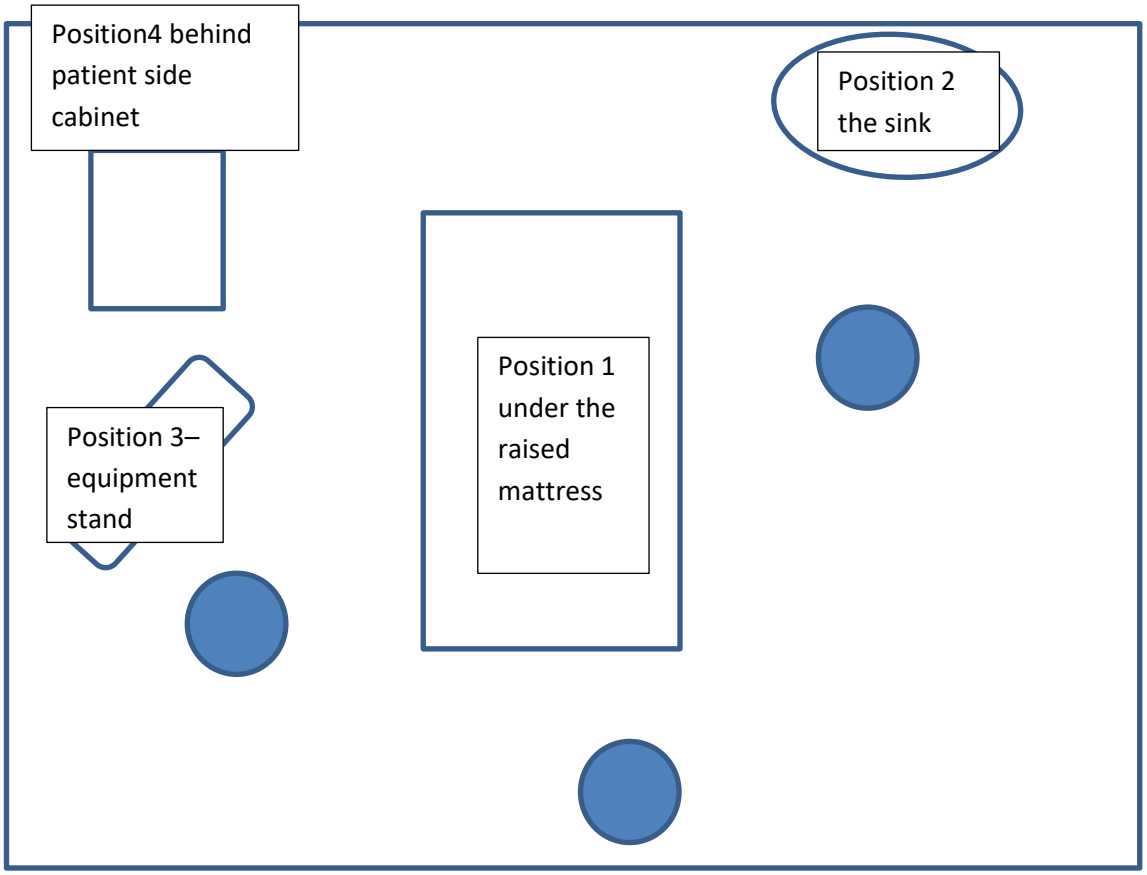
Position 3- the sink

Position 4 – the equipment in the corner

Room with the UVC system operating

Figure 1B Diagram of the room on Day 2





Key : blue circles (UV-C emitters)

Position 1 Under raised mattress (Full exposure)

Position 2 Sink (Partly shade)

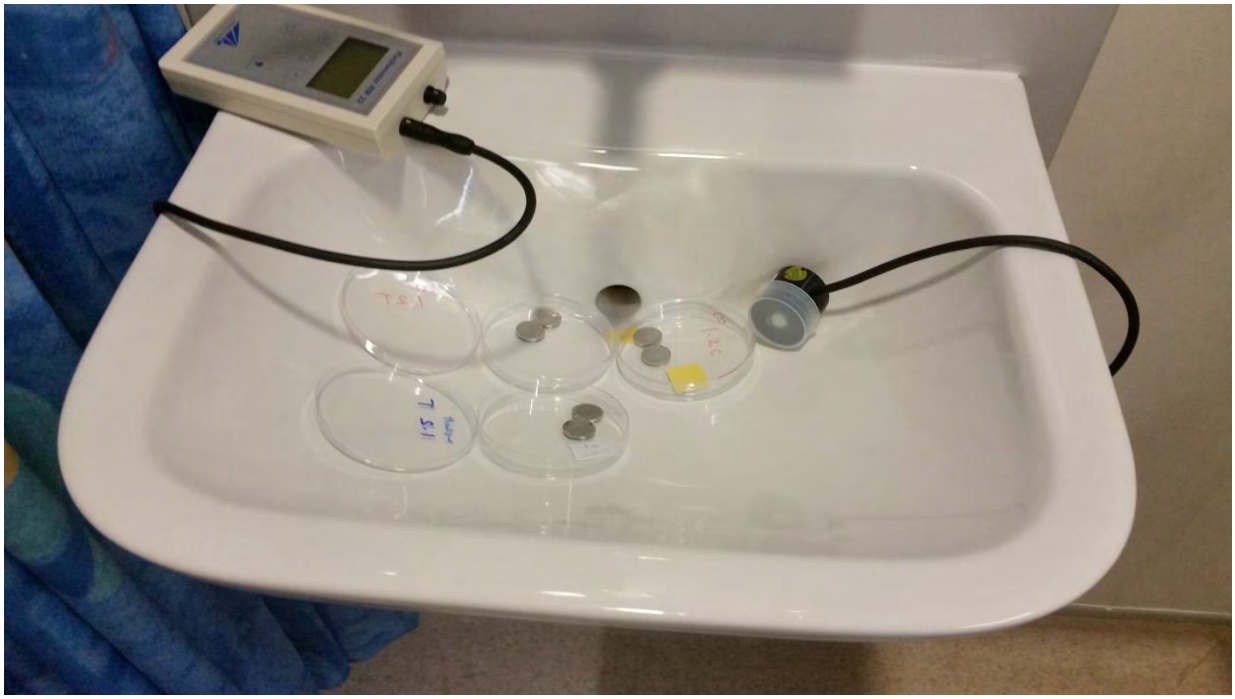
Position 3 Equipment stand (No shade)

Position 4 Behind locker in the corner of the room (Full shade)

Photographs of day 2

Photograph 1

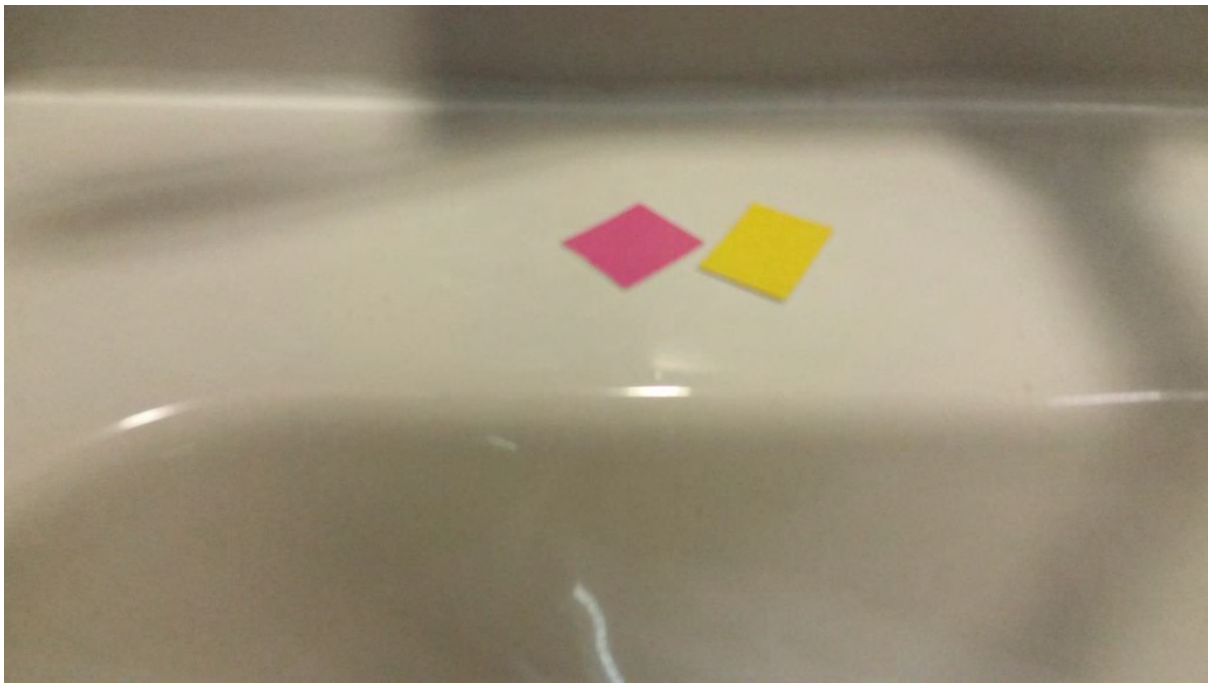
Position 1 underneath the raised mattress



Photograph 2 position 2 the sink

Photograph 3 position 3 frequent touch area

Photograph 4 position 4 floor



Photograph 5 Pre exposure (yellow) and post colour change (pink)

2.0 Results

2.1 Reproducibility of the technique

Using the control unexposed coupons; the reproducibility of the technique was assessed. The mean of eight replicates for each run was very similar and were not significantly different. This is shown below with the log of the mean count of the organisms recovered from the coupons in tables 1-3.

Table 1 Log mean count of *K. pneumoniae* (un-exposed control coupons)

	RUN 1 (n=8)	**RUN2 (n=8)**	**RUN3 (n=8)**	Overall mean (n=24)
Log mean count	4.91	4.29	4.22	4.47
SD	0.36	0.14	0.21	0.38

Table 2 Log mean count of MRSA (un-exposed control coupons)

	RUN 1 (n=8)	**RUN2 (n=8)**	**RUN3 (n=8)**	Overall mean (n=24)
Log mean count	6.09	5.53	5.72	5.78
SD	0.22	0.90	0.46	0.29

Table 3 Log mean count of *C. difficile* (un-exposed control coupons)

	RUN 1 (n=8)	**RUN2 (n=8)**	**RUN3 (n=8)**	Overall mean (n=16)
Log mean count	No data	4.23	4.68	4.46
SD	No data	0.17	0.18	0.32

The reproducibility was acceptable with all means for the different runs being within 1-2 SD's of the overall mean.

Of note:- There was no data available for the first run of the *C. difficile* controls because of jar failure. In all future calculations, the overall mean for *C. difficile* was used for comparison.

2.2 Antimicrobial effect of UVC

There was a log reduction of organism numbers observed for all bacteria tested. There was a higher log reduction (3.5- 4.0 log reduction) for the Gram positive organism (MRSA) (Table

5) with a 2.5-3.0 log reduction for the Gram negative organism (*K. pneumoniae)* (Table 4). *Clostridium difficile* was tested on two occasions, in different room sizes and one with light soil added and one with no soil added. There was a 1.5 – 2.0 log reduction when there was a light soil (Table 6) and between 0.5 -1.6 log reduction when there was no soil (Table 8). The slight difference in results could have been due to the size of the room and the amount of UVC emitted affecting the log reduction.

On both occasions there was a notable colour change in the dosimeter and this showed a similar trend to the log reduction in numbers observed.

Table 4; Log reduction in *Klebsiella pneumoniae* over three runs of the UVC emitter.

	RUN 1 Log reduction	RUN2 Log reduction	RUN3 Log reduction	Mean/SD Run1/2/3 (n=6)
Position1	4.34	2.40	0.28	2.34 (2.03)
Position2	4.19	4.13	0.82	3.05(1.93)
Position3	3.31	3.51	1.03	2.62(1.37)
Position4	1.94	0.62	1.03	1.19(0.67)

Table 5 Log reduction in MRSA over three runs of the UVC emitter

	RUN 1 Log reduction	RUN2 Log reduction	RUN3 Log reduction	Mean/SD Run1/2/3 (n=6)
Position1	4.38	4.57	1.68	3.54(1.62)
Position2	5.35	4.61	1.87	3.94(1.84)
Position3	4.79	3.56	2.10	3.48(1.35)
Position4	2.87	2.68	1.33	2.29(0.84)

Table 6 Log reduction in *C. difficile* over three runs of the UVC emitter.

	RUN 1 Log reduction	RUN2 Log reduction	RUN3 Log reduction	Mean/SD Run1/2/3 (n=6)
Position1	1.12	1.27	0.39	0.93(0.47)
Position2	1.79	1.44	1.87	1.70(0.23)
Position3	1.23	2.40	2.51	2.05(0.71)
Position4	No data	0.41	0.29	0.35(0.08)

2.3 Day 1 *C. difficile only (no inferring substances)*

The room was different than that of the room used on day 2 screening. This is shown in Figure 1A.

The mean organism counts for the control coupons not exposed to UVC are shown in Table 7.

Table 7 Log mean count of *C. difficile* (un-exposed control coupons)

	RUN 1 (n=8)	**RUN2 (n=8)**	**RUN3 (n=8)**	Overall mean (n=24)
Log mean count	4.14	4.51	4.49	4.50
SD	0.22	0.22	0.15	0.21

The reproducibility was acceptable with all log mean counts for the different runs being within 1-2 SD of the overall mean.

The log reduction for *C. difficile* spores on day 1 without soil are shown in the Table 8

Table 8 Log reduction in *C. difficile* over three runs of the UVC emitter.

	RUN 1 Log reduction	RUN2 Log reduction	RUN3 Log reduction	Mean /SD Run 1/2/3
Position1	2.13	1.33	1.34	1.60(0.50)
Position2	0.05	0.59	0.71	0.45(0.35)
Position3	1.88	1.02	1.16	1.35(0.46)
Position4	1.63	1.43	1.17	1.41 (0.24)

There was a similar log reduction for positions 1,3 and 4 (1.3-1.6 log). These were all in full exposure or partial shade. There was a 0.5 log reduction in the shade (behind the stand).

2.4 Comparison of mean log reduction with Dosimeter readings

The dosimeter colour change went from the base line yellow to pink and this could be detected by the naked eye. However, each of the colour change dosimeter indicators were read with a portable recorder, an X- rite RM 200QC and the readings were done within 20 mins after the UVC cycle had ended. The colour change was measured in LAB colour scale where the colour change value was presented in delta E. The higher the value the stronger the colour change. A deltaE value of 2 is generally said to be visible to the human eye. The

arbitrary readings are recorded in table 9 and compared with the log reduction of organism numbers. Where there was a higher log reduction the intensity of colour change from yellow to pink was higher. The trend can also be seen in Figure 2.1 and 2.2 where the log value of the dosimeter reading was compared to the log reduction of organism numbers. The raw data for the dosimeter readings are shown in the appendix.

Figure 2.1 Trend of log reduction in *C. difficile* (no interfering substance) numbers and log value of colour change in dosimeter indicator (day1).

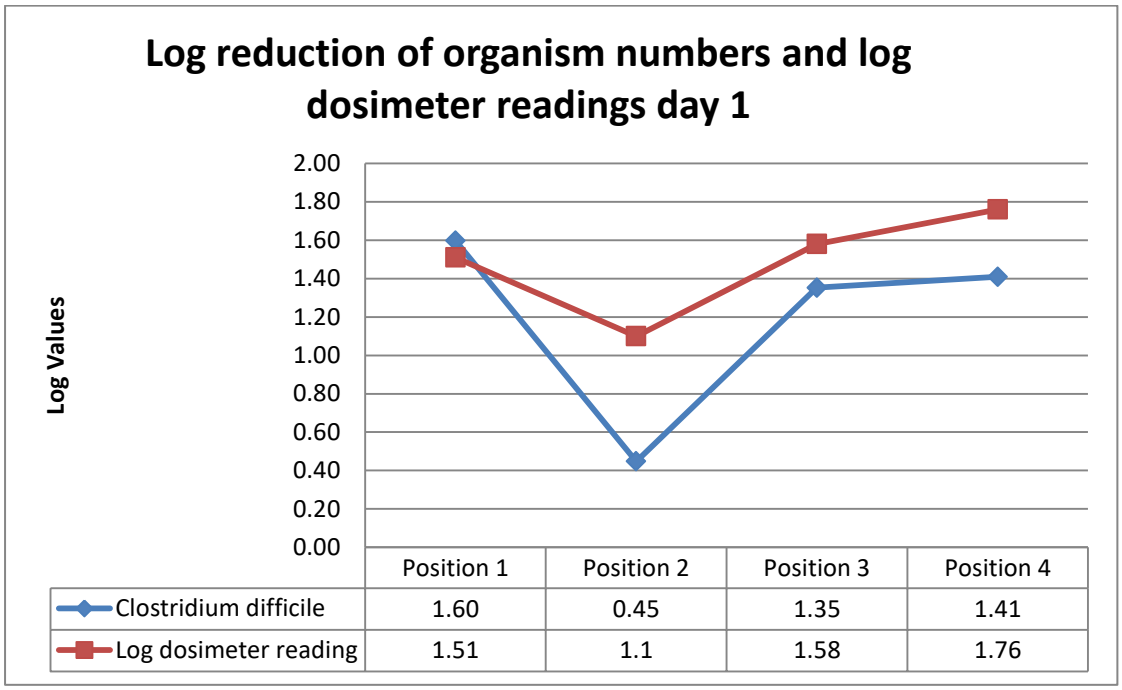


Table 9 comparison of mean log reduction compared to the dosimeter readings Day 1

	Clostridium difficile	Dosimeter reading	Log dosimeter reading	UVC radiometer reading
	Mean/SD	Mean/SD		mJ/cm2
Position 1	1.60 (0.46)	32.57(2.35)	1.51	62
Position 2	0.45 (0.35)	12.57 (4.15)	1.10	19
Position 3	1.35 (0.46)	38.30 (14.22)	1.58	NA
Position 4	1.41 (0.24)	57.50 (0.36)	1.76	1590

Figure 2.1 Trend of log reduction in organism numbers and log value of colour change in dosimeter indicator (day2).

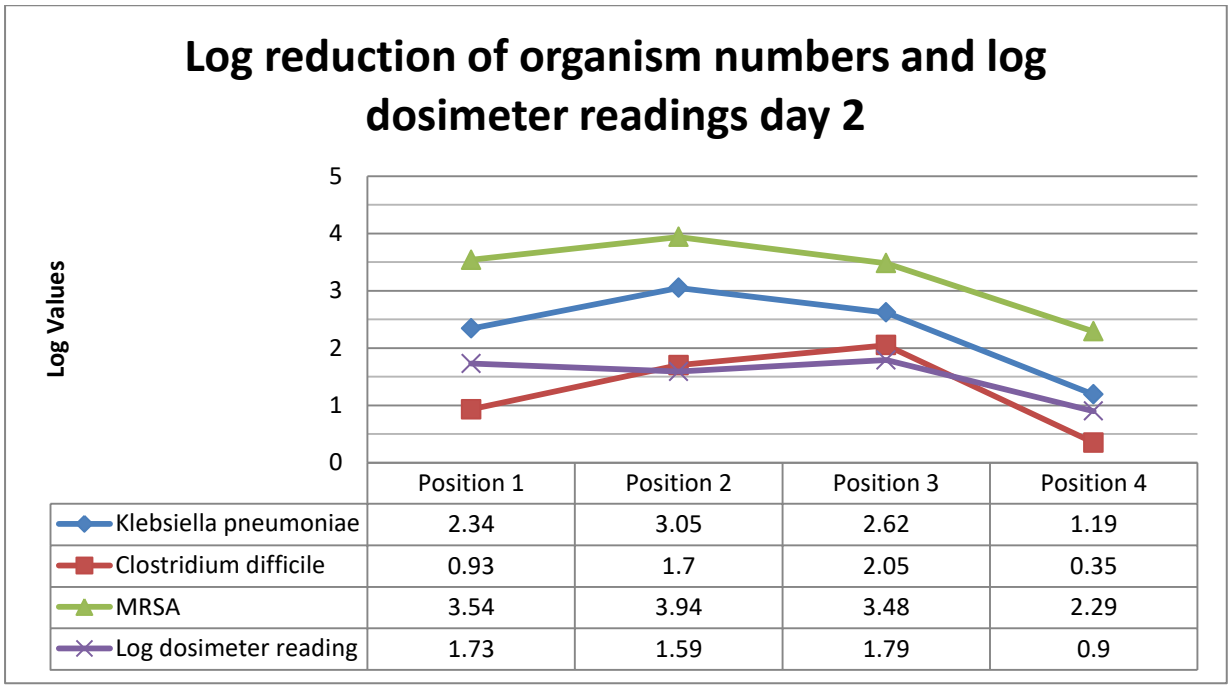


Table 10 comparison of mean log reduction compared to the dosimeter readings Day 2

	Klebsiella pneumoniae	*Clostridium difficile*	MRSA	Dosimeter reading	Log dosimeter reading	UVC radiometer reading
	Mean/SD	Mean/SD	Mean/SD	Mean/SD		mJ/cm2
Position 1	2.34 (2.03)	0.93(0.47)	3.54(1.62)	54.3 (14.52)	1.73	NA
Position 2	3.05(1.93)	1.70(0.23)	3.94(1.84)	39 (7.07)	1.59	NA
Position 3	2.62(1.37)	2.05(0.71)	3.48(1.35)	61.5 (7.81)	1.79	955
Position 4	1.19(0.67)	0.35(0.08)	2.29(0.84)	7.9(4.82)	0.90	10

3.0 Discussion

The log reductions seen with the three different organisms tested on day 2 did vary depending upon the organism type and the amount of shading present in the position the coupons were placed. Where there was full exposure to UVC (position 1, 2 and 3) as indicated by the dosimeter readings, in the presence of low soil, the log reduction was greater in MRSA >*K. pneumoniae* > *C. difficile* showing a log reduction of 3.66 >2.67 > 1.56 respectively. In position 4 where there was shading, there was still a small amount of observed UVC detected by the UVC radiometer (10 mJ/cm2) and 7.9 in delta E value on the dosimeter. This was reflected in the log reductions observed in the organisms being 2.29 > 1.19 > 0.35 for MRSA > *K. pneumoniae*, and *C. difficile* respectively. This trend was clearly displayed in Figure 2.1 which demonstrates that log reduction in organism numbers could be related to the log of the colour change.

This trend was reproduced on day 1 with *C. difficile* (no soil) only. There was a greater colour change and log reduction in organism numbers where there was more exposure to UVC varying between 0.5 and 1.5 log reduction.

This was a snap shot study undertaken to determine whether any relationship could be observed between the colour change in the dosimeter and log reduction in organism numbers. Three different bacteria strains were chosen: a Gram positive organism (MRSA) that was resistant to multiple antibiotics, a Gram negative multi-drug resistant organism (*K. pneumoniae*) and a spore forming Gram positive organism *C. difficile*. The two vegetative bacteria MRSA and *K. pneumoniae* showed a higher log reduction than the spore former *C. difficile* which is to be expected. There did appear to be a relationship between the dosimeter colour change and the log reduction but more experiments would have to be carried out to state that there was definite correlation.

Some of the observed differences could be explained by variables which could be better controlled in a more exacting environment. Some of these variables are:

- Distance from the UVC emitter
- Blocking UVC by the petri dish lid or edge
- Degree of shading caused by the position of dosimeter
- Inability to place the organisms in the exact position
- Degree of soiling on the coupons
- Numbers of replicates

4.0 Conclusion

This study demonstrates that there is a relationship between the level of reduction in organism numbers, amount of shading and colour change in the dosimeter indicator. In a light soil dosimeter readings, delta E values, between 39 and 62 related to 2.6 -3.9 log reduction in vegetative cells and 1-2 log reduction in spore forming bacteria.

5.0 Appendix

Raw data for Dosimeter results

The deltaE values are colour measurement values which indicates how strong the colour change of the dosimeters were. In general, a deltaE value of 1-2 is considered to be visible for the human eye (RISE, Sweden).

DAY 1				
	Run 1, deltaE	Run 2, deltaE	Run 3, deltaE	Radiometer measurement(mJ/cm2):
Position 1	34,6	30	33,1	62
Position 2	8,1	16,3	13,3	19
Position 3	49,5	22,3	43,1	NA
Position 4	57,1	57,6	57,8	1590
DAY 2				
	Run 1, deltaE	Run 2, deltaE	Run 3, deltaE	Radiometer measurement(mJ/cm2):
Position 1	37,8	60	65,1	NA
Position 2	34	44	41,4	NA
Position 3	52,5	66,5	65,5	955
Position 4	13,3	4,1	6,2	10

Report produced by:-

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Signed V Edwards-Jones Claes Lindahl

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