


Special Issue Research Article

Toward a Test Protocol for Surface Decontamination Using a Mobile Whole-room UVGI Device[†]

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ABSTRACT

Mobile whole-room UVGI devices are used in healthcare settings to control surface-borne pathogens. Unfortunately, no standard method comparing the efficacy of these devices is available. We assessed the effect of shadows on UVC 254 nm inactivation. The evaluation of a mobile whole-room UVGI device used spores of *Bacillus atrophaeus* as a surrogate for *Clostridium difficile* and *Staphylococcus aureus* as a surrogate for MRSA. Inactivation after 10 min of exposure varied significantly depending on whether the spores received direct UV exposure (4.3 log reduction), both direct and reflected UV exposure (3.0–4.0 log reduction) or reflected UV exposure alone (<1.0 log reduction). The susceptibility (z -value) for inactivation of *B. atrophaeus* spores on a glass surface was estimated to be $0.00312 \text{ m}^2 \text{ J}^{-1}$. *Staphylococcus aureus* microbial log reductions were approximately 5.5 for direct UV exposure, 3.6–5.2 for both direct and reflected UV exposure and approximately 2.75 for only reflected UV exposure. Our measurement of reflected dose ranged from 0.46% to 1.47%. Based on our findings, *B. atrophaeus* spores should be considered as a model organism for testing the impact of shadows on mobile whole-room UVGI device inactivation. Optimizing the reflected component of whole-room UVGI is important, especially for UVC-resistant organisms.

INTRODUCTION

Pathogens can persist for prolonged periods on surfaces in the hospital. *Methicillin-resistant Staphylococcus aureus* (MRSA), *Clostridium difficile* (*C. difficile*), *Klebsiella* species, *Candida auris* (*C. auris*) and *Vancomycin-Resistant Enterococcus* (VRE) are major concerns (1). The use of ultraviolet germicidal irradiation (UVGI) inactivates microbes in air and on surfaces (2). The Ebola Virus outbreak in 2015 heralded the use of mobile whole-room UVGI devices as an added layer of decontamination. Care in the use of mobile whole-room UVGI is required to overcome shadowed areas. Surfaces should be manually cleaned prior to

UVGI use (3). Sagripanti's team studied the UVC 254 nm inactivation of Lassa, Vaccinia and Ebola viruses which were inoculated and dried on nonporous glass slides (4,5). SARS-CoV-2 is reported to be viable on various surfaces for a number of days (6). Each of these pathogenic microbes can be transmitted from fomites due to inadequate cleaning of touched surfaces in hospital rooms. Studies have shown UVGI, primarily 254 nm produced by Hg lamps, can readily inactivate a wide range of these pathogens including MRSA, *C. difficile*, Coronavirus, *Ebola virus*, *C. auris* and SARS-CoV-2 (7–10). To interrupt transmission to patients, hospitals are using mobile whole-room UVGI for surface decontamination (11,12). Manufacturers of mobile whole-room UVGI devices claim that their equipment can kill or inactivate pathogens on surfaces by irradiating non-occupied patient rooms. These mobile whole-room UVGI devices come in a variety of configurations and costs. To date, there is no standardized method to compare the efficacy across devices.

In order to provide a framework for a test protocol, we conducted a pilot study to test the effectiveness of mobile whole-room UVGI using a hardy reference organism, bacterial spores of *Bacillus atrophaeus* (*B. atrophaeus*, formally known as *Bacillus subtilis* var. niger) as a surrogate for *C. difficile*. Further, we wanted to understand the degree to which shadows and reflections impact the effectiveness of this UVGI intervention. We would expect that the mobile whole-room UVGI device would be highly effective for killing or inactivating pathogens on room surfaces *except* under the following two conditions:

1 If the pathogen were not within the line of sight of the UV lamps.

When the pathogens reside in the shadows, a key parameter controlling disinfection efficacy would be surface reflectance—that is, the fraction of incident UV radiation that reflects off surfaces. Reflectivity varies from one surface to another.

2 If the pathogen is contained within a medium (e.g. sputum or blood) through which the UVC radiation has difficulty penetrating.

For this scenario, key parameters controlling disinfection efficacy would be the composition of the medium and its thickness.

Clearly, these two conditions could occur concurrently. We conducted this preliminary study to access the effect of shadows.

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MATERIALS AND METHODS

Experimental chamber. Description. All testing was done in a 10 ft by 15 ft floor and a 10 ft high ceiling, experimental chamber that was designed to be a prototype hospital room. The walls and ceiling are covered with a pebbled, hard-finish, white plastic wallboard. The reflectivity of this wallboard was measured to be 10% at 254 nm. However, when viewed in a dark room under UV light, the fluorescence of the plastic wallboard was obvious. Because the reflectivity was measured with a Lambda 900 spectrophotometer (Perkin Elmer, Waltham MA), an instrument with a single monochromator located before the sample, any radiation due to fluorescence would have been included as reflectivity at the excitation wavelength; thus, the true reflectivity of the wallboard is <10%.

Whole-room UVGI device. We used the Ultraviolet Devices, Incorporated (UVDI) of Valencia, CA, provided model NLA-110 UV Mobile Room Sanitizer (Fig. 1). This mobile whole-room UVGI device contains four ¾ in. diameter, 64 in. long, 325 W, low-pressure mercury lamps (Phillips model TUV 325 HO XPT SE). These measurements indicated that the whole-room UVGI, NLA-110 used 1370 W of electrical power (343 W per 325 W lamp), the current was 11.4 A at 120 V and the power factor was 0.99 as measured by a Kill A Watt EZ Power Monitor (P3 International Corp., NY, NY). The manufacturer's instruction manual states that each lamp emits 145 W of 254 nm UV. The axis of each of the lamps is vertical with one lamp on each of the four sides of the device. We placed this device in the geometric center of the floor of the experimental chamber (Fig. 2).

Biological testing. Spores of *B. atrophaeus* were used. *B. atrophaeus* is hardy, survives well indoors and is commonly used to evaluate the efficacy of biological decontamination methods. It is a surrogate for studies of *C. Difficile* and serves as a surrogate for microbes that are more UVGI resistant.

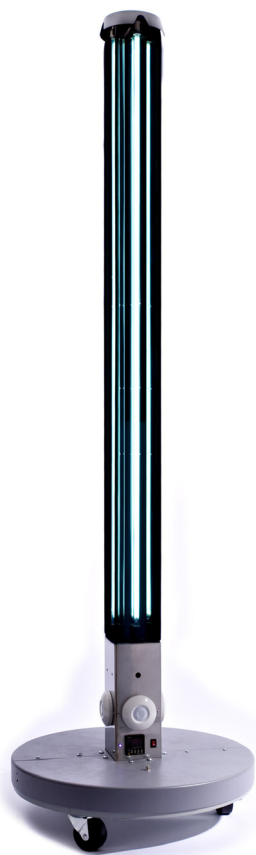


Figure 1. UVDI Model NLA-110 Mobile Room Sanitizer.

For the tests described herein, we used a suspension of *B. atrophaeus* spores in 1X PBS that had a concentration of 10^8 CFU mL⁻¹. Single-use portions of this suspension were stored in a freezer at -80 °C until used. Using a micropipette, we deposited 100 µL of the suspension on 1 in. by 3 in. glass test coupons that had been previously cleaned and then sterilized in an autoclave. The micropipette was then used to spread out the liquid so that it covered approximately one-half of the test coupon. All test coupons were dried in a biosafety cabinet for about 60 min, after which all of the liquid had evaporated. Test coupons for a given experiment were placed in the chamber at predetermined locations, while control coupons were kept inside the biosafety cabinet and not exposed to UVGI.

A two-inch diameter, 6 ft long aluminum tube simulating a patient bedrail was mounted vertically on the floor, 7 in. from the center of one of the chamber's 10 ft wide walls. The unexposed side of a coupon was held against the tube with the long edge of the coupon parallel to the axis of the tube. The sampling plan is shown in Table 1.

The whole-room UVGI device, which was oriented so that one of the lamps was in alignment with the aluminum tube, was turned on for 10 min (see grid diagram, Fig. 2). After this exposure, the test coupons were returned to the biosafety cabinet where the surface of each coupon was washed with 1 mL of 1X PBS by pipetting the fluid over the seeded surface 20 times to elute surviving bacteria. Rinsate from each test coupon was serially diluted and plated on tryptic soy agar in triplicate. The number of serial dilutions, which varied per sample location, was chosen to yield between 25 and 350 CFU per plate in order to keep the counting error within a reasonable range. All tests were replicated three times in order to evaluate experimental error.

During the course of the study, we received permission from our biological safety committee to test *Staphylococcus aureus* (*S. aureus*). Based on the same methodology, tests on *S. aureus* were run.

Irradiance measurements. Irradiance measurements were made with a model P9710-1 optometer and model UV-3718-2 UV detector (Gigahertz-Optik GmbH, Türkenfeld, Germany) at each test location. The detector was placed in the locations where the test coupons had been placed while being irradiated. The face of the probe was oriented directly at and perpendicular to the center of the lamp of the whole-room UVGI device. The irradiance was measured and recorded every second for 10 min at each location. The dose was calculated by multiplying the average irradiance by the exposure time. This procedure was repeated in triplicate in order to evaluate experimental error.

To further explore the direct and reflected UV dose, the NLA-110 Mobile Room Sanitizer was kept in the middle of the room (see Fig. 3), in the same orientation for all trials Gigahertz-Optik used for all data logging purposes. The data logged continuously for a complete cycle. The cycle time was set to 10 min, and a sensor was affixed to the aluminum tube 1 m high. Eight locations were measured—Front, Back, Right, Left, Front left, Front right, Back left and Back right. Each location was 1m away from the center of the whole-room UVGI device, with the corner measurements on the diagonal of the whole-room device. The data collected were with the sensor facing toward the whole-room UVGI device (“direct”) and away from the whole-room device (“reflected”) for each of the eight locations. The data were downloaded and saved. The dose was calculated based on the average between start and end was multiplied by the duration in seconds.

Calculations. The ratio of the number of CFUs per volume of liquid used to wash an exposed coupon (U) to the number of CFUs per volume of liquid used to wash an unexposed control coupon (U_o) is defined as fraction of bacteria surviving (f):

$$f = \frac{U}{U_o} \quad (1)$$

The number of log reductions (n) is defined as the difference between the logarithms of the CFUs per volume for unexposed and exposed test coupons:

$$n = \log_{10} U_o - \log_{10} U = -\log_{10} f \quad (2)$$

Thus, $n = 4$ corresponds to 4 log reductions, which is equivalent to 0.01% of bacteria surviving and 99.99% of bacteria inactivated; that is, starting with 10 000 CFUs in the wash from an unexposed coupon, only one CFU would remain in wash for the exposed coupon.

Statistical analysis. A one-way ANOVA analysis was performed for the number of log reductions for six experimental groups

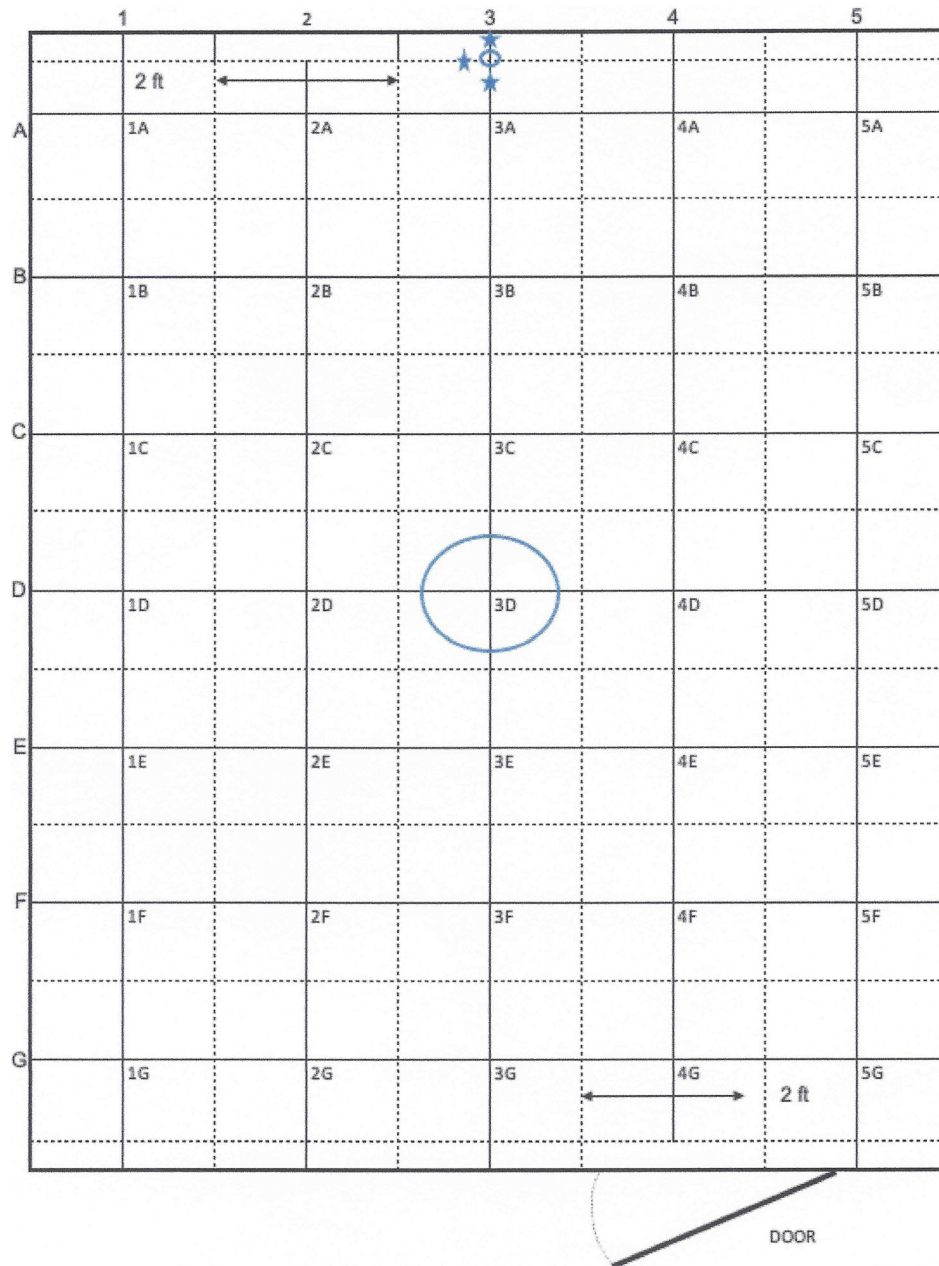


Figure 2. Grid plan view for UV measurements. Starred (*) locations are where the glass test coupons are placed. Large circle in the center represents location of the mobile whole-room UVGI device.

(locations), each composed of three replicate tests. A *P*-value of less than 0.05 indicates that the number of log reductions for at least one of the groups was significantly different at the 95% confidence level than at least one of the other groups. Tukey's multiple comparison tests were then performed on all possible pairs of groups to determine which of the pair members were statistically different from its other pair member. The same analysis was repeated using the three replicate measurements of dose for the experimental groups.

RESULTS

The number of log reductions for *B. atrophaeus* spores on glass test coupons due to whole-room UVGI for 10 min is shown in Fig. 4:

The degree of decontamination observed for the tests varied. At a height of 1 m in front of the tube, the number of log

reductions was 4.3. On the floor at the front and side of the tube, the number of log reductions was between 3.0 and 4.0, which is only somewhat lower. However, the number of log reductions at these three locations was considerably higher than the locations shown in Fig. 4. The Fig. 2 locations—at a height of 1 m on the rear or side of the tube and on the floor at the rear of the tube—the number of log reductions was all less than 1.0, and on the floor at the rear of the tube, it was only 0.11, about 1/40th of what it was at the front of tube at a height of 1 m.

Based on a statistical analysis using a one-way ANOVA analysis and Tukey's tests, each of the three locations having the highest number of log reductions was found to be significantly higher at the 95% confidence level than the three locations having the lowest number of log reductions. For the three locations

Table 1. Glass test coupon placement plan along a vertical aluminum tube.

Glass test coupons placed at height of 1 m

Test coupons were attached to the tube at a height of 1 m above the floor at the following three locations

1. On the front of the tube facing the UVGI device where the glass test coupons were exposed primarily to direct UV radiation
2. On the rear of the tube where the glass test coupons were exposed to only reflected UV radiation.
3. On one side of the tube where the glass test coupons were exposed to both the reflected and direct UV radiation

Glass test coupons floor level placement

Test coupons were placed on the floor at the base of the tube with the short edge of the coupon in contact with the tube at the following three locations

1. On the front of the tube facing the UVGI device where the glass test coupons were exposed primarily to direct UV radiation
2. One the rear of the tube where the glass test coupons were exposed primarily to reflected UV radiation
3. On one side of the tube where the glass test coupons were exposed to both reflected and direct UV radiation.

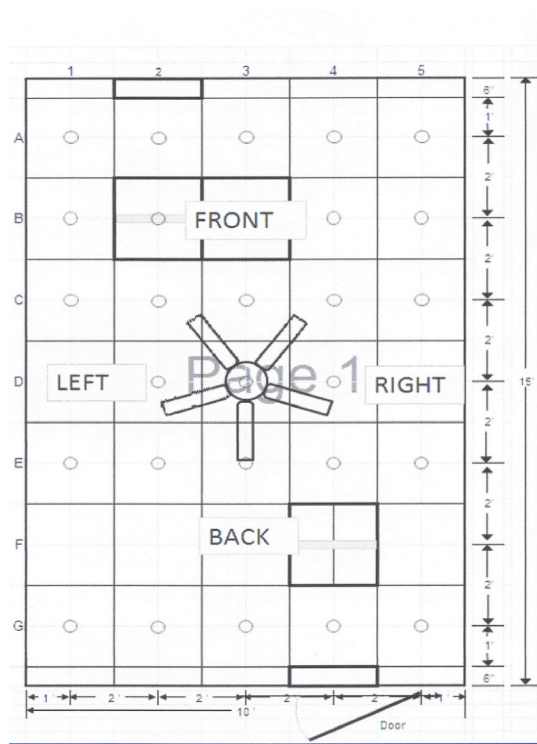


Figure 3. UV direct and reflected measurement grid. [Color figure can be viewed at wileyonlinelibrary.com]

having the lowest number of log reductions, the null hypothesis that they were equal could not be rejected. For the three locations having the highest number of log reductions, the null hypothesis could also not be rejected with the exception that at the 95% confidence level, the number of log reductions at 1 m high in front of the tube was higher than on the floor at the side of the tube.

The UV dose accumulated by each test coupon over a 10 min exposure period is shown in Fig. 5.

At the front of tube at a height of 1 m, the 10 min dose was 0.4 J cm^{-2} . On the floor at the front and side of the tube, the dose was about 0.1 J cm^{-2} , somewhat lower. However, the other three locations—on the rear or side of the tube at a height of 1 m and on the floor at the rear of the tube, the doses were significantly less. At the side of tube at a height of 1 m and on the floor at the rear of the tube, it was only 0.006 J cm^{-2} , about $1/65^{\text{th}}$ of what it was at the front of the tube at a height of 1 m.

Based on a statistical analysis using a one-way ANOVA analysis and Tukey's tests, each of the three locations having the highest UV dose were found to be significantly higher at the 95% confidence level than the three locations having the lowest number UV dose. This result was expected and essentially the same as what was observed for the number of log reductions. Clearly, the number of log reductions should be highly correlated with UV dose. Figure 6 is a plot of the number of log reductions versus dose.

The inactivation of *B. atrophaeus* spores is assumed to follow this exponential decay formula

$$\frac{C_{\text{UV}}}{C_{\text{noUV}}} = \exp(-zD) \quad (3)$$

where C_{UV} and C_{noUV} are concentrations of bacteria when they are exposed and not exposed to UVGI, respectively, all else being the same. When the logarithms of experimental values of $C_{\text{UV}}/C_{\text{noUV}}$ are plotted against dose, the slope of the resulting line is equal to z , the UV susceptibility of microbe. The susceptibility (z -value) for inactivation of *B. atrophaeus* spores on a glass surface can be estimated from this plot. The z -value is equal to the slope of the line in Fig. 6 multiplied by the natural log of 10, which is $0.00312 \text{ m}^2 \text{ J}^{-1}$.

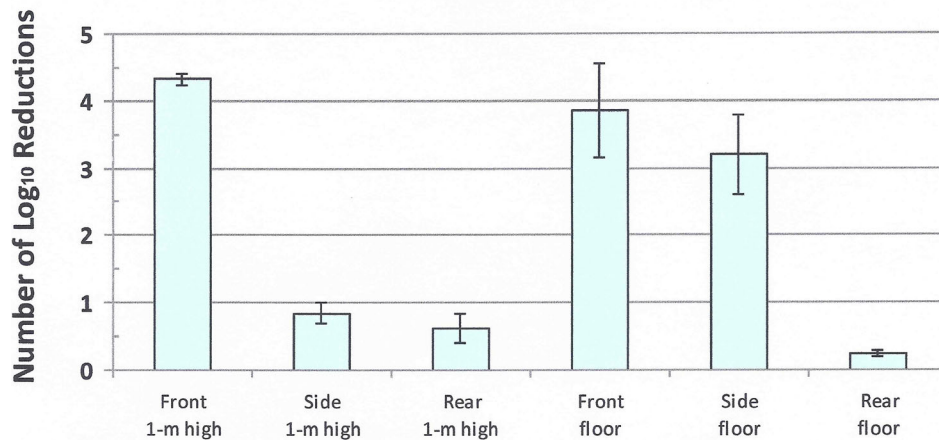
Similarly, tests of *S. aureus*, using the same protocol, resulted in microbial log reductions of approximately 5.5 for direct UV exposure, 3.6–5.2 for both direct and reflected UV exposure and approximately 2.75 for only reflected UV exposure (Fig. 7). The plot of microbial log reduction vs dose is shown in Fig. 8. These results show a similar trend but with a higher log kill due to the more susceptible microbial structure to penetration of the UVC (a cell wall versus a spore).

Our measurements of direct and reflected UVC dose showed expected influence of distance and reflectivity. In Fig. 3, we see the distance between the mobile whole-room UVGI device is shorter (Front, Back, Left Side and Right Side) resulting in slightly higher reflected dose vs. (Front Left, Front Right, Back Left and Back Right) which are off-axis and a longer path for the UV rays to travel. As with light, the intensity drops off at the square of the distance. We also see the impact of reflectivity at the shortest distance (Left side and Right Side). Overall, the reflected dose is low because of the $< 10\%$ reflectivity of the walls (Table 2).

DISCUSSION

For *B. atrophaeus* as shown in Fig. 4, there was a considerable difference between the number of microbial log reductions achieved

Surface Decontamination of *B. atrophaeus* spores (Mean for 3 tests with error bars for standard deviation)



Location Relative to Vertical Pipe and Height

Figure 4. Log reduction of *B. atrophaeus* spores based on location relative to vertical tube and height and 10 min UV exposure (The three left locations are attached to vertical tube 1 m above the floor, the three right locations attached to the tube (short edge) are on the floor). [Color figure can be viewed at wileyonlinelibrary.com]

Dose received at the slide surface over a 10-min exposure period (Mean for 3 tests with error bars for 95% confidence limits)

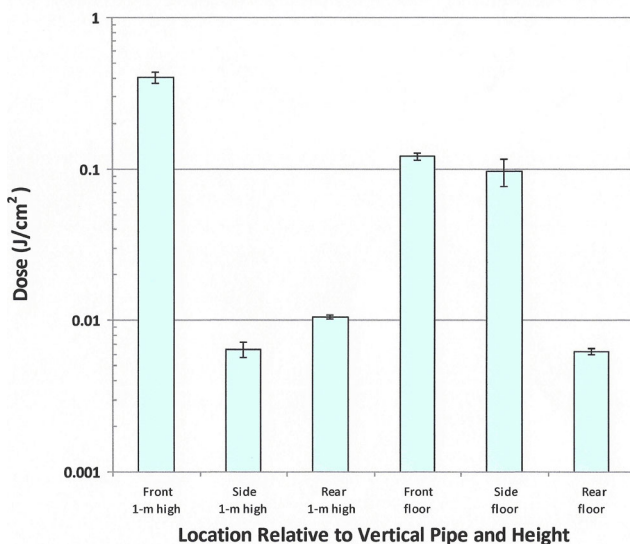


Figure 5. UV dose received at the glass slide at various locations over a 10 min exposure period. [Color figure can be viewed at wileyonlinelibrary.com]

for the locations having the three highest (3.2–4.3) and the three lowest (0.2–0.8) values. This difference was not surprising because most of the UV radiation at the front of the tube (both on the floor and 1 m high) and at the side of the tube on the floor is direct radiation from the UVGI device, whereas the test coupons at the rear of the tube (both on the floor and 1 m high) and the coupon at the side of the tube at a height of 1 m are exposed to only reflected UV radiation. The surface of the coupon on the side of the tube on the floor is horizontal, and it receives direct UV radiation, whereas the surface of the coupon on the side of the tube at a height of 1 m

is vertical and the coupon is oriented so that its edge faces the UVGI device, receiving mostly reflected UV radiation. The reason that the coupon receiving mostly reflected light has much lower log reduction is because at least 90% of the UV radiation undergoing a single reflection off a wall would be expected to be absorbed. Figure 5, a plot of UV dose versus location, also indicates that locations in the shadows get significantly lower dose than locations receiving direct UV radiation. This finding held true for *S. aureus* (see Fig. 7).

Locations where UV radiation has difficulty reaching may be low-touch locations less likely to transfer pathogens. Exceptions are patient bed rails and under patient overbed tables which represent high touch surfaces. It also needs to be emphasized that this result is very venue-specific, depending to a large extent on the reflectivity of room surfaces at 254 nm and placement of furniture and medical equipment. Highly UVC 254 nm reflective paints are available to increase reflectivity in shadowed areas compared to standard surface coatings (13, 14). These reflective UVC paints should be tested to determine how well they enhance whole-room UVGI in the shadows.

Based on Fig. 6, the z -value for *B. atrophaeus* spores on a glass surface, which was calculated from slope of the line in Fig. 6, is equal to $0.0031 \text{ m}^2 \text{ J}^{-1}$. However, the data points do not fit this line very well ($R^2 = 0.4$). This is in part likely due the extremely large range of doses measured. If the data point at a dose of 0.43 is ignored, the fit is much better ($R^2 = 0.95$), and the z -value is significantly higher at 0.0075. This wide range likely due to the small, reflected dose received from the back. The dose could be increased by a greater surface reflectance or a longer exposure time. (Our other work showed the z -value for *B. atrophaeus* spores in air was measured to be equal to $0.018 \text{ m}^2 \text{ J}^{-1}$, which is slightly more than double the z -value of $0.0075 \text{ m}^2 \text{ J}^{-1}$. (15)

Based on Fig. 7, for *S. Aureus*, the impact of shadows and reflected dose was not as pronounced, only the Rear floor

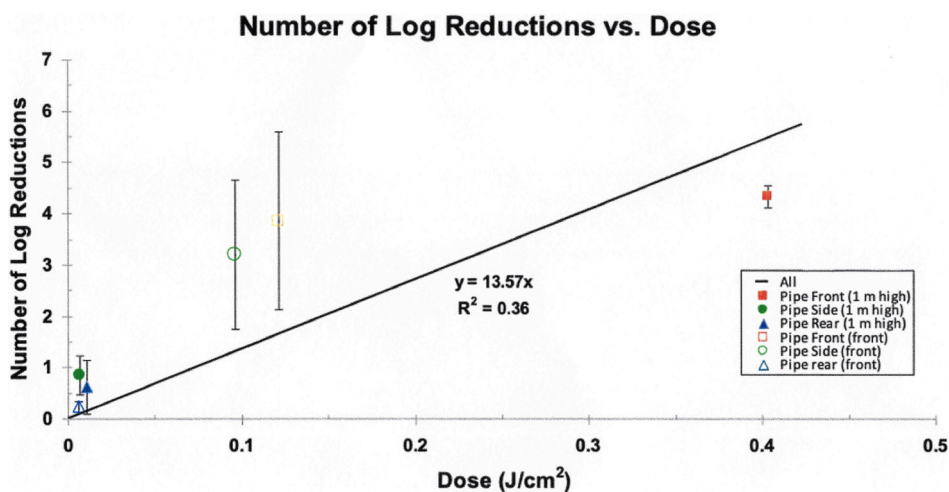


Figure 6. Log reduction of *B. atrophaeus* spores vs. dose (10 min exposure).

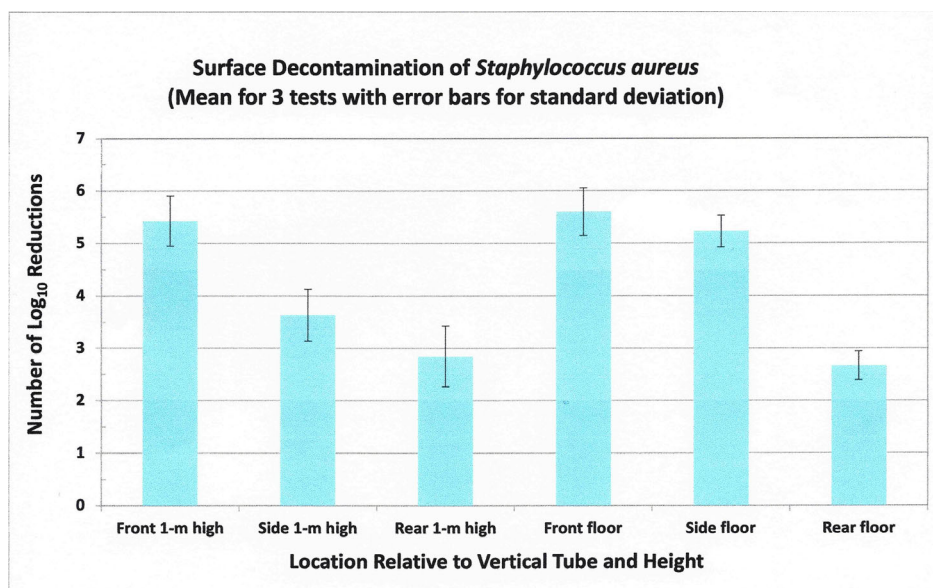


Figure 7. Log reduction of *S. aureus* based on protocol locations and 10 min UV exposure.

showed less than 3 log reductions followed by Rear 1 m high, slightly less than 3 log reductions. The most pronounced impact may be for the spore form bacteria, *C. difficile*. Finding a means to boost the reflected dose to inactivate *C. difficile* would enhance the ability to inactivate more susceptible MSRA. Another area of balance would be UV exposure time. The 10 min exposure used in our study could be increased to a 15 min period. Optimizing these factors in combination with manual cleaning of surfaces is needed in order to quickly and safely turnaround a patient room after discharge to receive a new patient (16).

CONCLUSION

A standardized test method for determining the performance of mobile whole-room UVGI devices is needed, and our method provided a step toward this protocol. Our study confirms that

shadow effects are a significant limitation of UVGI surface disinfection, but the appropriate use of highly reflective materials (e.g. UVC 254 nm reflective paints or surfaces) could offer a way to mitigate this limitation. Future recommendations would need to explore the balance between upper-room UVGI applications which seek to control reflections in occupied spaces and use of mobile whole-room UVGI use in non-occupied rooms. The hardness of a test organism to withstand and survive UVGI exposure is needed, and *B. Atrophaeus* could serve this purpose. Exposure time is a critical to achieving a desired 3 log kill, with surface reflectance enhancement needed to achieve this goal as well. Moving the whole-room UVGI device into a different position to impact the shadowed surfaces should be studied. Further exploration is also needed to understand the impact of furniture in a room. Finally, a simple means to check the delivered dose on room surfaces can build confidence that the intervention is working to the degree expected.

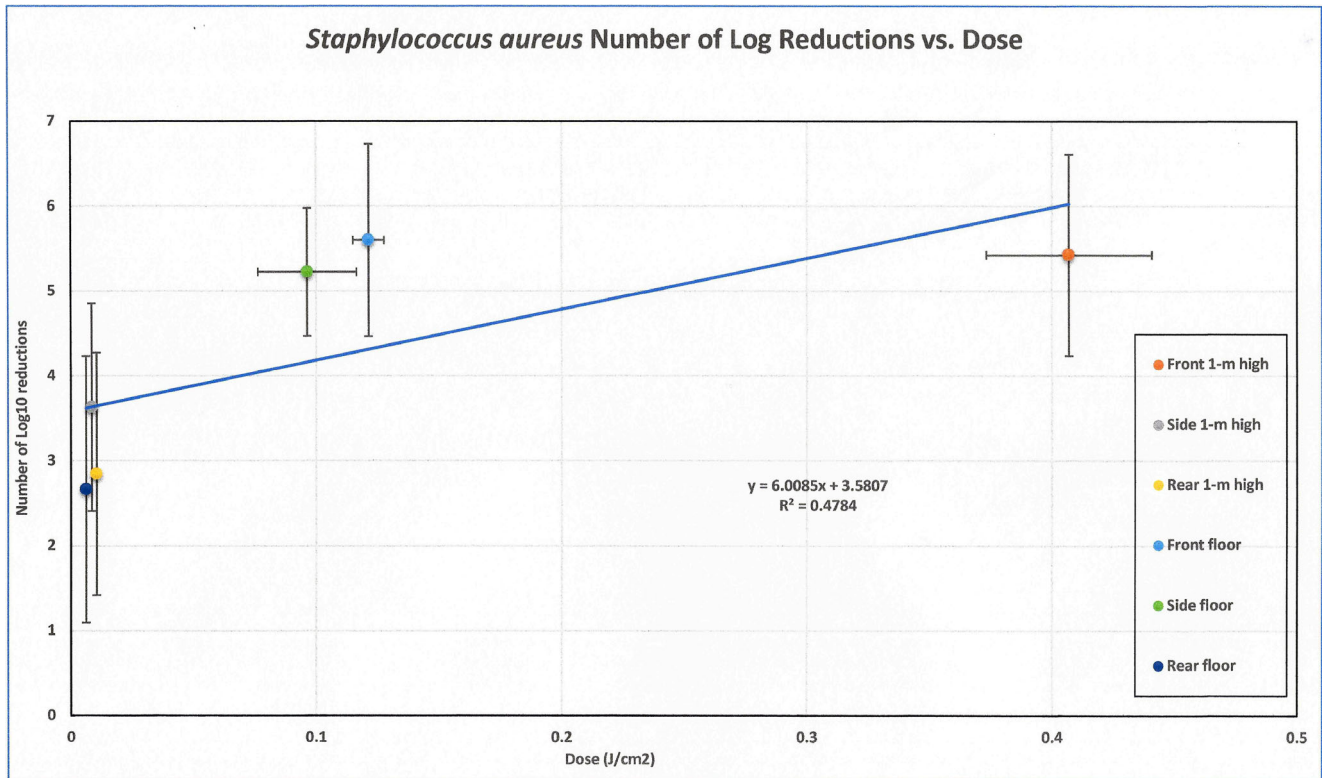


Figure 8. Log reduction of *S. aureus* vs. UV dose (10 min exposure).

Table 2. Direct and reflected dose from mobile whole-room UVGI device.

	Dose (J cm ⁻²)		Reflected dose as a percentage of the direct dose
	Direct	Reflected	
Front	1.537	0.007	0.46%
Back	1.297	0.007	0.54%
Right side	1.332	0.019	1.43%
Left side	1.429	0.021	1.47%
Front left	1.058	0.011	1.04%
Front right	1.039	0.011	1.06%
Back left	1.058	0.010	0.95%
Back right	1.040	0.008	0.77%

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