ORIGINAL ARTICLE

Effect of Variation in Test Methods on Performance of Ultraviolet-C Radiation Room Decontamination

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OBJECTIVE. To determine the effect of variation in test methods on performance of an ultraviolet-C (UV-C) room decontamination device.

DESIGN. Laboratory evaluation.

METHODS. We compared the efficacy of 2 UV-C room decontamination devices with low pressure mercury gas bulbs. For 1 of the devices, we evaluated the effect of variation in spreading of the inoculum, carrier orientation relative to the device, type of organic load, type of carrier, height of carrier, and uninterrupted versus interrupted exposures on measured UV-C killing of methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile* spores.

RESULTS. The 2 UV-C room decontamination devices achieved similar $\log_{10}$ colony-forming unit reductions in the pathogens with exposure times ranging from 5 to 40 minutes. On steel carriers, spreading of the inoculum over a larger surface area significantly enhanced killing of both pathogens, such that a 10-minute exposure on a 22-mm^2 disk resulted in greater than 2 log reduction in *C. difficile* spores. Orientation of carriers in parallel rather than perpendicular with the UV-C lamps significantly enhanced killing of both pathogens. Different types of organic load also significantly affected measured organism reductions, whereas type of carrier, variation in carrier height, and interrupted exposure cycles did not.

CONCLUSIONS. Variation in test methods can significantly impact measured reductions in pathogens by UV-C devices during experimental testing. Our findings highlight the need for standardized laboratory methods for testing the efficacy of UV-C devices and for evaluations of the efficacy of short UV-C exposure times in real-world settings.

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Automated ultraviolet-C (UV-C) room decontamination devices are increasingly used as an adjunct to standard cleaning and disinfection in healthcare facilities.[1] Under experimental conditions, these devices have demonstrated efficacy in killing a variety of bacterial pathogens, including *Clostridium difficile* spores.[2–10] In hospital rooms, use of the devices has been shown to reduce the burden of pathogens on surfaces.[2–4,6–8,11–13] There have also been some reports of use of UV-C devices associated with reductions in healthcare-associated infections.[14–16]

In addition to factors such as cost and ease of use, healthcare facilities considering the purchase of a UV-C room decontamination device must weigh claims regarding the effectiveness of different devices. However, few data are available on the comparative effectiveness of different UV-C devices.[5,8] Moreover, in contrast to the situation for surface disinfectants,[17] there are currently no standardized methods recommended for testing of UV-C devices. Thus, a variety of methods have been used in experimental testing. For example, inoculated surfaces have included glass, steel, and Formica carriers or bench countertops, spreading of the inoculum has varied from areas of 4 mm^2 to 25 cm^2, and inoculated carriers have been oriented in parallel or perpendicular to the bulbs.[2–10] Although it is known that UV-C efficacy is affected by distance and shading, it is not known if variation in other test conditions affect pathogen killing. Here, we compared the effectiveness of 2 UV-C room decontamination devices and evaluated the effect of variation in test methods on performance of 1 of the devices.

METHODS

C. difficile and Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strains

One strain of each pathogen was studied. The *C. difficile* strain was American Type Culture Collection (ATCC) strain 43598. Spores were prepared and stored as previously described.[18] The MRSA strain was a clinical isolate of pulsed-field gel electrophoresis type USA800. MRSA and *C. difficile* were cultured on selective media as previously described.[8]

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The UV-C Disinfection Devices

The Tru-D device (Lumalier) has been described previously.[3–5] The Clorox Healthcare Optimum-UV System (Clorox) has a 36 inch–diameter circular base and a height of 76 inches. The device is a wheeled unit containing four 64-inch low-pressure mercury lamps emitting 254-nm UV-C light. The device's cycle time is determined by the manufacturer on the basis of room size and configuration. The system contains a motion sensor connected to a safety-rated relay, aborting the cycle if someone enters the room during use.

Comparison of the Optimum-UV and Tru-D Devices for Killing of the Pathogens

We compared the efficacy of the 2 devices against the pathogens on steel disk carriers in a 10×10 foot room as described previously.[5] For each pathogen, 10-μL aliquots containing 1×10^6 colony-forming units in phosphate-buffered saline were spread to cover 1-cm^2 area of stainless steel carriers and allowed to air dry. The carriers were placed perpendicular to the vertical lamps (ie, horizontal) 4 feet from the devices at a height of 4 feet and exposed to cycles of 5, 10, 20, or 40 minutes. For comparison, the Tru-D reflected doses of 12,000 (vegetative cycle) and 22,000 (spore cycle) $\mu Ws/cm^2$ in single patient rooms require approximately 20 and 45 minutes, respectively.[3] To quantify viable organisms, treated and untreated control carriers were submersed in 1 mL of phosphate-buffered saline and vortexed vigorously, and dilutions were plated onto selective media and processed as described previously.[3,5,8] Because initial experiments demonstrated similar efficacy of the devices, subsequent experiments were conducted only with the Clorox Healthcare Optimum-UV System.

Effect of Inoculum Dispersal on Killing of the Pathogens by the Optimum-UV System

To assess the impact of dispersal of the inoculum, 10-μL aliquots of the pathogens were inoculated onto 10-mm^2 steel disk carriers (Cole Palmer) either without manual spreading (~4-mm^2 area) or with spreading (10-mm^2 area) or onto 22-mm^2 steel disk carriers with spreading. The carriers were placed perpendicular to the lamps 4 feet from the device at a height of 4 feet in the direct field of radiation and exposed to cycles of 5 or 10 minutes. Reductions were compared with untreated control carriers.

Effect of Orientation of the Carriers Relative to the UV-C Lamps

We tested the effect of the angle of UV-C incidence by altering the orientation of 22-mm^2 steel disk carriers relative to the Optimum-UV lamps. The carriers were positioned in parallel with the vertical lamp (ie, vertical orientation directly facing the lamp), perpendicular to the lamp, or at a 45° angle from the lamp. The carriers were placed 4 feet from the device at a height of 4 feet and exposed to 10 minutes of UV-C.

Effect of Different Types of Organic Load

We examined the effect of 3 commonly used simulated organic materials (5% and 10% fetal calf serum and ASTM E2197 standard organic load consisting of 5% tryptone, 0.4% mucin, and 5% bovine serum albumin) on killing of MRSA.[18] The 10-μL inoculum of MRSA prepared in each of the test organic materials was spread to cover 22-mm^2 steel disk carriers or allowed to dry after placement as a central droplet (~4-mm^2 area). Carriers were exposed to 3 minutes of UV-C.

Effect of Carrier Type

We examined reduction in the pathogens inoculated onto steel disks, plastic (Nunc), glass slides (Fisher Scientific), and Formica (Diller). For each carrier, a 10-μL inoculum was spread to cover 22 mm^2. The carriers were placed 4 feet from the device at a height of 4 feet and exposed to 5 minutes of UV-C. Log reductions were enumerated against untreated control carriers of the same material.

Effect of Variation in Carrier Height

We tested killing of MRSA at different heights at a distance of 6 feet from the Optimum-UV device. Steel disk carriers were placed on the floor as well as 1, 3, 4, and 6 feet above the floor and exposed to UV-C for 5 minutes.

Effect of Interrupted vs Uninterrupted Dosing

Because some manufacturers recommend running 1 cycle on each side of the bed, we examined the impact of interrupted UV-C cycles on germicidal activity against MRSA. Steel disk carriers were placed 4 feet from the device at a height of 4 feet. Reductions in MRSA were compared for 4 different exposure cycles: (1) uninterrupted 16-minute exposure; (2) 3 exposures of 5.33 minutes each with each exposure interrupted by a 1-minute pause; (3) 3 exposures of 5.33 minutes each with each exposure interrupted by a 20-minute pause; and (4) 8 exposures of 2 minutes each with each exposure interrupted by a 1-minute pause between cycles.

Statistical Analysis

A 1-way analysis of variance was performed to compare the mean log reductions. A post hoc Tukey honest significant difference test was used to test pairwise differences between group means. For each assessment of variation in test methods, a comparison group was chosen as the standard for comparison: spreading of the inoculum, reference equals 4 mm^2; orientation of steel disk carriers, reference equals horizontal orientation perpendicular to the lamps; organic load, reference

equals no organic load; carrier material, reference equals steel; height, reference equals 4 feet; and cycle interruption, reference equals uninterrupted 16-minute exposure. For statistical analysis, we used R software.[19]

RESULTS

As shown in Figure 1, there were no significant differences between the 2 devices in reduction of either pathogen after exposures of 5, 10, 20, and 40 minutes. For MRSA, a greater than 3 log reduction was achieved within 5 minutes. For *C. difficile* spores, log reductions increased with increasing exposure time, with 20 minutes of exposure required to achieve a reduction of approximately 2 log.

As shown in Figure 2, spreading of the inoculum over a greater surface area significantly enhanced killing of both pathogens. For *C. difficile* spores, spreading of the inoculum to cover 22 mm^2 resulted in a reduction of approximately 2 log within 10 minutes of exposure at 3 feet. For MRSA, spreading of the inoculum to cover 22 mm^2 resulted in a reduction of approximately 5 log within 10 minutes. Positioning of the carriers vertically in parallel with the lamp also enhanced killing of both pathogens in comparison with a perpendicular orientation (Figure 3).

Figure 4 shows the effect of different types of organic load on UV-C efficacy against MRSA. The ASTM standard organic load and 10% fetal calf serum significantly reduced killing of MRSA when the inoculum covered a 4-mm^2 surface area, whereas 5% fetal calf serum did not. However, none of the organic load suspensions significantly affected killing of MRSA when the inoculum was spread to cover 22 mm^2.

As shown in Figure 5, the log reductions of the pathogens achieved on plastic, Formica, and glass slides were not significantly different from the reductions on steel disks. Of note, when the 10-μL inoculum was not manually spread, the reduction of pathogens was significantly greater on glass slides than on steel disks (data not shown); it was observed that natural spreading of the inoculum occurred on glass but not on the steel disk. At 6 feet from the device, there were no significant differences in log reductions of MRSA achieved at different heights ranging from floor level to 6 feet above floor level ($P = .88$). There were also no significant differences in log reductions of MRSA with 16 minutes of UV-C exposure that was uninterrupted versus administered in interrupted doses.

DISCUSSION

In experimental testing, we found that the Tru-D and Clorox Healthcare Optimum-UV devices were equally effective for killing of MRSA and *C. difficile* spores. Using the Optimum-UV device, we demonstrated that variation in experimental testing conditions can have a significant impact on the measured efficacy of UV-C. Killing of the pathogens was significantly enhanced by spreading of the inoculum over an

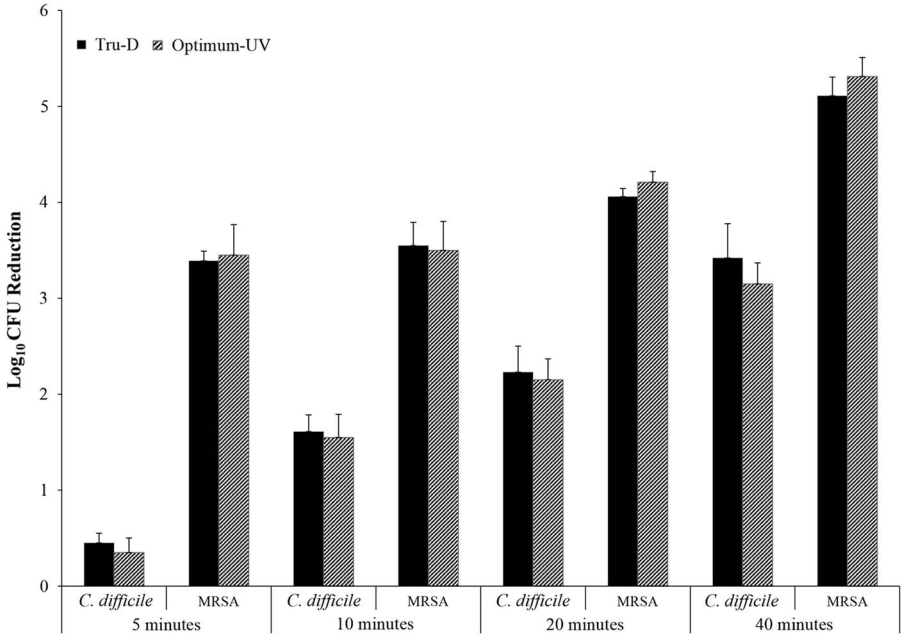

FIGURE 1. Efficacy of the Tru-D versus Clorox Healthcare Optimum-UV System for killing of *Clostridium difficile* spores and methicillin-resistant *Staphylococcus aureus* (MRSA). Steel disk carriers were inoculated with 1×10^6 colony-forming units (CFU) of the pathogens in 10 μL of phosphate-buffered saline and the inoculum was spread to cover the 10-mm^2 surface area of the disk. The carriers were placed 4 feet from the devices at a height of 4 feet and irradiated for 5, 10, 20, or 40 minutes. The means of data from triplicate experiments are presented. Error bars indicate standard error.

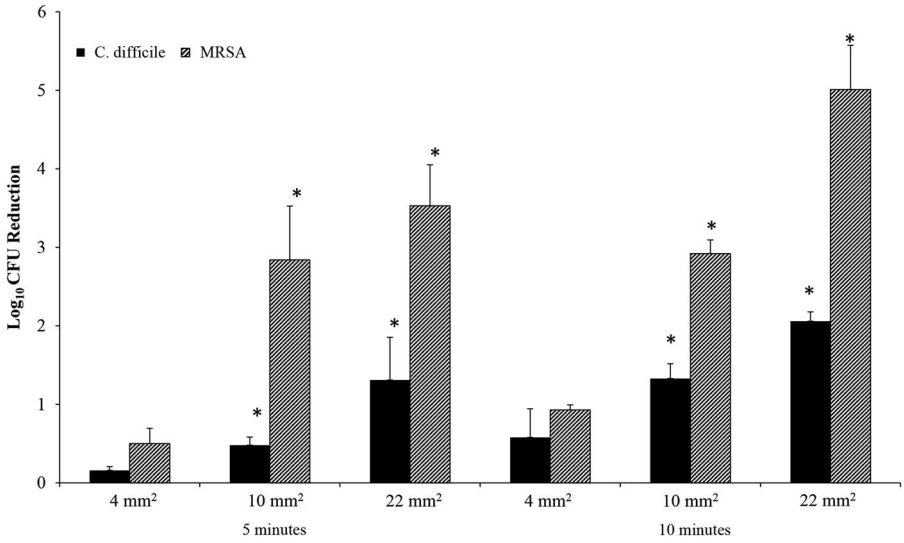

FIGURE 2. Effect of inoculum dispersal on killing of *Clostridium difficile* spores and methicillin-resistant *Staphylococcus aureus* (MRSA) by the Optimum-UV Device. Steel disk carriers were inoculated with 1×10^6 colony-forming units (CFU) of the pathogens in 10 µL of phosphate-buffered saline and the inoculum was either not spread (~4-mm^2 area on a 10-mm^2 disk), spread to cover the surface area of a 10-mm^2 disk, or spread to cover the surface area of a 22-mm^2 disk. The carriers were placed 4 feet from the device at a height of 4 feet and irradiated for 5, 10, 20, or 40 minutes. The means of data from triplicate experiments are presented. Error bars indicate standard error. Asterisk indicates $P < .01$ in comparison with the smaller surface area.

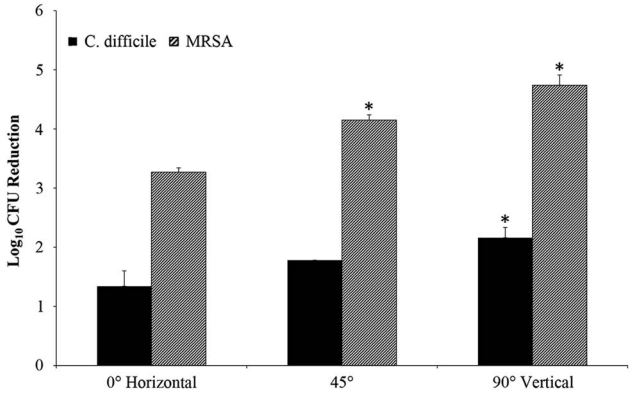

FIGURE 3. Effect of orientation of the carriers relative to the ultraviolet-C lamps on killing of *Clostridium difficile* spores and methicillin-resistant *Staphylococcus aureus* (MRSA) by the Optimum-UV Device. Steel disk carriers were inoculated with 1×10^6 colony-forming units (CFU) of the pathogens in 10 µL of phosphate-buffered saline and the inoculum was spread to cover the entire 22-mm^2 surface area. The carriers were adhered to glass slides and positioned in parallel with the vertical lamp (ie, 90° vertical and directly facing the lamp), perpendicular to the lamp (ie, horizontal), or at a 45° angle from the lamp. The carriers were placed 4 feet from the device at a height of 4 feet and irradiated for 10 minutes. The means of data from triplicate experiments are presented. Error bars indicate standard error. Asterisk indicates $P < .01$ in comparison with the horizontal carriers.

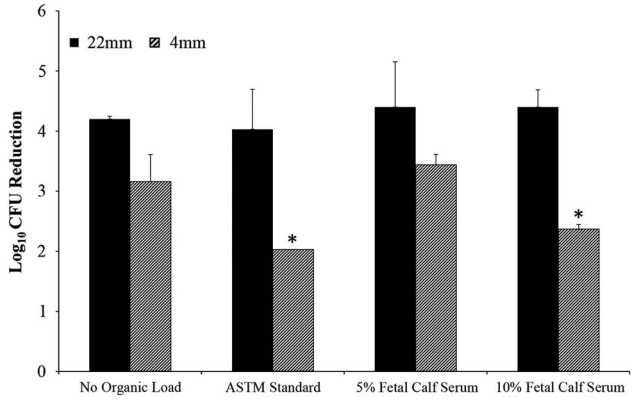

FIGURE 4. Effect of different types of organic load on killing of methicillin-resistant *Staphylococcus aureus* (MRSA) by the Optimum-UV Device. Steel disk carriers were inoculated with 1×10^6 colony-forming units (CFU) of MRSA in 10 µL of the organic load solution and the inoculum was either allowed to dry as a central droplet (~4-mm^2 area) or spread to cover the entire 22-mm^2 surface area. The carriers were placed 4 feet from the device at a height of 4 feet and irradiated for 3 minutes. The means of data from triplicate experiments are presented. Error bars indicate standard error. Asterisk indicates $P < .01$ in comparison with reductions in the absence of organic load.

increased surface area and orientation of carriers in parallel rather than perpendicular with the UV-C lamps. The choice of organic load also impacted the measured efficacy of UV-C. Our findings highlight the need for development of standardized methods for testing the efficacy of UV-C devices. Such standardization is needed to facilitate comparative

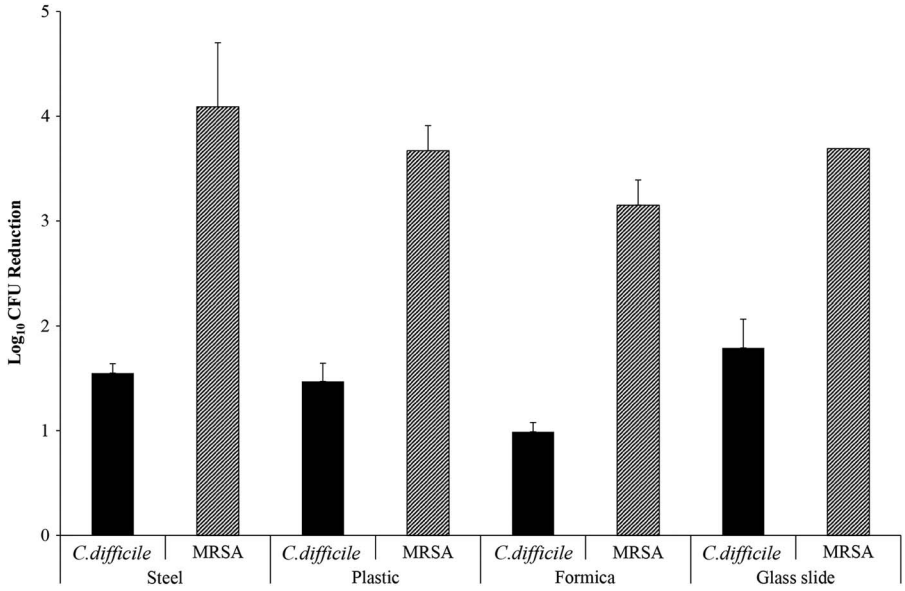

FIGURE 5. Effect of different types of carriers on killing of *Clostridium difficile* spores and methicillin-resistant *Staphylococcus aureus* (MRSA) by the Optimum-UV Device. Carriers were inoculated with 1×10^6 colony-forming units (CFU) of the pathogens in 10 μL of phosphate-buffered saline and spread to cover the entire 10-mm^2 surface area. The carriers were placed 4 feet from the device at a height of 4 feet and irradiated for 5 minutes. The means of data from triplicate experiments are presented. Error bars indicate standard error.

effectiveness evaluations, particularly since there may be significant differences in performance of different UV-C devices.[8]

The most important practical implication of our results is that relatively short UV-C cycles could potentially be effective in reducing pathogens, including *C. difficile* spores, on surfaces. We found that 20 minutes was required to achieve a 2 log reduction in *C. difficile* spores spread to cover 10 mm^2, whereas only 10 minutes was required to achieve a similar reduction in spores spread to cover 22 mm^2. Similarly, Rutala et al[10] found that a 10-minute UV-C exposure was sufficient to reduce *C. difficile* spores by approximately 3 log when the spore inoculum was spread to cover a Rodac template (~25 cm^2). Such cycle times would be much shorter than the approximately 45-minute cycles currently recommended for the Tru-D when used in rooms of patients with *C. difficile* infection.[3–4] Given that pathogens are typically present in relatively low concentrations on hospital surfaces,[3,8] further studies are indicated to validate the effectiveness of relatively short UV-C exposure times in reducing the burden of *C. difficile* spores in isolation rooms.

Our findings have some additional practical implications. Since organic load may reduce efficacy of UV-C, our findings support the recommendation that surfaces be cleaned prior to UV-C exposure.[4,9] The finding that interrupted and uninterrupted UV-C exposures were similarly effective suggests that the practice of running 2 cycles in different locations in the room will not adversely affect performance.[11–12] Finally, positioning of objects such as call buttons or tables in close proximity to UV-C devices has been recommended to take advantage of the fact that efficacy is reduced as distance from the device increases.[3–8] Our results suggest that orientation of objects such that the largest surface area is in parallel with the UV-C lamps may enhance efficacy.

Our study has some limitations. We evaluated only 2 pathogens and tested only 1 strain of each. Moore et al[20] reported that spores of an epidemic ribotype 027 strain were less susceptible to UV-C than a ribotype 001 strain. Similarly, our group reported some variability in killing of different strains of *C. difficile* and vancomycin-resistant *Enterococcus* by UV-C.[3] To standardize testing of chemical disinfectants against *C. difficile* spores, the Environmental Protection Agency has recommended that 1 clinically relevant strain (ATCC 43598) be used and that a standard method be used for spore preparation.[21] The *C. difficile* strain used in our study was ATCC 43598. Additional studies are needed to assess the impact of different methods of preparation and handling of *C. difficile* spores because factors such as triggering of germination or heat activation could have a significant impact on UV-C efficacy.[22–23]

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Potential conflicts of interest: C.J.D. reports that he has served on advisory boards for Clorox and 3M and has received research grants from 3M, EcoLab, GOJO, and Clorox. All other authors report no conflicts of interest relevant to this article.

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