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Concise Communication

A do-it-yourself test protocol using commercial *Bacillus atrophaeus* spores to evaluate the effectiveness of ultraviolet-C light room-decontamination devices

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Abstract

We developed a do-it-yourself test protocol using commercial *Bacillus atrophaeus* spores to assess the efficacy of ultraviolet-C (UV-C) light room-decontamination devices. Overall, 4 UV-C devices reduced *B. atrophaeus* by ≥3 $\log_{10}$ colony-forming units in 10 minutes, whereas a smaller device required 60 minutes. Of 10 in-use devices, only 1 was ineffective.

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Ultraviolet-C (UV-C) light room-decontamination devices are not regulated by the US Food and Drug Administration (FDA), and there are no standard test protocols to demonstrate efficacy.[1–3] This is an important concern because variations in test methods can markedly impact reductions of pathogens achieved by UV-C,[1,4] and devices may vary substantially in efficacy.[5] Standardized test protocols could improve consumer confidence when assessing the claims of manufacturers.[3,6,7] Such testing requires technical expertise and has typically been performed by commercial laboratories or research groups.

For several reasons, it would be advantageous for healthcare facilities to have access to do-it-yourself test protocols to evaluate UV-C efficacy. First, in-house testing could be used to compare devices being considered for purchase. Second, in-use devices could be tested intermittently to ensure appropriate performance. Third, there can be substantial discrepancies in results obtained by commercial laboratories and research groups using similar methods (authors' unpublished data). Finally, some manufacturers' service agreements prohibit testing of devices by outside parties. Here, we developed a do-it-yourself test protocol for UV-C room decontamination devices that would require limited or no onsite microbiological expertise.

Methods

Test organisms

Bacillus atrophaeus (ATCC 9372) and *Geobacillus stearothermophilus* (ATCC 12980) spores were purchased from Mesa Laboratories (Lakewood, CO) as spore suspensions or on preprepared 8×12-mm steel disks inoculated with 10^3 or 10^6 colony-forming units (CFU) of spores. For comparison, a clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolate and *Clostridioides difficile* American type culture collection (ATCC) strain 43598 were tested.[4] MRSA and *C. difficile* carriers were prepared by spreading 10 µL containing 6 $\log_{10}$ CFU onto 20-mm steel disks. *C. difficile* spores were prepared as previously described.[4]

Devices tested

We included 5 low-pressure mercury UV-C room decontamination devices in laboratory testing: Tru-D (Professional Disposables International, Woodcliff Lake, NJ), Rapid Disinfector (Steriliz LLC, Rochester, NY), UVDI-360 Room Sanitizer (UltraViolet Devices, Santa Clarita, CA), Guardian (Camillus LLC, North Canton, OH), and VORTEX-UV Portable UVC Room Sanitizer (MRSA-UV LLC, West Palm Beach, FL). The VORTEX-UV device is a small device intended for decontamination of small rooms up to ~6×6 m (~20×20) feet in 15 minutes. For larger rooms, concurrent use of multiple devices is recommended. We tested 10 additional UV-C room decontamination devices used in hospitals against *B. atrophaeus*.

Test protocol

A 3-$\log_{10}$ or greater reduction in test organisms in comparison to untreated controls was considered an indication of effective decontamination.[4] In initial experiments, 10 minutes of UV-C exposure from the UVDI-360 Room Sanitizer consistently reduced *B. atrophaeus*, *G. stearothermophilus*, and *C. difficile* spores by >3 $\log_{10}$ CFU. Therefore, the test protocol was defined as 10 minutes of exposure to steel disk carriers aseptically adhered to petri-dish bottoms positioned parallel to lamps 0.914 m from the device at the midpoint of the lamps. *B. atrophaeus* was selected as the indicator organism because cultures were easier to process than

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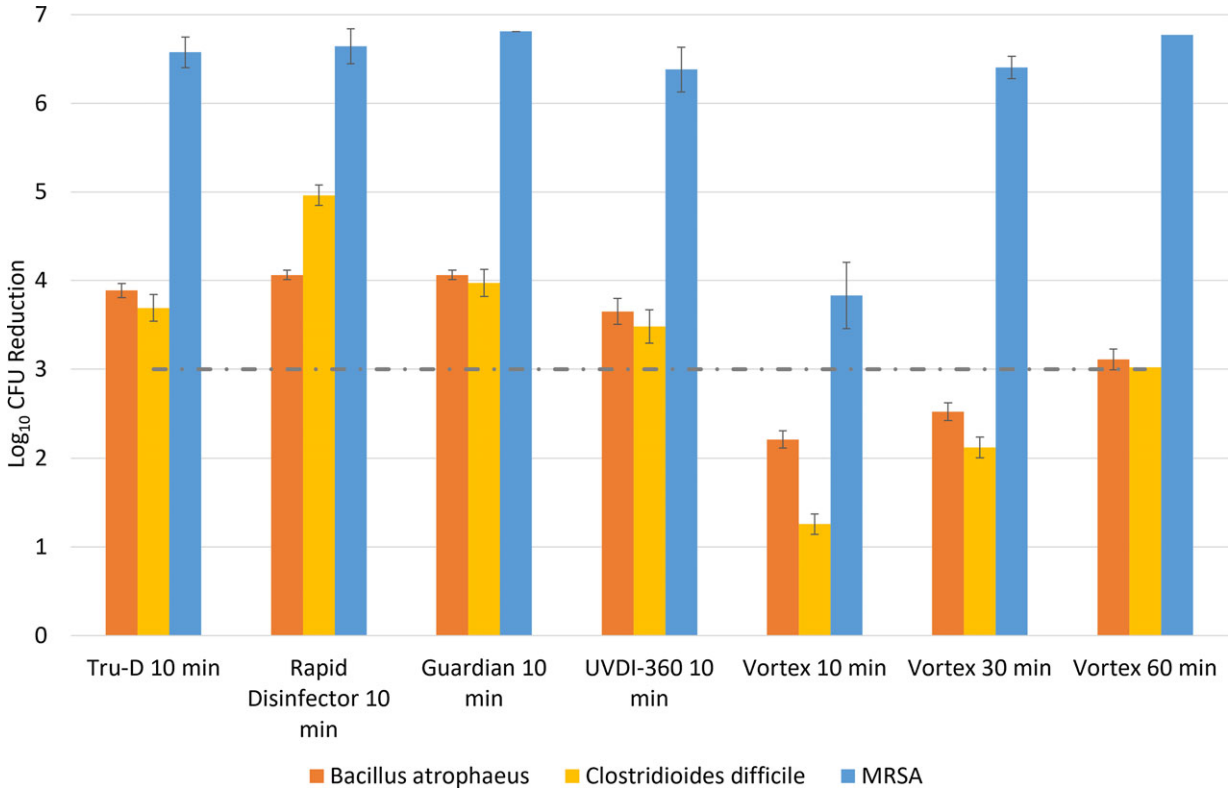

Figure 1. Log_{10} reductions in recovery of the test organisms on steel disk carriers after exposure to ultraviolet-C light from room decontamination devices. The carriers were placed 0.91 m from the device and oriented parallel to the bulbs. MRSA, methicillin-resistant *Staphylococcus aureus*. Note. CFU, colony-forming unit. The dashed line indicates a 3-log_{10} reduction, which was considered an indication of effective decontamination.

G. stearothermophilus cultures. A detailed description of the proposed do-it-yourself test protocol is provided as Supplementary Material (online).

After identification of standard test conditions, the devices were tested against 6-log_{10} CFU disks. Additional testing was conducted with longer cycle times of up to 60 minutes if a 3-log_{10} reduction in spores was not achieved in 10 minutes. Each test was repeated in triplicate.

For *B. atrophaeus*, we assessed whether 10-minute UV-C cycles would consistently reduce 3-log_{10} CFU disks to undetectable levels. For each device, 10 disks exposed to UV-C were aseptically transferred to tubes containing 2 mL trypticase soy broth that were incubated for up to 7 days. The percentages of disks with positive cultures were calculated.

Testing of devices used in hospitals

In addition to the 5 devices included in laboratory testing, 10 UV-C room decontamination devices used in hospitals were tested against *B. atrophaeus* using the standard test protocol. For devices not achieving a ≥3-log_{10} reduction, the manufacturer was contacted to ensure that maintenance was up to date.

Microbiology

Disks were processed to quantify MRSA and *C. difficile*.[5] For *B. atrophaeus* and *G. stearothermophilus*, disks were processed as described in the Supplementary Materials (online). Log_{10} CFU reductions were calculated by subtracting viable organisms recovered from treated versus untreated carriers.[5]

After UV-C exposure, the 3-log_{10} CFU *B. atrophaeus* disks were transferred to tubes containing 2 mL trypticase soy broth. The tubes were incubated at 30°C for up to 7 days. Aliquots from all tubes were plated on trypticase soy agar and were assessed for growth of *B. atrophaeus*.

Results

As shown in Figure 1, the Tru-D, UVDI-360, Rapid Disinfector, and Guardian devices reduced MRSA and the spore-forming organisms by >6 log_{10} and >3 log_{10} CFU, respectively. The VORTEX device did not reduce the spore-forming organisms by ≥3 log_{10} when operated for 10 or 30 minutes but did when operated for 60 minutes.

Figure 2 shows the percentage of the 3-log_{10} *B. atrophaeus* disks with negative cultures after exposure to a 10-minute cycle of UV-C. For the Tru-D, UVDI-360, Rapid Disinfector, and Guardian devices, all 10 treated disks had negative cultures, whereas only 1 of 10 disks treated with a 10-minute cycle with the VORTEX device had a negative culture.

Of the additional 10 devices used in hospitals, 1 (10%) did not achieve a 3-log_{10} reduction in *B. atrophaeus* using the proposed protocol or the manufacturer's recommended protocol. For this device, 10 of 10 disks with 3 log_{10} CFU of *B. atrophaeus* had positive cultures after UV-C exposure. The manufacturer stated that the bulb was not due for replacement.

Discussion

Infection prevention personnel often face uncertainty when assessing claims regarding the efficacy of UV-C light devices.[6]

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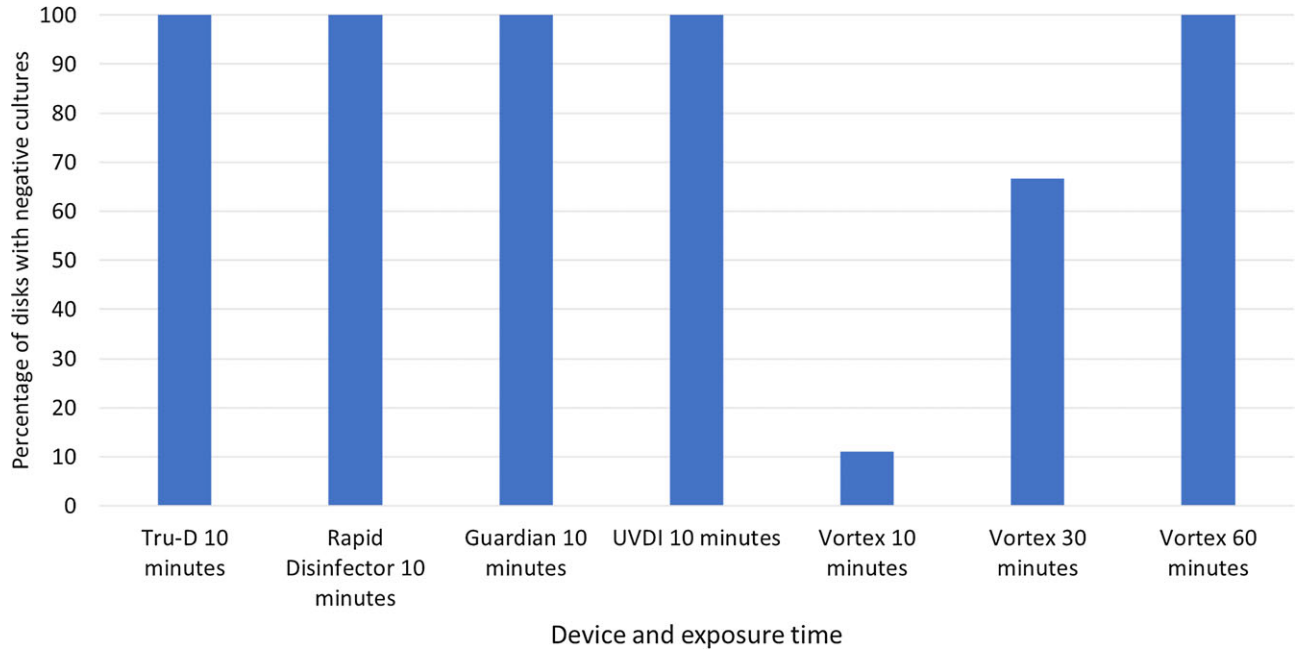

Figure 2. Percentage of the steel disks inoculated with 10^3 colony-forming units of *Bacillus atrophaeus* spores with negative cultures after exposure to a 10-minute cycle of ultraviolet-C light from room-decontamination devices.

To address this uncertainty, we propose a do-it-yourself test protocol analogous to the use of biological indicators to assess sterilization technologies. Our findings provide proof of concept that this protocol could be useful for onsite evaluation of UV-C light room-decontamination devices.

The proposed test method requires limited or no onsite microbiological expertise. The use of *B. atrophaeus* spores from commercial vendors specializing in production of biological indicator spores provides standardization of the test organism. After UV-C exposure, the disks can be placed in sterile containers and mailed to the vendor for nonquantitative (10^3 CFU disks) or quantitative (10^6 CFU disks) cultures. Alternatively, cultures can be processed on site by microbiology laboratories. The process for nonquantitative cultures is particularly straightforward, and *B. atrophaeus* is nonpathogenic with no safety concerns.[8]

The inexpensive VORTEX device was less effective than the standard room devices. However, with a 60-minute cycle the device achieved a $\geq$3 $\log_{10}$ CFU reduction in *B. atrophaeus*. Thus, for devices that have relatively low UV-C output, similar results may be achieved with longer cycle times and/or by placing multiple devices in the room.

Our study had several limitations. The proposed test protocol was not intended to address all variables that impact UV-C performance.[4] Rather, it was well suited for direct comparison of devices and assessment of in-use devices. Others have proposed test protocols in simulated patient rooms.[3] Colorimetric indicators and irradiance measurements using a radiometer could be useful as adjunctive methods to assess UV-C dose delivery.[9,10] Therefore, we have included information and results for these methods as Supplementary Material (online). Finally, additional studies are needed to validate the proposed protocol and to determine whether mailing specimens has an impact on culture results.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/ice.2023.24

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