

Effectiveness of a Portable, Large-Area Ultraviolet Germicidal Device

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Abstract

Effective disinfection of the hospital environment is a key component in the prevention of healthcare-associated infections. The objective of this project was to evaluate the effectiveness of an ultraviolet germicidal device in reducing the concentrations of culturable bacteria on indoor surfaces. The ultraviolet germicidal device was installed and operated in four experimental trials conducted in a microbiology research chamber. Agar plates inoculated with known concentrations of two test microorganisms were placed on benches inside the chamber at two distances, 1.5 meters and 3.0 meters from the machine, for exposure times of 5 minutes, 10 minutes, and 20 minutes. With test agar plates directly exposed to ultraviolet radiation, percent reductions were all >99.9% compared with the laboratory control plates. However, with indirect UV exposure, the edge of the plastic petri dishes provided some protection from the UV source, as indicated by the presence of colonies along the edge of the agar plates. Additional research will be conducted to further characterize the device for optimal use in surface decontamination and to determine its effectiveness in reducing airborne culturable bacterial concentrations.

Key words healthcare-associated infections, ultraviolet germicidal device, *Staphylococcus aureus*, *Escherichia coli*, surface decontamination

Introduction

The estimates of direct medical costs of healthcare-associated infections (HAI) to U.S. hospitals range from \$28 to \$45 billion annually (Scott, 2009). Contaminated environmental surfaces may play an important role in the transmission of emerging pathogens, such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and *Clostridium difficile*, to hospital patients (Nerandzic, Cadnum, Pultz, & Donskey, 2005; Rutala, Gergen, & Weber, 2010). Therefore, effective disinfection of the hospital environment is a key component of HAI prevention. One method of disinfection is ultraviolet (UV) radiation. UV-C radiation (wavelength 100-280 nm) is the most harmful to living organisms because of its damaging effect to the nucleic acids, DNA and RNA (WHO, 2002). UV-C irradiation inactivates nucleic acids by abnormally chemically bonding adjacent thymine and cytosine bases, forming thymine dimers. Previous studies have shown varying degrees of susceptibility of all groups of microorganisms to UV irradiation, including bacterial endospores and protozoan cysts (Blatchley & Peel, 2001). UV radiation has been used for the control of airborne transmission of pathogens in hospital operating rooms for more than 50 years. However, its use has been mainly for disinfection of air through duct and upper-room air irradiation (Blatchley & Peel, 2001).

More recently, UV irradiation has been applied to the disinfection of surfaces, and portable devices incorporating germicidal UV lamps have been designed and tested (Katara, Hemvani, Chitnis, Chitnis, & Chitnis, 2008; Nerandzic et al., 2005; Owens et al., 2005; Rutala et al., 2010). A new device has been developed by Hypermed, Inc. that combines air and surface disinfection capabilities, the Portable, Large-Area Ultraviolet Germicidal (UVG) Device. The objective of this project was to evaluate the effectiveness of the UVG Device in reducing the concentrations of culturable bacteria on indoor surfaces. Four experimental trials were performed in the microbiology research chamber located at the University of Nevada, Las Vegas.

Experimental Design

The UV unit was evaluated to determine its efficacy in reducing culturable concentrations of surface-associated bacteria. The UVG Device was installed and operated in a total of four experimental trials in

the research chamber to determine the effect of the device on culturable surface-associated bacteria. In the first three trials, agar plates inoculated with known concentrations of two test microorganisms were placed on benches inside the chamber at two distances, 1.5 m and 3.0 m, from the machine. The plates were uncovered, and the UVG Device was activated in open mode (no air flow) for exposure times of 5 minutes, 10 minutes, and 20 minutes. Covered, shielded chamber control plates were placed in the chamber next to test plates, and laboratory control plates were also inoculated, but not placed in the chamber. Ozone concentrations were measured in the chamber during each of the three experiments. The agar plates were incubated, and colony forming units (CFU) were enumerated. CFU data were analyzed, and the reductions in CFU were calculated for each of the test bacteria at the two distances and the three exposure times. Results obtained showed that the edge of the plastic petri dishes provided some protection from the UV source, as indicated by CFU present along the edge of the agar plates, and that this effect was greater at a distance of 3.0 m from the UV source than 1.5 m from the source. Therefore, one additional trial was performed consisting of exposure for 10 minutes, but with the benches holding inoculated plates tilted so that the entire plates were subjected to UV radiation.

Materials and Methods

Ultraviolet Germicidal Device

The Portable, Dual-Mode, Large-Area Ultraviolet Germicidal (UVG) Device (Hypermed, Inc., Las Vegas, NV) is equipped with 8 UV light bulbs (Philips 25 Watt UV Germicidal Bulb) (Figure 1). In the open mode, the UVG Device bulbs extend horizontally to disinfect surfaces within 3.0 m of the device. In closed mode, a fan operating at 57 cubic meters per minute draws air past UV bulbs in the central core, disinfecting the air.

Test Organisms and Culture Preparation

The test microorganisms were *Staphylococcus aureus* (ATCC #6538), a gram-positive bacterium, and *Escherichia coli* (ATCC #25922), a gram-negative bacterium (American Type Culture Collection, Manassas, VA).

For each experiment, stock cultures of *S. aureus* and *E. coli* were used to inoculate one 125 ml flask containing 25 ml of tryptic soy broth (TSB, Difco Laboratories, Sparks, MD) for each microorganism. The cultures were incubated overnight (37°C, 60

rpm) in an environmental shaker (G24 Environmental Incubator Shaker, New Brunswick Scientific Co. Inc.). The following morning, a working culture was prepared for each microorganism by adding 1 ml of the overnight culture to a 250 ml flask containing 100

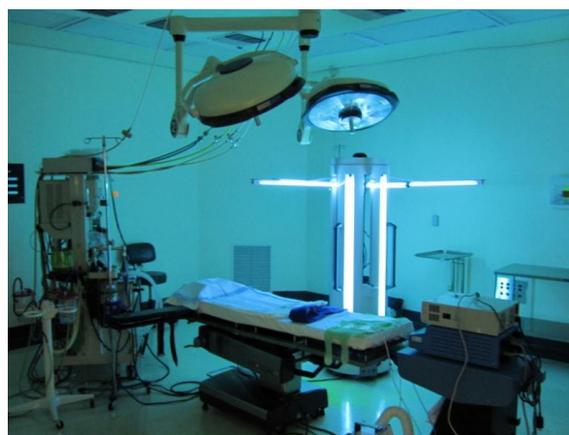


Figure 1. The Portable, Dual-Mode, Large-Area UVG Device shown closed (top photo) and operational in open mode (bottom photo).

ml of TSB and incubated in the environmental shaker (37°C, 200 rpm). The cultures were checked periodically for the desired optical density (OD) at a 600 nm wavelength using a spectrophotometer

(Spectronic Genesys 5, Milton Roy). At $OD_{600nm} = 1.1$, the cultures were harvested by centrifugation (Hermle Z360K Centrifuge, National Labnet Co.) for 5 minutes at $4200 \times g$. The supernatants were discarded and the pellets were resuspended to the original volume using sterile 0.01M potassium phosphate buffer (PB). The cultures were washed two times by centrifugation as described above and resuspended in sterile PB. The washed suspensions were serially diluted and spread plated onto tryptic soy agar (TSA, Difco Laboratories) plates (laboratory controls) to determine the culturable concentrations of the suspensions. Replicate chamber control and test agar plates were then inoculated by serial dilution and spread plating to achieve approximate concentrations of 10^3 , 10^2 , and 10^1 CFU per plate. Inoculated plates were transported to the chamber and exposed to UV light as described below. After testing was completed in the chamber, laboratory and chamber control plates were incubated with test plates for 24 hours at 37°C , and CFU were enumerated.

Test Chamber

Trials were conducted in a test chamber designed to resemble a residential indoor environment, and previously used in decontamination studies (Buttner et al., 2004). The chamber, which measures 4.0 m by 4.0 m by 2.2 m high, has a sheet vinyl tile floor. The interior walls, exterior walls, and ceiling are covered with gypsum wallboard and coated with interior latex paint. The chamber is equipped with a heating, ventilation, air conditioning (HVAC) system utilizing rectangular bare metal ductwork and sized to simulate a residential system. During these experiments, the HVAC was not operated during testing, and the chamber was used under "static" conditions. An anteroom equipped with a HEPA-filtered air shower attached to the chamber entrance was used to reduce mixing of air resulting from entering and exiting the chamber during experiments. For all activities in the research chamber, technicians wore full-face respirators and non-woven protective clothing. Upon completion of trials, the chamber was disinfected with a 0.5% sodium hypochlorite solution.

UVG Device Tests

For trials 1-3, inoculated chamber control and test agar plates were placed on benches in the test chamber, at distances of 1.5 m and 3.0 m from the UVG Device. Chamber control plates remained covered and were wrapped in aluminum foil, while test plates were uncovered. For each test

microorganism, a total of 20 test plates were placed, 10 plates ($5 = 10^3$ CFU, $5 = 10^2$ CFU) at 1.5 m from the device, and 10 plates ($5 = 10^3$ CFU, $5 = 10^2$ CFU) at 3.0 m from the device. For each test microorganism, a total of 9 chamber control plates were placed ($3 = 10^3$, $3 = 10^2$, $3 = 10^1$ CFU) at a distance of 1.5 m from the device. For each trial, there were a total of 58 agar plates in the chamber (9 chamber control plates \times 2 microorganisms, and 20 test plates \times 2 microorganisms). After exiting the chamber, the UVG Device was activated in open mode for a period of 5 minutes. At the end of the exposure time, the device was deactivated and the exposed agar test plates were covered and collected with the chamber control plates. The experiment described above was repeated for 10-minute and 20-minute exposure times.

For trial 4, the test conditions were identical, except plates were placed on benches tilted towards the UV source in the test chamber, at distances of 1.5 m and 3.0 m from the UVG Device. There were a total of 58 agar plates in the chamber (9 chamber control plates \times 2 microorganisms, and 20 test plates \times 2 microorganisms). After exiting the chamber, the UVG Device was activated in open mode for a period of 10 minutes. At the end of the exposure time, the device was deactivated and the exposed agar test plates were covered and collected with the chamber control plates. Temperature and relative humidity were recorded at the beginning and end of testing. Ozone was monitored continuously during testing with a C-30ZX ozone monitor and DL-3 Data Logger (Eco Sensors, Inc., Santa Fe, NM).

Data Analysis

The reduction in surface concentrations of the two test microorganisms was determined by culture analysis. CFU were enumerated for laboratory and chamber control plates, averaged, and compared with mean CFU from test plates. The percent reduction was calculated for each test microorganism and at each distance for the 10-minute exposure time to evaluate the effectiveness of the UVG Device in reducing surface bacterial concentrations in the chamber.

Results

For trials 1-3, the concentration of both *E. coli* and *S. aureus* test cultures after harvesting, washing and spread plating was 1.6×10^9 CFU/ml, as determined by triplicate laboratory control plates. The CFU enumerated on the chamber control plates that were

covered in aluminum foil before exposure to UV light were nearly identical to the laboratory control plates, with concentrations ranging from 1.5×10^9 to 1.8×10^9 CFU/ml. No ozone was detected in the chamber in any of the three tests (lower detection limit, 0.1 ppm). The temperature and relative humidity at the beginning of testing were 23.9°C and 35%, respectively, and at the end of testing the temperature and relative humidity were 25.0°C and 40%, respectively.

The CFU enumerated on the test plates were averaged, converted to CFU/ml of the inocula, and the mean values obtained from trials 1-3 were used to determine the percent reduction compared with the laboratory control plates (Table 1). The data showed that longer UV exposure time resulted in greater percent reductions in CFU, and shorter distances from the UV source also resulted in greater percent reductions. In addition, greater reductions were observed with *S. aureus* than with *E. coli*.

The edge of the plastic petri dishes provided some protection from the UV source, as indicated by CFU present along the edge of the agar plates. The effect was greater at a distance of 3.0 m from the UV source than 1.5 m from the source. Therefore, a fourth trial was performed to determine if percent reductions were greater if the entire plate was subjected to UV radiation.

For trial 4, the concentrations of *E. coli* and *S. aureus* test cultures after harvesting, washing and spread plating were 1.3×10^9 CFU/ml and 1.5×10^9 CFU/ml, respectively, as determined by triplicate laboratory control plates. The CFU enumerated on the chamber control plates that were covered in aluminum foil before exposure to UV light were 1.8×10^9 for *E. coli* and 1.5×10^9 CFU/ml for *S. aureus*, indicating that UV exposure had no effect on their culturability. No ozone was detected in the chamber during the 10-minute test (lower detection limit, 0.1 ppm). The temperature and relative humidity measured were 21.1°C and 23%, respectively, at both the beginning and end of testing.

With test agar plates directly exposed to UV radiation, there were no CFU enumerated after UV

exposure for 10 minutes at distances of 1.5 m and 3.0 m from the source (Figure 2). Compared with the laboratory control plates, all percent reductions were greater than 99.9%.

Discussion

Results from all trials indicate that exposure time, distance from UV source, and interference between UV source and test surfaces effected percent reductions of CFU. Overall, longer exposure time and shorter distances between the UV source and test surfaces resulted in greater percent reductions. In trials 1-3, the edge of the plastic petri dish prevented some of the UV radiation from reaching the agar surface of the plate, as indicated by CFU present along the edges. Results from trial 4 suggest that percent reductions are greatest when the entire surface is subjected to UV radiation and no interference is present. The percent reductions observed in trial 4 indicate at least a 3 order of magnitude decrease in culturable bacteria after UV light exposure for 10 minutes with the UVG Device. It is possible that greater reductions may occur; however, only two concentrations of the test microorganism were tested in this phase of the study, with a corresponding limit of detection of 3 orders of magnitude reduction in culturable bacteria.

The 10-minute exposure results observed in this study were comparable to those observed in other studies that evaluated UV surface decontamination devices (Nerandzic et al., 2005; Owens et al., 2005; Rutala et al., 2010). However, bacterial spores have been shown to be more resistant to UV irradiation than vegetative cells. One device achieved a 2-3 log reduction in culturable *Clostridium difficile* spores and methicillin-resistant *Staphylococcus aureus* after 45 minutes of exposure (Nerandzic et al., 2005). A shorter exposure of 20 minutes was effective against vegetative bacteria, but disinfection of spores was reduced. Similarly, another device was tested that reduced culturable vegetative bacteria by over 3 logs in 15 minutes, but 50 minutes was necessary for a 3-log reduction of *C. difficile* spores (Rutala et al., 2010). The effectiveness of the UVG Device was not evaluated with bacterial spores in this study.

Table 1. Percent reduction in colony forming units (CFU) obtained after exposing agar plates inoculated with the two test microorganisms to UV light for different times and distances.

UV Exposure Time (minutes)	Distance from UV Source (feet)	Percent Reduction	
		<i>E. coli</i>	<i>S. aureus</i>
5	5	94.9	97.1
	10	56.3	69.4
10	5	96.8	98.5
	10	78.1	86.3
20	5	98.8	99.8
	10	90.0	97.0

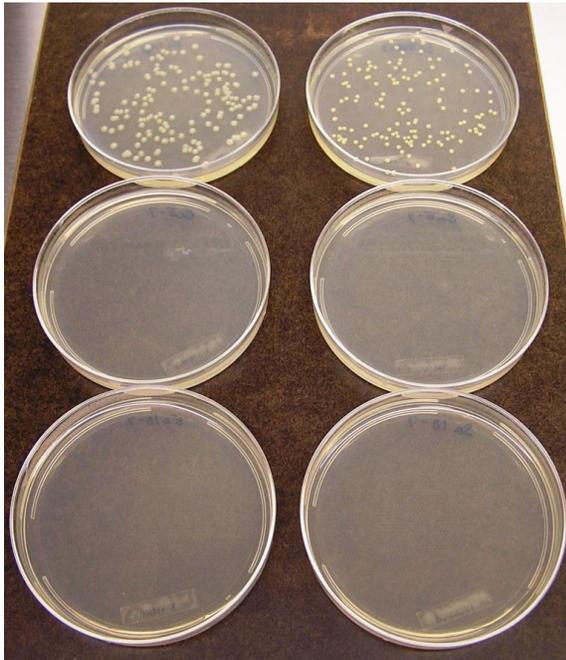


Figure 2. Photograph of agar plates after UV exposure for 10 minutes. Top row: *E. coli* (left) and *S. aureus* (right) colonies growing on plates that were covered and wrapped in aluminum foil. Middle row: No growth on plates that were exposed to UV at a distance of 1.5 m from the source. Bottom row: No growth on plates that were exposed to UV at a distance of 3.0 m from the source.

A limitation of this research was that the radiation dose at the plate surfaces was not determined in this study. Eight 250-watt bulbs were used in the UVG Device. Previous disinfection device tests have indicated effective surface doses ranging from 3,000-36,000 $\mu\text{W}/\text{cm}^2$, depending on the output of the device and the microorganism being tested (Nerandzic et al., 2005; Owens et al., 2005; Rutala et al., 2010). A germicidal UV bulb with 40-watt power was shown to have efficient inactivation of

bacteria to a distance of up to 2.44 m on either side of the bulb with an exposure time of 30 minutes (Katara et al., 2008).

In summary, the UVG Device tested in this study achieved a reduction of vegetative bacterial cells >99.9% up to a distance of 3.0 m with an exposure time of 10 minutes. The edge of the plastic petri dishes provided some protection from the UV source; therefore, direct irradiation of surfaces was required. Future studies will characterize the UV-C radiation dose per area of surface for the UVG Device in the test chamber, and additional microorganisms, including bacterial spores, will be tested to determine the effective operation of the unit for surface disinfection. In addition, research will be conducted in which microorganisms will be aerosolized in control and test events in the chamber, and the UVG Device will be operated in closed mode (with air flow through the machine) to determine the effectiveness of the device in reducing airborne culturable bacterial concentrations. This study demonstrated the effectiveness of the UVG Device in reducing concentrations of culturable bacteria on indoor surfaces, a key component in the prevention of health-care associated infections in hospital environments. Therefore, potential applications of this technology are deployment of the UVG Device for at least five minutes in the following hospital settings: (1) in operating rooms between surgeries, repeated as necessary to overcome any blind-spots; (2) in intensive care units during patient changeover and when patients are out of the room undergoing tests; (3) in regular patient rooms during patient changeover; and (4) in emergency rooms during patient changeover.

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