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Monitoring Ultraviolet Lamps in Biological Safety Cabinets with Cultures of Standard Bacterial Strains on TSA Blood Agar

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Abstract

Background: Ultraviolet radiation is often used to decontaminate the interior surfaces of biological safety cabinets (BSCs). The Centers for Disease Control and Prevention (CDC) recommends a UV lamp intensity of 40 μW/cm^2 at the center of the work area to ensure surface decontamination. A commonly-used source of UVC in BSCs—the G30T8 lamp—provides approximately 125 μW/cm^2 one meter from the lamp. However, since many BSCs have an interior height of less than one meter, UV intensities at the work surface should be considerably higher because UV radiation follows an inverse square law. Few laboratories have the instruments necessary to measure UVC intensity. The Web site of the Atlantic Ultraviolet Corporation lists UV dosages required to kill a wide range of microorganisms. This study investigates the possibility of using readily available strains of 4 common bacteria to monitor the output of UV lamps in BSCs.

Methods: Inoculated plates with 4 bacterial strains were exposed to UV radiation in a plate carrier device exposed for different times and in different locations in the BSC. Half the plates were covered with a strip of aluminum to serve as an unexposed growth control. Reduced UV intensities were simulated using Petri dish sleeves that provided a range of UV intensities from 20 μW/cm^2 to 510 μW/cm^2. Various UV intensities were measured in different areas of the BSC, and compared with calculated and estimated values. Various times to kill the bacteria were measured based on bacterial strain, UV intensity, exposure time, and location in the BSC.

Results: In a blinded study in which the UV intensities were estimated from the killing times of the bacteria and compared with actual measured values, 4 of the 6 measured values were in the estimated ranges, and 2 estimated ranges were slightly below the measured values.

Conclusion: Our results support the hypothesis that cultures of known bacterial strains could be used to closely estimate the UV lamp output.

As an aid in the decontamination of surfaces in a biological safety cabinet, ultraviolet radiation of wavelength 254 nanometers (nm) is often used. This is in the range of wavelengths known as UVC. The Centers for Disease Control and Prevention (CDC) recommends that, if used in a BSC for surface decontamination, to ensure the energy output is sufficient to kill microorganisms the lamp be tested periodically, and the intensity should not be less that 40 microwatts per square centimeter (μW/cm^2) at the center of the work area.[1] The manufacturer of a BSC may also advise periodic monitoring of UV and/or periodic replacement of the UV lamp, in which case the user would be expected by accreditation agency requirements to follow such recommendations. Probably the most commonly used source of UVC in BSCs is the G30T8 (G30W) lamp. Having a nominal lamp power of 30 watts, these typically provide a germicidal UV intensity (irradiance or radiation output) of approximately 125 μW/cm^2 one meter from the lamp.[2] In many BSCs, the distance from the lamp to the floor of the cabinet is less than one meter, so intensities at the work surface considerably greater than 125μW/cm^2 should be expected as UV radiation follows an inverse square law.

Few clinical microbiology laboratories possess the instrumentation to measure UVC intensity, and it is not always obvious from the certification or recertification stickers on BSCs whether or not this was done by the person who last checked the BSC's performance. In addition, some recommendations are for monitoring at intervals shorter than the annual BSC recertification checks of airflow.[3]

The Web site of the Atlantic Ultraviolet Corporation lists UV dosages required to kill a wide range of microorganisms.[4] No units of energy are given, but are probably μW seconds/cm^2 (ie, intensity × duration of exposure). The UV dose for *Escherichia coli* and *Staphylococcus aureus* is 6,600, for *Enterococcus fecalis* it is 10,000, and for *Pseudomonas aeruginosa* it is 3,900.

The study reported here investigated the possibility of using readily available standard strains of these 4 bacteria to monitor the output of UV lamps in BSCs. Killing times at various UV intensities were determined.

Materials and Methods

For measurements of UV intensity, an International Light Model IL1400 photometer was used with an IL UVC detector #SEL240/TD (International Light, Newburyport, MA) (**Image 1**). This instrument gives a direct readout of intensity in μW/cm^2.

The bacterial strains used were 4 of those required for in vitro susceptibility testing by the Clinical and Laboratory Standards Institute, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus fecalis* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853.[5] These were maintained on tryptic soy agar (TSA). For inocula for exposure tests the bacterial strains were inoculated into tryptic soy broth (TSB) and incubated to a turbidity equivalent to the 0.5 McFarland standard. Fifty μL of this was diluted into 1 mL of TSB, and 1 μL of this used to inoculate half the agar surface using disposable calibrated loops and streaking in

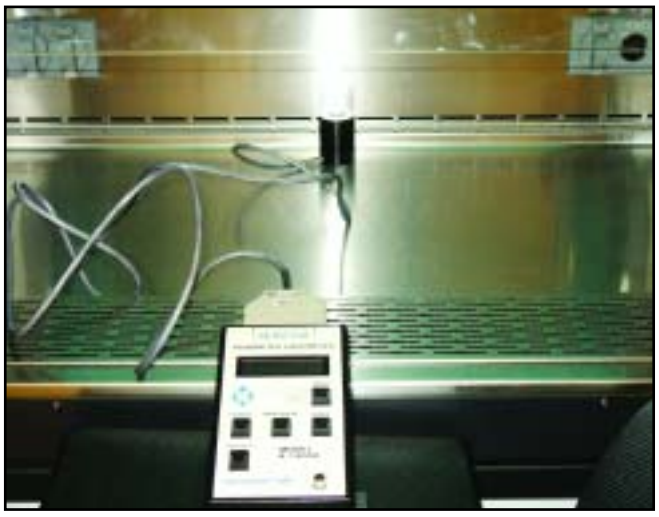

Image 1_The IL1400A meter, with the UVC detector in position at the center rear of the authors' BSC.

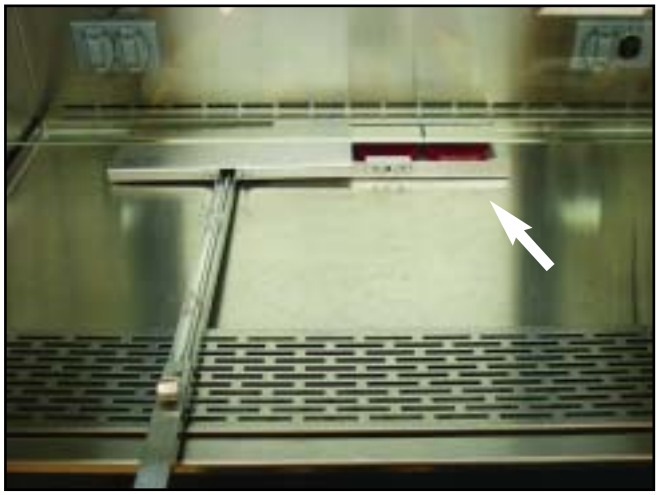

Image 2_Two of 3 inoculated Petri dishes being exposed at the center rear of the BSC.

Image 3_Close up of 2 of 3 Petri dishes being exposed in the BSC.

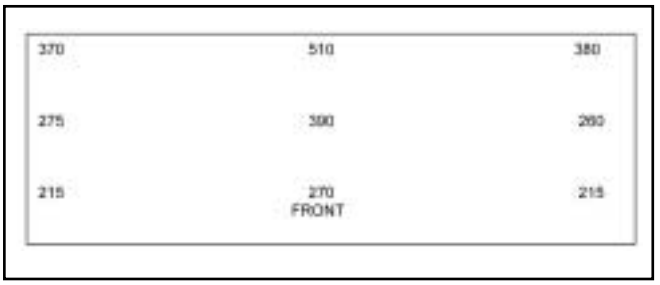

Figure 1_Measured UV intensities (μW/cm^2) at floor level in the authors' BSC, with the detector positioned vertically. The biological safety cabinet's floor dimensions are 36" wide, 18" deep.

3 directions at 60° to each other. Initial studies were performed with plain TSA, and later with TSA with 5% sheep blood. After exposure, the plates were placed in the dark and placed in a 35°C incubator within 1 hour of exposure. Growth was recorded after 18 to 24 hours and after 48 hours.

Inoculated plates were exposed to UV radiation in a plate carrier device designed and constructed by 1 of the authors (BJH) (**Image 2, 3, 4**). This enabled 3 plates to be positioned at the rear of a BSC and exposed for different times by sideways movement of the cover. A strip of aluminum was used to cover half the plates to serve as an unexposed growth control. A sliding rod in the handle that locked into the plate-carrying tray enabled the tray to be placed in position and withdrawn from the BSC with minimal exposure of the operator's hand to UV. An opaque glove was worn on the left hand as an additional precaution against UV exposure.

The UV lamps were switched on for at least 5 minutes before any readings or exposures were made. To achieve reduced UV intensities (ie, to simulate reduced UV lamp output), the material used for the sleeves in which the Falcon brand of sterile Petri dishes #1029 are supplied was used (Becton Dickinson and Company, Franklin Lakes, NJ). One layer of this material blocked approximately 33% of the transmission of UVC radiation, and by combining up to 8 layers over inoculated plates in the carrier tray, 8 reduced levels of UV intensity could be achieved, plus the initial exposure with no attenuation, giving a range of intensities from 510 μW/cm^2 to 20 μW/cm^2.

Exposure studies were performed in a Labconco Purifier Class II Model #36204 BSC (Labconco Corporation, Kansas City, MO) shown in (**Figure 1, Image 1, 2**). The UV lamp was a clean G30T8 with less than 500 hours of use. In this BSC the UV lamp is positioned on the rear wall of the cabinet and 53 cm (21") above the floor of the cabinet.

As a study to test the feasibility of using the killing times of known bacterial strains to estimate UV intensities, additional exposure studies were carried out in 6 other cabinets on the University of Toledo Health Science campus as a blinded study. The cabinets were selected by convenience sampling (ie, they were available for testing as no one was working in them at the time we were looking for BSCs to test). Five were class II BSCs and 1 was a small cabinet without forced air circulation, used for tissue culture work. These cabinets were tested without prior cleaning of the lamp. One author (MV) took the UV intensity readings in each cabinet at the center rear, and the other author (BJH) exposed the inoculated plates for 10, 20, and 40 seconds at the center rear. One author (BJH) examined the plates after incubation for growth and estimated the UV intensities from the killing times for *S. aureus, E. coli,* and *E. fecalis*. These were then compared with the actual measured intensities.

Results

The measured UV intensity at the center at the rear of the authors' BSC was 510 μW/cm^2. The expected intensity, calculated from the inverse square law and not taking into account

any reflection from the walls of the BSC, was 450 µW/cm^2. Measured values at other locations in this BSC are shown in **Figure 1**. In other BSCs, the actual levels and their relationship to the highest intensity (directly below the center of the lamp) may be different due to reflected UV, cabinet dimensions, and type of lamp and its location, age, and cleanness, etc.

Table 1 shows the results of the killing at different UV exposure intensities and times for the 4 bacteria. All 4 were killed by exposure for 10 seconds at 510 µW/cm^2. *Enterococcus fecalis* was the most resistant to UV, and *P. aeruginosa* was the most susceptible.

Table 2 shows the results of exposure of *S. aureus, E. coli,* and *E. fecalis* for 10, 20, and 40 seconds, and the biological estimates versus measured intensities for 6 BSCs on the health science campus.

Discussion

As expected, the results in **Table 1** show an inverse relationship between exposure intensity and time of exposure for killing. While the dosages (exposure intensity × time) to kill are less than the published data cited earlier,[4] the relative amounts are consistent. The G30T8 UV lamp produces UV intensity of 125 µW/cm^2 at 1 meter from the lamp and after 8,000 hours of use, the UV output is 80% of that of a new lamp.[2] Lamps from other manufacturers may vary slightly (plus or minus) from these ratings. From the inverse square law, a new, clean, properly-functioning G30T8 lamp can be expected to produce a UV intensity of approximately 250 µW/cm^2 at 70 cm from the lamp, and approximately 450 µW/cm^2 at 53 cm. In practice, the intensities achieved at the interior surfaces of BSCs will be affected by factors such as reflected radiation and angle of incidence. Even so, these levels should be well in excess of the CDC's recommended minimum of 40 µW/cm^2. At the CDC's recommendation of an intensity of at least 40 µw/cm^2, the *P. aeruginosa* would require at least 45 seconds of exposure, the *E. coli* and the *S. aureus* at least 90 seconds of exposure, the *E. fecalis* at least 120 seconds of exposure.

Actual exposure times to kill, as measured at the center rear of the BSC, or at the actual center of the floor area, will vary depending on the bacteria used. Times should be in the range of 20 to 40 seconds of exposure for complete killing at the inoculum level used in this study, even towards the end of useful life of the lamp (ie, 8,000 hours operating time and 80% of the initial UV output). Kill times in excess of 120 seconds would suggest levels less than the recommended 40 µW/cm^2.

In the blinded study in which the UV intensities were estimated from killing times and compared to the actual measured values, 4 of the 6 measured values were in the estimated ranges, and 2 estimated ranges were slightly below the measured values. This supports the hypothesis that cultures of known bacterial strains could be used to closely estimate the UV lamp output.

It should be noted that in a 2000 position paper, the American Biological Safety Association stated, quoting the CDC, National Institutes of Health (NIH), and the National Sanitation Foundation (NSF), that UV lights within a BSC are neither recommended nor required.[3] However, in this position paper, it is stated that UV lamps should be checked periodically (approximately every 6 months) to ensure the

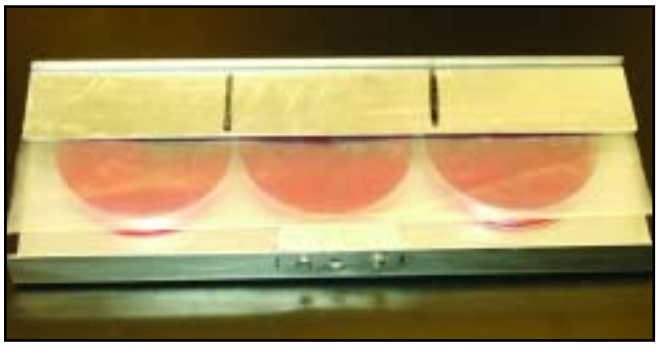

Image 4_Three Petri dishes in the carrier, covered with 2 layers of plastic attenuating the UV intensity to 240 µW/cm^2.

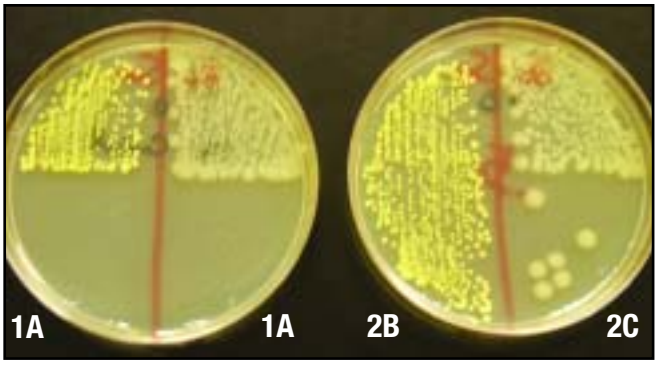

Image 5_*Staphylococcus aureus* (left) and *Escherichia coli* (right) on each plate of TSA. Upper half of each plate was shielded from the UV.
Plate 1 - 510 µW/cm^2 for 10 seconds
Plate 2 - 45 µW/cm^2 for 80 seconds
1 A - complete killing on exposed area of plate
2 B - no killing on exposed area of plate
2 C - marked reduction of number of colonies on exposed area of plate

Table 1_Killing of Bacteria at Different UV Exposure Times and Intensities

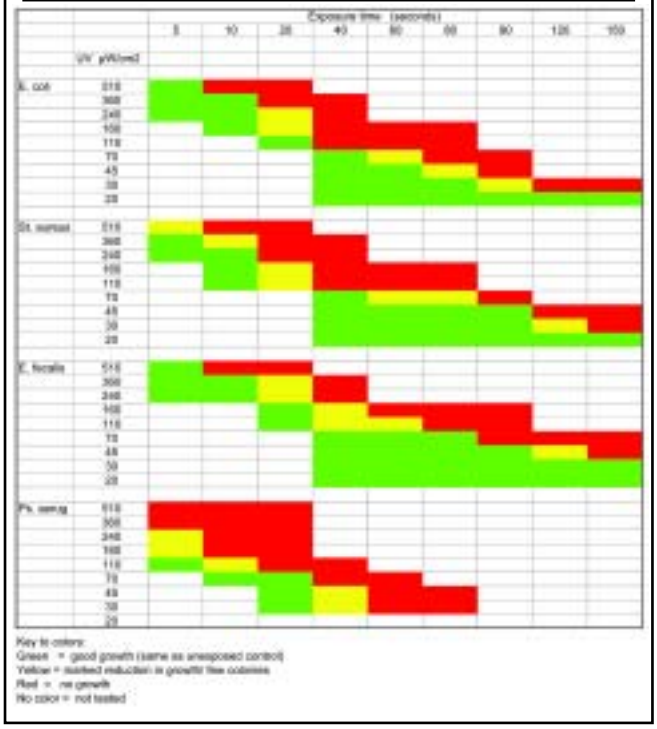

Table 2_Results of Exposure at 10, 20, and 40 Seconds, and the Biological Estimates Versus Measured Intensities for 6 BSCs on the Health Science Campus

appropriate intensity of UV light is being emitted for germicidal activity. No required intensity level is given.

The results of the study reported here show that clinical microbiology laboratories needing to monitor UV lamp output but without having calibrated UVC metering equipment can use readily-available materials (ie, cultures on TSA blood agar of known ATCC strains they already have in the laboratory for other purposes). Inoculated plates can be placed at the desired locations in the BSC, each Petri dish lid opened to cover half the medium (the plastic is opaque to UVC) to provide a growth control, and the exposures timed. From the time to kill in **Table 1**, UV intensity at the test locations in the BSC can be estimated.

At least one state's health department (New York) suggests that plate irradiation testing may help meet the requirement that the energy output of UV lamps in BSCs not be less than 40 $\mu W/cm^2$ (at the surface to be decontaminated) and monitored at least annually.[6] However, no information is given in that suggestion (Quality Assurance Standard 21—Guidance) as to organisms to expose nor exposure times to use. The study presented here gives clinical microbiology laboratories a practical yet simple and documented procedure to meet monitoring requirements with a plate irradiation bioassay. LM

Acknowledgement: Robyn Atkinson, PhD, Wadsworth Center, New York State Health Department, provided details of the New York State Regulations and Standards and the Web site address for those.

1. Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets. 2nd Ed. 2000 CDC/NIH Atlanta, GA.
2. UV Lamps for Germicidal Applications General Electric Company. Available at: www.gelighting.com. Accessed February 4, 2006.
3. Position Paper on the Use of Ultraviolet Lights in Biological Safety Cabinets. 2000 American Biological Safety Association, Mundelein, IL.
4. Microorganisms Deactivated by Ultraviolet Light Exposure. Atlantic Ultraviolet Corporation, Hauppauge, NY. Available at: www.ultraviolet.com. Accessed July 28, 2006.
5. Performance Standards for Antimicrobial Susceptibility Testing M100-S16 16th Informational Supplement. 2006. Clinical Laboratory Standards Institute, Wayne, PA.
6. Clinical Laboratory Evaluation Program, Laboratory Standards. Quality Assurance Standard 21 Guidance. Wadsworth Center, New York State Department of Health. Available at: www.wadsworth.org/labcert/clep/Survey/standardsmenu.htm. Accessed August 28, 2006.