



# Biological Monitoring of Ultraviolet Germicidal Irradiation in a Biosafety Level 3 Laboratory

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## Abstract

Ultraviolet germicidal irradiation (UVGI) lamps are used in biological safety cabinets and laboratory containment rooms as methods of surface decontamination. Although some organizations have discouraged the use of UVGI for disinfection, many scientists continue to request this method. The objective of this study was to assess the reliability of using UVGI produced from UV ceiling lamps as an effective method to decrease microbial agent contamination from floor surfaces in a biosafety level 3 laboratory. Actively growing *Bacillus cereus* and *B. anthracis* vegetative cells or spores (Sterne strain-Veterinary Vaccine Formula) were used as biological indicators to assess UVGI effects within the containment laboratory. Studies were conducted using UVGI exposure times ranging from 15 minutes to 2 hours with varying inoculums ranging from  $10^3$  to  $10^9$  colony-forming unit per sample. Later in the study an UVGI radiometer was used to determine the UVGI intensity and to measure the correlation between the biological indicator results and mechanical instrument data. Study results showed that ceiling-mounted UVGI lamps were effective in reducing the viability of both *B. cereus* and *B. anthracis* vegetative cells and spores after a minimum UVGI exposure time of 1 hour at an intensity as low as  $8 \mu\text{W}/\text{cm}^2$ . Additionally, an UVGI radiometer could be used to determine UVGI effectiveness and a UVGI intensity of  $8 \mu\text{W}/\text{cm}^2$  to  $42 \mu\text{W}/\text{cm}^2$  corresponded with the increased disinfection observed.

## Introduction

Ultraviolet germicidal irradiation (UVGI) has been shown to be an effective disinfecting or sterilizing agent against both vegetative and spore forms of bacteria, as well as other microbial agents (Blatchley et al., 2001; Dietz et al., 1980). The antimicrobial effect of UVGI is accomplished by the adsorption of energy in the Ultraviolet-C (UV-C) range of 100-280 nm (ABSA Position Paper, 2000) which chiefly affects purines and pyrimidines of nucleic acids. This alteration in nucleic acids leads to a block in transcription, thereby preventing replication of

the cell. Mercury vapor lamps, also referred to as germicidal lamps, are the most common lamps used for disinfection purposes since they emit 90% of their radiation in the UV-C wavelength of 254 nm (Davis et al., 1980).

Numerous studies have been conducted to document the effects of UVGI exposure against microbial agents. These include the decontamination of air in hospital rooms (Banrud et al., 1999; Botzenhart et al., 1976; Rudnick, 2001), the disinfecting of biological safety cabinet interior surfaces (Fleming et al., 1995), and the sterilization of consumable products (Varnam, 1991). Microbial agent-specific studies using UVGI for decontamination have been reported for rhinovirus (Myatt et al., 2003), *Mycobacterium tuberculosis* (Ko et al., 2002; Nicas et al., 1999; Riley et al., 1976), and *Serratia marcescens* (Ko et al., 2002). A recent report also found UVGI to be an effective method to inactivate organisms in the cooling coils and drip pans in the ventilation systems of buildings (Menzies et al., 2003). Recently, the Environmental Technology Verification Program was established by the U.S. Environmental Protection Agency's National Homeland Security Resource Center to test UV lights in treatment systems as a means to protect against biological contamination in buildings and other public places ([www.epa.gov/etv](http://www.epa.gov/etv)).

Although there are many positive benefits for using UVGI, as noted above, a debate continues on the application of this technology in research laboratories (Dietz et al., 1980). The inability of UVGI to penetrate objects, problems caused by shelving and equipment shadowing, and the occupational risks of workers to UVGI exposure (Talbot et al., 2002; U.S. HEW, 1972) are the main issues that continue to draw questions about the use of this technology as a reliable and safe method for disinfection within the laboratory. In December 2000, the American Biological Safety Association (ABSA) published a position paper on the use of ultraviolet lights in biological safety cabinets (BSC) and stated that "UV lights are not recommended for use in a biological safety cabinetry." In this position paper the Centers for Disease Control and Prevention also stated that "UV lamps are not required in BSC" (ABSA Position Paper, 2000). However, since many individuals will continue to use UVGI for disinfection

purposes, methods for monitoring the effectiveness of this process will need to be available. Additionally, to advance the applications of ultraviolet technology, the International Ultraviolet Association was recently established, in part to encourage research into using this methodology ([www.iuva.org](http://www.iuva.org)). This study reports on a method for the biological monitoring of UVGI ceiling lamps for decontamination purposes in a biosafety level-3 (BSL-3) laboratory.

## Materials and Methods

### BSL-3 Laboratory Design and UVGI Lamp Placement

The BSL-3 laboratory evaluated was composed of four separate rooms with UVGI lamps located as illustrated in Figure 1. The UVGI lamps (Model G30T8, Sylvania Manufacturing Co.) were 36 inches long, 1 inch in diameter, and emitted 30 watts of irradiation at a wavelength of 254 nm. The ceiling height in all rooms was 9

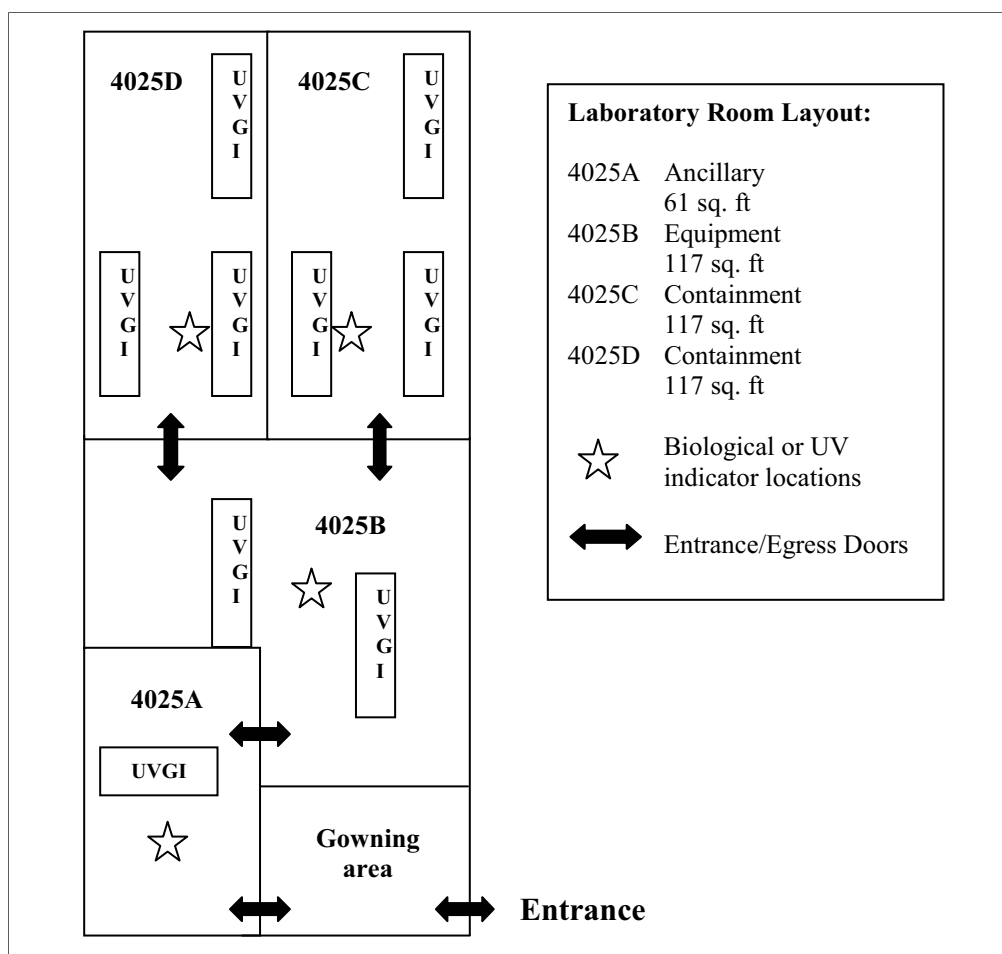
feet. The lamps in each room were on separate timer mechanisms, which were placed on various settings ranging from 15 minutes to 2 hours. The humidity in the rooms was < 60% and the temperature ranged from 68°F to 72°F during the evaluation. Air in the laboratory was high-efficiency particulate air (HEPA) filtered, single-pass 100% outside air supplied.

### Radiation Measurement

UVGI was measured using an UVX Radiometer (Ultra-Violet Products, Upland, CA) fitted with a sensor to measure irradiation at a wavelength of 254 nm. A 12-foot probe extension allowed for readings to be taken across the entire floor area in each working suite. The UVX instrument had three application ranges which cover a span from 0  $\mu\text{W}/\text{cm}^2$  to 20  $\text{mW}/\text{cm}^2$ , 0 to 200  $\mu\text{W}/\text{cm}^2$ , and 0 to 2000  $\mu\text{W}/\text{cm}^2$ . The instrument was calibrated to zero before readings were taken. Readings were recorded as  $\mu\text{W}/\text{cm}^2$  per manufacturer's instructions.

**Figure 1**

Schematic representation of ultraviolet germicidal irradiation lamp (UVGI) placement in the containment laboratory.



## Biological Testing

For biological testing, cultures of *Bacillus cereus* (ATCC 86100) or *B. anthracis* (Sterne strain-Veterinary Vaccine formulation, Colorado Serum Company, Denver, CO) were grown at 37°C for 24 to 48 hours on sheep blood agar (SBA) plates (Remel, Lenexa, KS). Inoculum suspensions of the actively growing *B. cereus* or *B. anthracis* vegetative cells were made in sterile phosphate buffered saline (PBS) (Sigma Scientific Co., St. Louis, MO). The *B. anthracis* spore suspension (Sterne strain-Veterinary Vaccine formulation) was used directly from the vaccine vial. A 0.1 ml amount of each inoculum suspension was pipetted onto a SBA plate and spread using a sterile disposable inoculating loop. The inoculum was subsequently 10-fold serially diluted in PBS to determine viable bacteria per ml. For maximum UVGI exposure, the inoculated SBA plates were placed on the floor and centered directly under the germicidal lamp(s) in each room (Figure 1). This placement was necessary in rooms 4025 C and D due to equipment and shelving along the walls. The lids were removed and the plates were exposed to the UVGI for times ranging from 15 minutes to 2 hours. Additionally, unexposed control plates of the same inoculum, held at room temperature during the tests, were also prepared to determine the viable bacterial or spores per ml inoculum. Bacterial counts from exposed and control plates were tabulated after overnight incubation at 37°C in ambient air and expressed as colony forming units (cfu) per ml. Colonies that grew on the SBA plates were verified by colonial morphology and Gram stain as *Bacillus* spp. A greater than 3- $\log_{10}$  (0.1% viability) reduction in bacterial growth was defined as adequate reduction in viability following UVGI exposure. (Blatchley et al., 2001).

## Results

The results of 20 months of monitoring using varied amounts of either *B. cereus* or *B. anthracis* vegetative cells as inoculum are shown in Table 1. The data showed that a 2-hour exposure to UVGI ceiling lamps in each room of the BSL-3 laboratory was effective in reducing the numbers of *B. cereus* or *B. anthracis* vegetative cells present on the exposed SBA plate in all areas. No differences in survival rates were seen between the different inoculum sizes or between *B. anthracis* or *B. cereus* vegetative cells. In all cases, colonies growing were embedded in the agar.

The effect that exposure time to UVGI had on inactivation of the *Bacillus* spp. vegetative cells is shown in Table 2. A greater than 3- $\log_{10}$  reduction of vegetative cells was noted in all areas at 15-, 30-, 60-, and 120-minute exposure periods. However, as the number of UVGI ceiling lamps in the lab rooms decreased, increased bacterial counts were noted. In no case was complete sterilization of the plates accomplished.

Table 3 shows the comparisons of a 1-hour ultraviolet

germicidal irradiation (UVGI) exposure (as determined by radiometric readings) for effects on *B. anthracis* vegetative cells of varying inoculum size. As the data indicate, no significant difference was seen in the bacterial viability between the two inoculum sizes tested. Table 4 shows a direct comparison of *B. anthracis* vegetative cells and *B. anthracis* spores to UVGI exposure (as determined by radiometric readings). These comparisons showed that with both vegetative cells or spores used as biological indicators, the cumulative effects of UVGI intensity generated over a 1-hour time period and thus the total disinfectant effect created were dependant upon the number of germicidal ceiling lamps located in each room.

## Discussion

The ability to monitor the disinfecting properties of ultraviolet germicidal irradiation (UVGI) using a biological method would be a useful tool for those who utilize a biosafety level 3 (BSL-3) laboratory. Fleming et al. (1995) have shown UVGI lamps to be effective to sterilize exposed surfaces of the BSC, when using the proper controls. More recently, First et al. (2005) demonstrated the safety of personnel working in rooms with UVGI in place and being utilized above a 6.5-ft. height. The present study examined the feasibility and reliability of using a biological method to monitor UVGI as a method to decrease bacterial contamination from floor surfaces in a BSL-3 laboratory while using ceiling-mounted UVGI lamps.

The proper placement and number of lamps installed in each room of the containment laboratory were important considerations during the design process. This lamp placement, along with determination of adequate exposure time to UVGI, was needed so that effective irradiation levels could be accomplished. UVGI over a 2-hour exposure time was shown to be effective in decreasing vegetative *B. anthracis* and *B. cereus* cells in all rooms of the BSL-3 laboratory at the locations studied. Further studies showed that a 1-hour UVGI exposure time would suffice in all BSL-3 laboratory rooms within a well-defined exposure area, but that an increased exposure time in the room having one lamp might be considered to ensure adequate disinfection.

As expected, UVGI effectiveness increased as exposure time increased, and the reduction in bacterial and spore viability was more pronounced in rooms containing three lamps compared to those with two or one lamp(s). Direct comparison between the biological indicators and radiometric measurements showed that the intensity of the UVGI was dependant upon the number of germicidal lamps installed in each room and decreased accordingly, as might be expected, with fewer lamps present. The direct comparisons allowed for utilization of the radiometer alone for future measurements of the germicidal activity

**Table 1**

Biological testing to show effectiveness of ultraviolet germicidal irradiation using various bacterial vegetative cell concentrations.

<i>Bacillus</i> spp. concentration (median, range) <sup>c</sup>	Testing frequency <sup>d</sup>	Number Positive samples/room <sup>b</sup>			
		4025A	4025B	4025C	4025D
1.25 x 10 <sup>3</sup> (5x10 <sup>2</sup> to 2x10 <sup>3</sup> )	11	2	2	2	1
2 x 10 <sup>6</sup> (1x10 <sup>6</sup> to 3x10 <sup>6</sup> )	2	1	0	0	0
3 x 10 <sup>7</sup> (2x10 <sup>7</sup> to 4x10 <sup>7</sup> )	5	3	2*	3	3**
3 x 10 <sup>8</sup> (2x10 <sup>7</sup> to 4x10 <sup>9</sup> )	2	1	1	1	0

a Study was conducted over a period of 20 months using an exposure time of 2 hours.  
 b Number of samples showing at least one colony or more. One plate was placed into each room per evaluation. The colony counts per plate for positive results were < 30 cfu/ml. In all cases, colonies growing were embedded in the agar.  
 c Colony forming units/ml was determined from positive control plates. Both *B. cereus* and *B. anthracis* vegetative cells were used in the evaluation.  
 d Number of times testing was done at this inoculum level over the 20-month period. Radiometric readings were not taken during this time period.  
 \* One of the lamps failed to activate.  
 \*\* These evaluations were not done.

**Table 2**

The effect that exposure time had on the inactivation of vegetative *Bacillus anthracis* or *B. cereus* cells following ultraviolet germicidal irradiation exposure.

Room No.	No. Lamps	Colony Counts <sup>a</sup>			
		15 <sup>b</sup>	30 <sup>b</sup>	60 <sup>b</sup>	120 <sup>b</sup>
4025 A	1	>300	>300	100	5 <sup>c</sup>
4025 B	2	>300	100	15 <sup>c</sup>	2 <sup>c</sup>
4025 C	3	>300	41	1 <sup>c</sup>	5 <sup>c</sup>
4025 D	3	>300	30	5 <sup>c</sup>	9 <sup>c</sup>

a One plate was placed into each in each room per evaluation. Expressed in colony forming units (cfu) per plate. The inoculum used was 3.4 x 10<sup>6</sup> cfu/ml.  
 b Exposure time expressed in minutes.  
 c Colonies embedded in agar.

**Table 3**

Comparison of the effects of a 1-hour ultraviolet germicidal irradiation exposure on *B. anthracis* vegetative cells of varying inoculum sizes.

Location	No. Lamps	Inoculum <sup>a</sup>	Energy <sup>b</sup> Reading	Colony Counts
4025A	1	1	8	5
		6	13	13 <sup>c</sup>
4025B	2	1	14	4
		6	26	3
4025C	3	1	26	1
		6	31	0
4025D	3	1	25	1
		6	35	0

<sup>a</sup> Inoculum count x 10<sup>7</sup> cfu/mL per sample  
<sup>b</sup> Ultraviolet germicidal irradiation energy readings were taken at 254 nm and recorded in  $\mu\text{W}$  per cm<sup>2</sup>.  
<sup>c</sup> Colonies growing were embedded in the agar.

**Table 4**

Comparison of the effects of ultraviolet germicidal irradiation intensity on the inactivation of *B. anthracis* vegetative cells and spores.

Lab Room	Number Lamps	Energy Reading <sup>a</sup>	Counts in cfu/ml <sup>b,c</sup>	
			Cells	Spores
4025A	1	12	> 300	> 300
4025B	2	26	> 300	~ 300
4025C	3	34	~ 300	135
4025D	3	38	112	68

<sup>a</sup> UVGI readings were taken at 254 nm (recorded in  $\mu\text{W}$  per cm<sup>2</sup>) during a 1-hour exposure time.  
<sup>b</sup> In colony forming units (cfu)/plate. Inoculums were: 2 x 10<sup>8</sup> cfu/mL for *B. anthracis* vegetative cells and 7 x 10<sup>7</sup> cfu/mL for *B. anthracis* spores.  
<sup>c</sup> Counts are an average of two plates per room per inoculum.

on the UVGI ceiling lamps. An UVGI reading as low as  $8 \mu\text{W}/\text{cm}^2$  during a 1-hour exposure was adequate to produce a greater than  $3\text{-log}_{10}$  reduction in viable bacteria. Since the intensity of UVGI emitted decreases with use over time, periodic use of biological indicators or radiometric measurements will be needed to ascertain the effectiveness of the lamps throughout the course of utilization.

Since UVGI is a known exposure danger, and there are limitations when using this method, any laboratory proposing to use UVGI ceiling lamps should make certain that proper built-in safeguards and safety precautions are present. Known dangers of UVGI exposure to the eyes and skin are well documented (ABSA Position Paper, 2000; Talbot et al., 2002; US HEW, 1972). The Clinical Laboratory Improvement Act (CLIA) general checklist (Gen 70832, Phase 1) now requires that use of UVGI lamps be evaluated by asking the following question: "Are policies documented to prevent or reduce UVGI light exposure from instrument sources?" In the BSL-3 laboratory, a laboratory-specific UVGI Safety Plan should be initiated to reduce the danger of accidental UVGI exposure to laboratory personnel. Suggestions for a plan include:

- Posting of "Caution—UV ceilings lamps in use" warning signs
- Placement of UV-protection film on all door glass
- Installation of "lock-out" light switches so that when the regular lights are turned "on," the UV lamps are not activated
- Installation of door electrical connections so that when the door to a room is opened the UV lamps are deactivated
- Mechanisms for proper disposal of germicidal lamps containing mercury vapor
- A well-written, laboratory-specific Standard Operating Procedure (SOP) for the utilization of these lamps. The SOP should outline parameters such as the cleaning, monitoring, maintenance, and replacement of the UVGI lamps; timer settings for exposure lengths (minutes or hours); and when (end of day or between room uses), by whom (laboratorian or facilities manager), and how often (daily or weekly) the UV lamps should be activated.

Likewise, these safety parameters should be considered when utilizing UVGI in biological safety cabinets.

## Conclusions

This study showed the effectiveness of UVGI exposure on reducing bacterial cell counts on exposed floor surfaces in a BSL-3 containment laboratory. Effectiveness was dependant upon both the number of UVGI lamps in each room and on the exposure time used. A 1-hour exposure time with a measured UVGI intensity as low as  $8 \mu\text{W}/\text{cm}^2$  resulted in a greater than  $3\text{-log}_{10}$  reduction

(0.1% viability) in viable bacteria. As a result of this study, location-dependant exposure times are now included with the Standard Operating Procedures for the laboratory. When UVGI effectiveness decreases below that of the predetermined cut-off rate of  $3\text{-log}_{10}$  reduction in plate counts, the lamps should be cleaned (if found to be dirty) or replaced. Utilization of UVGI should not be considered as a replacement for para-formaldehyde or other accepted decontamination methods for a biological safety cabinet or a laboratory room. Additionally, as a supplement to UVGI, weekly disinfection of the floors with a fresh solution of 10% bleach or other disinfecting solution should still be considered.

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