# Effectiveness of UV Exposure of Items Contaminated with Anthrax Spores in a Class 2 Biosafety Cabinet and a Biosafety Level 3 Laboratory Pass-Box

Peter C. B. Turnbull, Anatolio E. Reyes, Michael D. Chute, and Alfred J. Mateczun

Biological Defense Research Directorate, Naval Medical Research Center, Silver Spring, Maryland

## Abstract

***Aims:*** *To determine the effectiveness and limitations of ultraviolet (UV) germicidal lamps in biosafety cabinets and pass-boxes, with particular reference to anthrax spores.*

***Methods and Results:*** *An estimated 50 colony forming units (CFUs) of spores of the Sterne strain of* Bacillus anthracis *were distributed on blood agar plates and on smooth and rough plastic surfaces, glass slides, galvanized metal strips, squares of writing paper, nitrocellulose/cellulose acetate strips, and membrane filters. After drying these were exposed in triplicate to the UV sources of a Class 2 biosafety cabinet and a biosafety level (BSL) 3 pass-box for various time periods. Viable* B. anthracis *could not be recovered from hard surfaces at 15 minutes (the shortest time period tested) when fully exposed to UV at intensities of ≥ 3.8 W/m² (the lowest intensity tested) in a Class 2 biosafety cabinet and a BSL3 pass-box. However, the penetrating ability of the UV was found to be extremely limited with even the microstructural elements of nitrocellulose or similar membranes able to substantially shield the spores. The UV failed totally to penetrate thin (66 µm) paper or Petri dish plastic, although it did penetrate plastic sandwich wrap.*

***Conclusions:*** *The UV from germicidal lamps in biosafety cabinets and pass-boxes is rapidly sporicidal for fully exposed anthrax spores on hard surfaces but is readily nullified by the slightest obstacle to direct exposure.*

***Significance and Impact of the Study:*** *The results fill a major information gap for those devising or following protocols for decontamination of items and materials in biosafety cabinets and pass-boxes. Such protocols need to be written around a full comprehension of the data presented here.*

### Keywords

UV; sterilization; anthrax spore; biosafety cabinet; pass-box

## Introduction

Electromagnetic radiation with wavelengths in the range 10-400 nm is termed ultraviolet (UV) light. As reviewed by Russell (1982), maximal germicidal activity lies in the range 240-280 nm within the UV-C range (200-280 nm), this being the wavelength range causing maximal dimerization of thymine molecules on DNA. Germicidal lamps are generally low-pressure mercury arc lamps that convert approximately 50% of their input power to light, about 85% of which is at 253.7 nm.

Despite the American Biological Safety Association position paper (Anon, 2000a) quoting the Centers for Disease Control and Prevention (CDC), the National Institutes of Health, and the National Sanitation Foundation that UV lights within a biological safety cabinet are neither recommended nor required (Harrington & Valigosky, 2007), BSCs are frequently fitted with germicidal lamps. In addition, biosafety level 3 laboratories (BSL3) often have pass-boxes—ultraviolet-bathed portals used for passing potentially contaminated items out of the laboratory. However, availability of information on which to base practical guidelines on exposure times for sterilizing items in cabinets after use or in pass-boxes is limited. The CDC recommends that, if used in a BSC for surface decontamination, the UV intensity should be ≥40 µW/cm² (≥0.4 W/m²) at the center of the work area (Anon, 2000b; Harrington & Valigosky, 2007). The American Ultraviolet Company (Lebanon, IN) and Atlantic Ultraviolet Corporation (Hauppauge, NY) websites list sterilizing dosages for a wide range of microorganisms, including *Bacillus anthracis*. American Ultraviolet also supplies radiant energy output data for a number of UV lamps together with guidance on calculating intensities at various distances. However, as pointed out by Harrington & Valigosky (2007), few laboratories using the UV lamps in their BSCs are, in practice, equipped to monitor the performance of their systems or the effectiveness of their sterilization procedures, and they proposed an agar plate culture-killing procedure for the benefit of such laboratories where the relevant UV instrumentation was not available.

Information on deterioration rates of UV lamps is largely dependent on the data supplied by the manufacturer; these generally state that lamps should be changed after 9,000 to 13,000 hours. A chart given on the Atlantic Ultraviolet Corporation web site shows a fall to 85% efficiency after 6,000 hours and 80% efficiency after about 15,000 hours. Users' procedures obviously need to be adjusted to compensate for this deterioration.

This study was initiated to generate data to assist laboratories that use UV in their biosafety cabinets and/or pass-boxes to design their decontamination procedures for *B. anthracis* in an informed manner.

## Materials and Methods

### Spores and Bacteria

Spores of the Sterne $34F_2$ vaccine strain of *B. anthracis* were prepared as described by Jones et al. (1996) with the resuspension in 65% isopropanol omitted. They were diluted to a working suspension containing 1,000 cfu/ml in sterile deionized water. An estimated 50 cfus (i.e., 50 µl) were spread over standard blood agar (BA) plates (trypticase soy agar with sheep blood; Remel Inc., Lenexa, KS) or were distributed over surfaces or membranes placed in sterile Petri dishes by spotting 50 µl volumes of the suspension over these surfaces/membranes.

For initial comparison, suspensions of *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 35150 in buffered saline were also diluted to approximately 50 colonies in 50 µl and this volume spread on BA plates. (These species were not included in the subsequent tests on inert surfaces and membranes.)

### Surfaces Utilized

Sterilized smooth plastic surfaces (Petri dish lids), rough plastic surfaces (floppy disks), glass slides, painted and unpainted galvanized metal strips, and squares of the note paper used in the laboratory and frequently passed out through the pass-box were inoculated as described above and the inoculum allowed to dry. Squares of Millipore HiFlow 07504 nitrocellulose/cellulose acetate with polyethylene terephthlate backing, 2.5 $cm^2$ in dimension, and 25 mm disks of polyethersulfone hydrophilic (PESH) filters (Millipore 0.22 µm Cat No. GPW P02500) were also inoculated and allowed to dry.

### Exposure and Recovery

The BA plates inoculated with *B. anthracis*, *E. coli,* and *S. aureus* were exposed in triplicate to the UV lamp of a Class 2 biosafety cabinet for 30 minutes. They were then incubated together with unexposed controls at 35°C for 48 hours.

After the contaminated surfaces, paper squares, or membranes had dried, they were exposed in triplicate to the UV lamps of the biosafety cabinet and a pass-box for various time periods from 15 minutes to overnight (17-20 hours). Nutrient agar contact plates were used to recover viable organisms from the contaminated hard surfaces. The plates were incubated at 30°C overnight, and colonies that had developed on the contact plates were counted. The exposed paper squares and membranes were placed, inoculated side down, on BA plates. After overnight incubation at 30°C, they were carefully peeled off the BA surfaces and the plates returned to the incubator for a further overnight incubation. Again, colonies that had developed were counted. Triplicate unexposed controls were included for all experiments.

The majority of experiments were done with UV sources that had been in place and in routine use in the cabinet (PerkinElmer Optoelectronics, Fremont, CA, code G64T5UCB) and pass-box (USHIO America, Inc., Cypress, CA, code G8T5) for an extended period of time. On completion of the experiments, new sources (Atlantic Ultraviolet Corp., code G64T5L in the case of the biosafety cabinet) were inserted for a number of repeat experiments.

### UV Monitoring

The UV intensities at the rear and front exposure positions in the cabinet and on the base of the pass-box were determined from calibration curves obtained by measuring the UV intensities at four distances (between 14.0 cm and 59.5 cm in the experimental BSC and 5.85 cm to 28.1 cm in the pass-box) from the UV source with a Zenith 30-0083 (185-320 nm) ultraviolet meter (Atlanta Ultraviolet Corp.). The distances between lamp and target in the BSC were 73 ± 1 cm at the front, 59 ± 0.5 cm at the rear, and 28 ± 0.5 cm in the pass-box.

## Results

No growth occurred on the BA plate cultures of the *B. anthracis*, *E. coli,* and *S. aureus* following 30-minute exposures after inoculation to UV intensities of 3.8 $W/m^2$ and 4.1 $W/m^2$. Unexposed controls grew normally as expected.

Viable organisms could not be recovered from plastic (both smooth and rough surfaces), glass, or metal (unpainted or painted) surfaces after 15 minutes at either the rear (4.1 $W/m^2$) or front (3.8 $W/m^2$) of the cabinet or in the pass-box (3.9 $W/m^2$). Viable *B. anthracis* was recovered in expected numbers from the unexposed controls.

In the case of paper, viable organisms again could not be recovered after 15 minutes when the inoculated side of the paper faced the germicidal lamp. When the reverse side of the paper faced the UV lamp, there was no die-off as compared with controls at 15, 30, or (the latest time tested) 60 minutes. This paper is 66 µm thick and, though not entirely opaque to visible light, was opaque to UV of the wavelength range detected and measured by the UV meter (meter reading of zero through the paper for an incident intensity of 3.62 $W/m^2$). Petri dish plastic was also opaque to the same incident UV light, but clear plastic polyethylene sandwich wrap reduced the intensity only to 3.20 $W/m^2$.

In contrast to the other materials, spores deposited on PESH and nitrocellulose membranes remained viable for extended periods (Table 1). The PESH filters, 178 µm

in thickness, were more protective than the HiFlow 07504 nitrocellulose/cellulose acetate with polyethylene terephthlate backing (152 µm thick), and, at the lower intensities (initial [old] light source), a high proportion of the spores were still viable on the PESH filters after overnight exposure. With the new light sources, the higher intensities did result in more rapid killing, but viable spores were still being found at 6 hours on the PESH filters (Table 1). An unpaired t-test showed differences between the two membranes under the old UV sources to be significant at 6 hours and overnight ($P < 0.001$) and at 2 hours ($P = 0.002$) and, under the new UV sources at 6 hours ($P = 0.011$) though not at 2 hours ($P = 0.14$). Under the new sources, virtually no spores survived on either membrane type after overnight exposure (Table 1).

Under the initial (old) UV source, the intensities at the rear and front of the cabinet were 4.1± 0.1 W/m$^2$ and 3.8± 0.1 W/m$^2$ (Table 1), respectively, and showed no observable decline over the 2-month period of the study. The intensity on the floor of the pass-box under the initial UV source was 3.9 ± 0.2 W/m$^2$. The new UV sources installed toward the end of the project raised the intensities to 6.4 ± 0.1 W/m$^2$, 6.0 ± 0.1 W/m$^2$, and 5.6 ± 0.2 W/m$^2$ at the rear and front of the cabinet and in the pass-box, respectively.

Twelve other cabinets in the Directorate's facility were found to have floor-level intensities ranging from 56% to 99% (mean 84%) of that of the study cabinet before the lamp was changed, supplying guidance as to levels that might be expected in normally used biosafety cabinets with UV sources that have not been changed in the recent past.

## Discussion

No information was available on the ages of the UV lamps in the facility cabinets, but there was reason to believe that most of them, including the one in the test cabinet, had been in place for >2 years. The lamp in the test cabinet is put to fairly heavy use, often being left on overnight after work has been done in the cabinet. Some measure of the loss of energy over time is apparent from the 1.56-fold increase in intensity following replacement of the lamp with a new one. However, even at the lower intensity of the old lamp, cultures of *B. anthracis*, *S. aureus,* and *E. coli* were killed by exposure for 30 minutes (the shortest time tested), and the *B. anthracis* spores on hard surfaces and on the exposed side of writing paper were rendered unrecoverable within 15 minutes (also the shortest time tested). An analogous situation was found in the pass-box. For reference purposes, the front and rear intensities of 6.0 W/m$^2$ and 6.4 W/m$^2$ respectively within the cabinet as determined in this study (Table 1) were somewhat higher than those calculated (3.6 W/m$^2$ and 5.3 W/m$^2$ respectively) from a supplier's estimate (J. Stines, American Ultraviolet Co., personal communication, 2008) for the G64T5L lamps used. Similarly, the intensities determined for the G8T5 pass-box lamps (Table 1) were higher than that calculated from the value given for this UV source on the

**Table 1**

Survival of *B. anthracis* (Sterne strain) Spores on Polyethersulfone Hydrophilic (PESH) and Nitrocellulose/Cellulose Acetate (HiFlow) Membranes Exposed to Biosafety Cabinet and Pass-box Germicidal UV Sources (means of triplicate viable counts)

| Site | Membrane | UV Intensity (W/m²)* | UV Source† | Exposure Time (hours) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0 | 2 | 6 | 17-20 |
| Cabinet front | PESH filter | 3.8 | Old | 34 | 21 | 22 | 20 |
| | | 6.0 | New | 7 | 6 | 3 | >0§ |
| | HiFlow | 3.8 | Old | 37 | 21 | nd | 2 |
| | | 6.0 | New | 23 | 1 | 0 | 0 |
| Cabinet rear | PESH filter | 4.1 | Old | 34 | 27 | 26 | 13 |
| | | 6.4 | New | 7 | 3 | 1 | 0 |
| | HiFlow | 4.1 | Old | 37 | 12 | nd | 0 |
| | | 6.4 | New | 23 | 0 | 0 | 0 |
| Pass-box | PESH filter | 3.9 | Old | 34 | 22 | 24 | 20 |
| | | 5.6 | New | 7 | 3 | 1 | 0 |
| | HiFlow | 3.9 | Old | 37 | 21 | nd | 0 |
| | | 5.6 | New | 23 | 15 | 0 | 0 |

*± 0.1 for the biosafety cabinet readings, ± 0.2 for the pass-box readings
†See text for explanation of old and new
§1 colony in 6 drop count drops
nd: not done

American Ultraviolet Company web site (2.2 W/m$^2$).

The ease with which the spores could be shielded from the bactericidal effect of the UV was demonstrated by their survival on the reverse side of the writing paper, a mere 66 µm thick, and within the PESH and nitrocellulose/cellulose acetate membranes, respectively 178 µm and 152 µm thick (Table 1). The poor penetrating ability of UV is well recognized and was manifest in these results. Newsom and Walsingham (1974) noted a marked difference in survival under UV of *Mycobacterium phlei* and spores of *B. globigii* dried on aluminum foil strips when foil pre-coated with horse serum was compared with uncoated foil. Gardner and Shama (1998) noted shielding of *B. subtilis* spores on cellulose filter paper and found that inactivation of the spores on Grade 6 paper, with a higher density of microfibrils, was slower than on Grade 2 filter paper with lower microfibril density. In the field, shielding from harsh sunlight by soil particles in surface soil at carcass sites has also been noted (Turnbull et al., 1998), and the scanning electron micrograph in Figure 2b in that publication (Turnbull et al., 1998) visually demonstrates how anthrax spores can embed in a nitrocellulose membrane. In the same location in a separate study (Lindeque & Turnbull, 1994), spores of a fully virulent strain of *B. anthracis* on heavily inoculated sterilized silk threads were reduced from 4+ growth to 1+ after exposure for 60 minutes to the UV source of a class 2 biosafety cabinet and were all but killed by exposure for 4 hours to the midday sun (intensities subsequently determined to be in the order of 1.8 W/m$^2$ 2 hours before and after a peak of 2.3 W/m$^2$ at 12h40). However, the possible shielding effect on the underside of the threads (which were not turned over during the experiments) had not been taken into account. The shielding effect also was probably not appreciated by Weinnzirl (1914) who concluded that the time required for direct sunlight to kill the spores of *B. anthracis* and other *Bacillus* species spread on sterile paper slips in Petri dishes varied from 2.5 to 8.5 hours, depending on time of year (the experiment was done at an unspecified location within the USA).

Gardner and Shama (1998) observed that the state of the spores is important, showing that the inactivation rate of wet *B. subtilis* spores was 6- to 10-fold greater than dry spores. However, they noted that other reports indicated that, for cells of non-sporing bacteria, inactivation of wet cells might be slower than that of dry cells. *B. anthracis* (Sterne strain) spores were found to have essentially identical UV inactivation kinetics to those of standard biodosimetry *B. subtilis* strains (Nicholson & Galeano, 2003).

Studies on the kinetics and mechanisms of UV germicidal action and its use for disinfection of water, other liquids, and surfaces of a variety of entities from foods to contact lenses and egg shells are numerous and have been well reviewed (Gardner & Shama, 1998; Nicholson & Galeano, 2003; Russell, 1982), and its use for disinfection of operating rooms discussed (Banrud & Moan, 1999). In contrast, publications on the practical effectiveness of UV in biosafety cabinets appear to be few in number.

The results presented here demonstrate that UV sources in biosafety cabinets and pass-boxes are rapidly sporicidal for anthrax spores when fully exposed on hard surfaces but that the penetrating ability of the UV is extremely limited with even the microstructural elements of nitrocellulose or similar membranes able to substantially protect the spores. The UV fails totally to penetrate thin (66 µm) paper. Procedures for decontamination of items and materials in biosafety cabinets and pass-boxes need to be written around a full comprehension of these features. For example, paper being moved through the pass-box should be exposed for approximately 15 minutes on one side and then turned over for exposure for a further 15 minutes on the other side.

It should be noted that these experiments were not set up to establish the D-values necessary to meet the accepted criteria for proving the sterilizing capabilities of the UV sources in the cabinets and pass-box used, but rather to demonstrate the effects of surface type and location of the contaminant in relation to the UV lamp on the effectiveness of UV sporicidal activity.

## Historical Postscript

Of added historical note is the paper of Ward (1892) who noted that "*a few c.c. of Thames water with many thousands of anthrax spores in it may be entirely rid of living spores by continued exposure daily for a few days to the light of the sun.*" He further demonstrated by using a stencil with a letter "E" cut out covering a gelatin plate culture of anthrax spores "*placed in the middle of a field at Cooper's Hill at 9:30 a.m. on Wednesday, November 30, and exposed to the clear, but low, sunshine which prevailed that day…until 3.30 p.m.… after…incubation—the letter E stood out sharply and clearly transparent from the faint grey of the rest of the plate of gelatin. Not a trace of anthrax could be found in the clear area…*"

## Acknowledgements

Funding for this project was provided under U.S. Navy Work Unit 80000.000.000.A0031. The authors are grateful to Jeff Stines, American Ultraviolet Company, Lebanon, IN, and Tibor Kovacs, Light Sources Inc., Orange, CT, for technical advice on UV lamp performance.

### Authors' Note

This article reports the results of research only. The views expressed are those of the authors and do not necessarily reflect the official policy or position of the

U.S. Department of the Navy, U.S. Department of Defense, or the U.S. Government. Questions or comments may be directed to the corresponding author, Michael Chute, via e-mail at Michael.Chute@med.navy.mil.

## References


Anon. (2000a). *Position paper on the use of ultraviolet lights in biological safety cabinets.* Mundelein, IL: American Biological Safety Association.

Anon. (2000b). *Primary containment for biohazards selection. Installation and use of biological safety cabinets* (2nd ed.). Atlanta: CDC/NIH.

Banrud, H., & Moan, J. (1999). The use of short wave ultraviolet radiation for disinfection in operating rooms. *Tidsskrift for den Norske Lægeforening: Tidsskrift for praktisk medicin, ny række, 119,* 2670-2673.

Gardner, D. W. M., & Shama, G. (1998). The kinetics of *Bacillus subtilis* spore activation on filter paper by u.v. light and u.v. light in combination with hydrogen peroxide. *Journal of Applied Microbiology, 84,* 633-641.

Harrington, B. J., & Valigosky, M. (2007). Monitoring ultraviolet lamps in biological safety cabinets with cultures of standard bacterial strains on TSA blood agar. *Laboratory Medicine, 38*, 165-168.

Jones, M. N., Beedham, R. J., Turnbull, P. C. B., & Manchee, R. J. (1996). Antibiotic prophylaxis for inhalation anthrax. *Salisbury Medical Bulletin, 87S,* 127-128.

Lindeque, P. M., & Turnbull, P. C. B. (1994). Ecology and epidemiology of anthrax in the Etosha National Park. *Onderstepoort Journal of Veterinary Research, 61,* 71-83.

Newsom, S. W. B., & Walsingham, B. M. (1974). Sterilization of the biological safety cabinet. *Journal of Clinical Patholology, 27,* 921-924.

Nicholson, W. L., & Galeano, B. (2003). UV resistance of *Bacillus anthracis* spores revisited: Validation of *Bacillus subtilis* spores as UV surrogates for spores of *B. anthracis* Sterne. *Applied and Environmental Microbiology, 69,* 1327-1330.

Russell, A. D. (1982). *The destruction of bacterial spores* (pp. 152-163). London: Academic Press.

Stines, J. (2008). American Ultraviolet Co. Personal communication.

Turnbull, P. C. B., Lindeque, P. M., Le Roux, J., Bennett, A. M., & Parks, S. R. (1998). Airborne movement of anthrax spores from carcass sites in the Etosha National Park, Namibia. *Journal of Applied Microbiology, 84,* 667-676.

Ward, H. M. (1892). Experiments on the action of light on *Bacillus anthracis*. *Royal Society Proceedings, 51,* 393-400.

Weinzirl, J. (1914). The germicidal action of sunlight upon bacterial spores. *American Journal of Public Health, 4,* 969-974.