

ORIGINAL ARTICLE

The inactivation and removal of airborne *Bacillus atrophaeus* endospores from air circulation systems using UVC and HEPA filters

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Abstract

Aims: To (i) evaluate the UV radiation in the 'C' band/high efficient particulate air (UVC/HEPA) instrument's potential to inactivate spores of *Bacillus atrophaeus* and selected *Bacillus* species and (ii) test whether a titanium dioxide coating inside the cylindrical HEPA filter improves the system's efficacy.

Methods and Results: Known amounts of dried spore preparations of *B. atrophaeus*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus stearothermophilus* and *Bacillus thuringiensis* were exposed to the UVC lamp within a cylindrical HEPA filter for different time lengths (30 min to 48 h) and with different air flow speeds (0–235 l s⁻¹). The log₁₀ reduction (range 5–16 logs) of colony forming units for spores exposed to the UVC compared with the unexposed spores was significant ($P < 0.0001$). The addition of a titanium dioxide (TiO₂) veneer to the interior surface of the HEPA filter significantly increased the inactivation of spores ($P < 0.0001$).

Conclusions: The UVC/HEPA unit could inactivate spores of *B. atrophaeus*, *B. cereus*, *B. megaterium*, *B. stearothermophilus* and *B. thuringiensis*.

Significance and Impact of the Study: The UVC/HEPA unit represents an effective method of decontaminating circulating air within an air-duct system as found in a building.

Introduction

Air quality within a large commercial building was once of little concern to the average person who worked within. However, since the 1980s it has been noted by the government and a few researchers to be a potential source of pollutants (Goswami *et al.* 1997). After the deliberate release of *Bacillus anthracis* spores in 2001, the quality and decontamination of air that flows in air-conditioning ducts has come to the forefront.

One objective of the Center for Biological Defense (CBD) is to examine prototype equipment and methods developed for the decontamination of air systems. These various instruments require extensive validation and testing prior to deployment. The CBD has the ability to test the efficacy of prototypes using surrogates in lieu of *B.*

anthracis, providing the developers with an independent assessment for consideration. The CBD tested an air filtration unit UVC/HEPA, Model 500R (Isolate Inc., Houston, TX, USA), that utilized ultraviolet (UV) light to inactivate/kill micro-organisms before they were trapped within the folds of a high efficient particulate air (HEPA) filter. This instrument was intended to clean the air flowing within a building's air-duct system and to provide a safer filter for handling by maintenance and repair personnel. The instrument was uniquely designed as an air-duct section that contains a cylindrical HEPA filter coupled with a fan. The entire unit can be connected to and become part of a duct system. Contaminated air is pulled by the fan into the interior or 'dirty side' of the filter, through the filter, and finally out to the exterior or 'clean' side. Any particulate matter and micro-organism

would be captured in the HEPA filter and away from personnel during maintenance or change out of the filter. Although desiccation stresses may inactivate micro-organisms captured on the HEPA filter, the filter itself does not necessarily kill living organisms. The entire unit and HEPA filter is designed to be easily accessible as it can be 'spliced' into the air-duct system. The interior of the cylindrical HEPA filter can be sealed from the outside air during filter change to prevent contamination of surrounding area and personnel.

A lamp emitting short-wave UV radiation in the 'C' band (200–280 nm) (UVC) was added inside the filter's 'dirty' side. Numerous reports state UVC effectively inactivates viruses, growing bacterial cells and spores (Chang *et al.* 1985; Nicholson *et al.* 2000; Nicholson and Galeano 2003; Caillet-Fauquet *et al.* 2004; Taghipour 2004; Blatchley *et al.* 2005; Koivunen and Heinonen-Tanski 2005; Newcombe *et al.* 2005). Reportedly, UVC more efficiently inactivates spores than UVB (290–310 nm) or UVA (320–400 nm) bands (Slieman and Nicholson 2000) and is equally affective against pigmented and nonpigmented spores (Moeller *et al.* 2005). UVC lamps internal to air-cleaning units have proved to be effective against fungal spores and bacterial vegetative cells found in the air (Riley 1994; Cundith *et al.* 2002; Kujuvdzic *et al.* 2006). Recent tests have coupled different bands of UV with titanium dioxide (TiO₂) and have successfully sterilized dental implants, different surfaces and drinking water (Kuhn *et al.* 2003; Lonnen *et al.* 2005; Riley *et al.* 2005). Researchers have shown that coating surfaces with titanium dioxide and exposing to UVC can inactivate micro-organisms in different air-flow systems (Keller *et al.* 2005; Vohra *et al.* 2005).

The dual purpose of this study was to (i) evaluate the UVC/HEPA instrument's potential to inactivate spores of *Bacillus atrophaeus*, a *B. anthracis* surrogate, before the spores were captured by the HEPA filter and (ii) test the instrument with and without a TiO₂ coating inside the cylindrical HEPA filter.

Materials and methods

Instrument information and conditions

The UVC/HEPA air-cleaning system unit Model UVC/HEPA-500HR (US Patent 6,783,578 B2) (Isolate Inc.) (Figs 1–5) utilizes a fan to pull air through the system, passing over the UVC light (253.7 nm) and through a tubular HEPA filter (30.5 cm long and 35.56 cm in diameter). The entire unit (51.44 cm high, 51.44 cm wide and 112.76 cm long) would normally be added to the ducts of a heating, ventilating, air-conditioning system or other system where air needs to be cleaned as the two

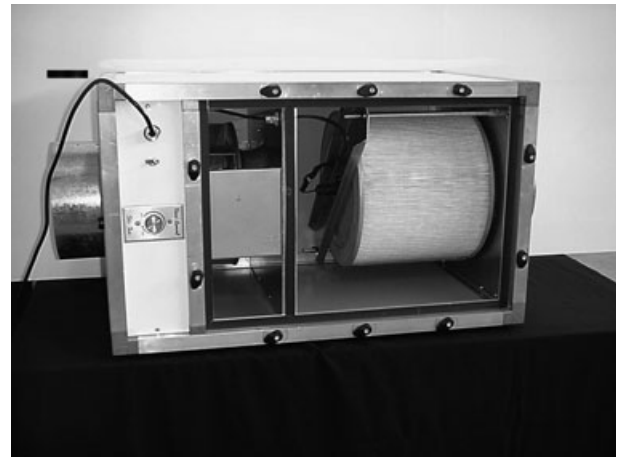


Figure 1 Side view of Model UVC/HEPA-500HR. The entire unit is 'spliced' into an air-duct system. Shown is the inside of the outer cabinet that holds the filter and fan components. Seen is the 'clean' side of the filter. The front panel is removed when changing out a filter or making repairs. The black bar is 10.16 cm long. The unit is 51.44 cm high and 51.44 cm wide. The large rectangular unit is 91.44 cm long and each duct collar is 10.16 cm long giving an overall length of 112.76 cm. The tubular HEPA filter is 30.48 cm long and 35.56 cm in diameter.

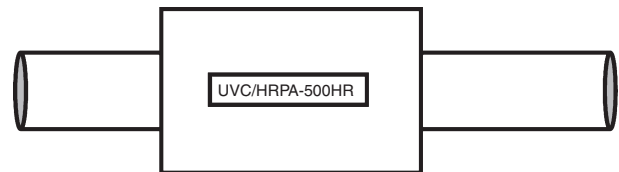


Figure 2 Location of UVC/HEPA-500HR in an air-duct system. The unit 'splices' into the duct system. A side panel on the unit allows easy access to the filter and fan components.

openings at either end are configured as air-duct sections. For all tests at CBD, the entire unit was fitted into a large Biosafety Cabinet to prevent accidental spread of spores and exposure to UVC light. Personnel wore gloves, long-sleeved lab coats and UV safety glasses when working with the unit. During tests, the release chamber was attached to the unit and blocked one's vision and physical access into the HEPA unit. In addition, the Biosafety Cabinet door was closed. Thus, personnel were not exposed to any UVC light. Dosimetry or testing exposure of personnel to UVC was not performed because all microbiology labs have lamps of similar wavelength, for example in Biosafety Cabinets or in Wood's lamps, and to our knowledge, dosimetry is not required under these circumstances. Although most tests were performed using the instrument's fan set to off (0 l s^{-1}), to avoid the loss of spores into the HEPA filter, other speeds were tested:

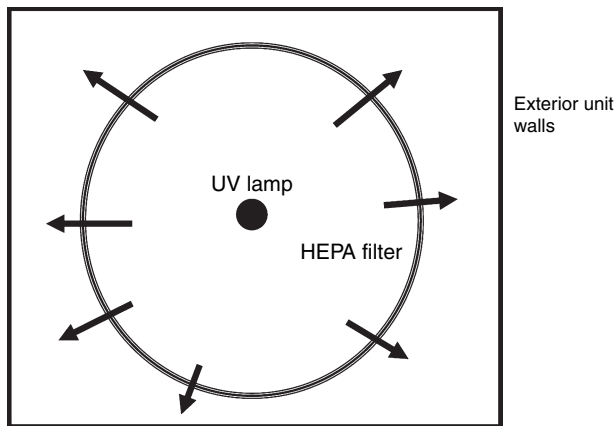


Figure 3 Diagram of cross section of UVC/HEPA-500HR unit. The arrows indicate the direction of airflow.

low ($47\text{--}70\text{ l s}^{-1}$), medium (141 l s^{-1}) and maximum (235 l s^{-1}). The UVC lamp (UVC 17616-5424178) (120 V, 60 Hz, 0.35 A) within the unit was provided by UVC Manufacturing and Consulting, Minden, NV, USA. The UVC lamp is a mercury vapour lamp that emits approximately 95% UVC at 253.7 nm with the balance in the visible wavelengths. Fluence was measured with a UV Sensor and Display (P/N 80000115 and P/N 80000117; Steril-Aire, Burbank, CA, USA). At the inner surface of the HEPA filter cylinder, the UVC fluence rate ranged from $1870\text{ }\mu\text{W cm}^{-2}$ with the fan off to $3720\text{ }\mu\text{W cm}^{-2}$ when the fan was at maximum speed. This increase in fluence is expected as the mercury vapour lamp is designed to emit more UVC when the fan is on and an air stream is flowing over the lamp. This allows the lamp's interior to reach a hotter temperature that will vapourize more of the mercury within the lamp. As more

mercury is vapourized, more UVC is produced. Thus the optimal fluence is recorded when the fan is on continuously as in a building's air system flowing within the air duct. This optimal fluence is quickly obtained as the air flows over the tubular lamp. The lamp is designed to emit a homogeneous output of power within the unit, so that the entire interior is exposed to the same amount of UVC. The time of UVC exposure ranged from 30 min to 48 h. All tests were performed in an air-conditioned facility with a temperature range of $68^{\circ}\text{F}\text{--}73^{\circ}\text{F}$ and relative humidity range of 46.0–56.0%.

Bacterial strains and preparation of spores

Most testing was performed using spores of *B. atrophaeus* (CBD 71) (formerly *Bacillus globigii*) obtained from the US Army at Dugway, UT, USA and previously validated as an acceptable surrogate for *B. anthracis* (Nicholson and Galeano 2003). Other tests were later performed with spores of *Bacillus cereus* (CBD 103), *Bacillus megaterium* (CBD 300), *Bacillus stearothermophilus* (ATCC 7953) and *Bacillus thuringiensis* (CBD 87). *B. atrophaeus* spores were prepared and dried by the US Army. These spores have been processed so that they are easily airborne, are $1\text{--}2\text{ }\mu\text{m}$ in size and remain as single spores in the dry state. They do not clump together just as the theoretical 'weaponized' *B. anthracis* spores would be. Dry amounts (range = $60.0\text{--}154.9\text{ mg}$) of the *B. atrophaeus* spores were placed into sterile Eppendorf tubes and kept at room temperature in an ARS Auto-Desiccator 'D-Box' (Sanplatic Corporation, Osaka, Japan) until use to ensure that they would remain dry. Phase-contrast light microscopy was performed using a Zeiss Axiostar 1122-100 Microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY,

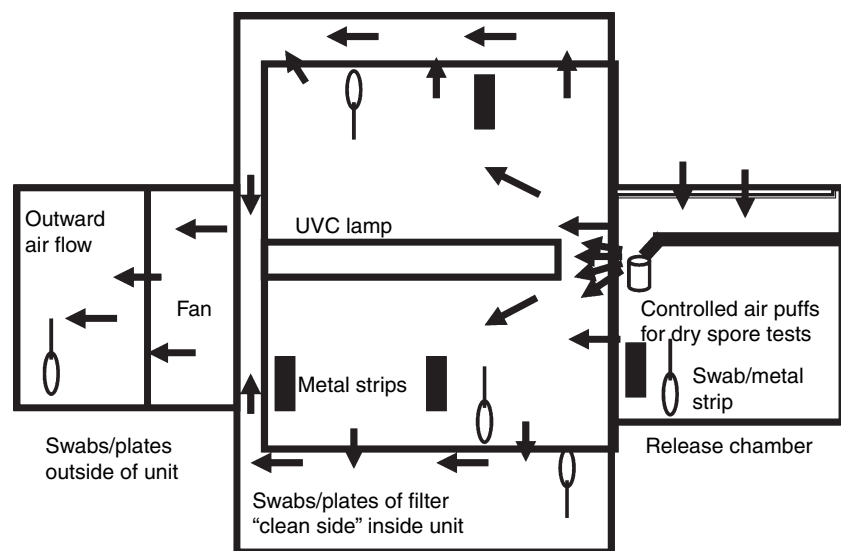


Figure 4 Diagram of side view of UVC/HEPA-500HR unit. The arrows indicate the direction of the airflow while the locations from where swab samples were taken and where metal strips/plates were placed during the tests are also indicated.

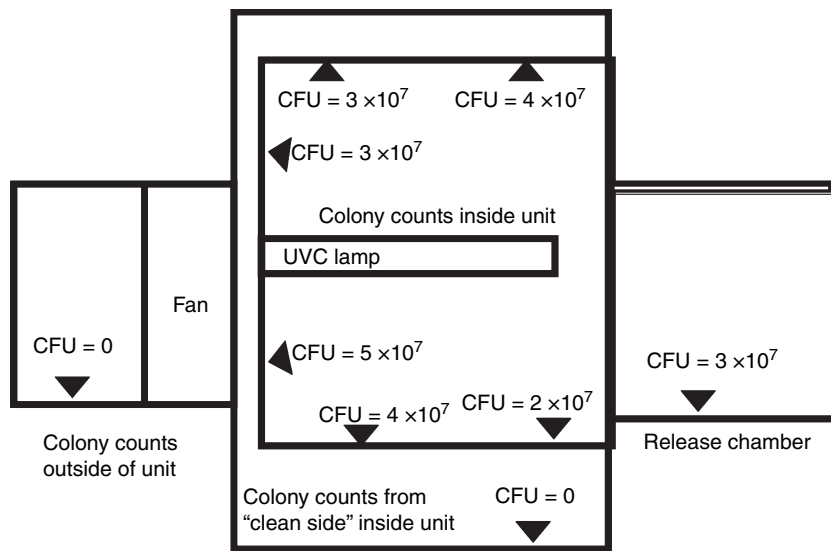


Figure 5 Locations within UVHEPA unit from where premoistened swabs were scrubbed. The final CFU given per site is given at each location and shows an even dispersal of spores within the instrument.

USA) to determine the absence of vegetative cells and to note the presence of spores at early stages of germination and dormant spores. The *B. atrophaeus* spore preparations contained no visible vegetative cells or spores in early stages of germination. The spores were tested with hydrochloric acid following established protocols (Sagripanti *et al.* 2007). An Eppendorf tube of spores (range = 66–80 mg) was sacrificed and checked for purity, approximate amount, acid survival and for their continued ability to germinate at the beginning of the testing period and every 2–4 months until the experiments were finished. For this, 100 μ l of 1x phosphate-buffered saline (PBS) was added to the dry spores, vortexed hard for 5 min, heat shocked at 65°C for 30 min and serially diluted and plated onto tryptic soy agar (TSA) (Remel, Lenexa, KS, USA). The plates were incubated at 30°C for 7 days or until colonies appeared. The counts from later tests were compared with the initial and other earlier tests to see if there was a decrease in the number of germinating spores. The remainder of the *B. atrophaeus* spores was stored at 4°C. Wet preparations of the *B. atrophaeus* spores were made by adding 10 ml 1x PBS to approximately 1 ml of dry spores and vortexed hard for 10 min. This was then stored at 4°C until use. To determine the approximate spore concentration, serial dilutions in 1x PBS of the spore suspension were heat shocked at 65°C for 30 min, plated onto TSA and incubated at 30°C up to 7 days. The resulting colony forming units (CFU) were enumerated. Phase-contrast light microscopy, acid survival and CFU counts were repeated with all subsequent tests and compared so that any decrease in the number of CFU would be noted.

For spore preparations of the other *Bacillus* species, bacteria were grown on modified nutrient sporulation medium including phosphate (Atlas 1993) for 48–72 h

at 30°C or until 90–99% phase-bright spores were observed by phase-contrast light microscopy. The balance of the cells seen were predominantly phase dark spores being in the early stages of germination or dead 'ghost' vegetative cells. Following the protocol recommended by the US Army, the spores with germinating and vegetative cells were harvested into microcentrifuge tubes, heat shocked at 65–80°C for 30 min to kill the vegetative cells, washed with 1 ml saline, vortexed hard for 5 min and centrifuged at 16 000 g for 15 min. Liquid was removed and the process repeated twice. Finally, 1 ml of saline was added to the spore pellet and the mixture was vortexed hard thrice in 5 min increments. Multiple serial dilutions were made to ascertain the estimated concentration and a portion of the spores were subjected to the acid survival test as above. In order to dry the spores, open Eppendorf microcentrifuge tubes containing 100 μ l aliquots of the harvested, heat shocked and washed spores were placed into the desiccator until all liquid was removed. After the spores were dried, the tubes were closed, covered with parafilm and vortexed to loosen the dried spores before storage in the desiccator. Before each test, the tube of spores to be used was first vortexed hard for 5 min for a minimum of three times until the contents appeared to be a fine dust within the tube. Preliminary tests using phase-contrast microscopy showed the spores to be in singles, pairs and small aggregates of four to approximately 10 cells (2–15 μ m) when the spores macroscopically looked like fine dust.

Tests with airborne spores

Dry spores (dry weight range = 60.0–154.9 mg) were released either directly inside the UVC/HEPA unit or into

an air chamber attached to the UVC/HEPA unit using thin tubing (0.5 mm diameter) to deliver 3 puffs of air (66 cm^3 at $1.05\text{--}1.41 \text{ kg cm}^{-2}$) into the microcentrifuge tube containing the dry spores (Fig. 4). The force of the air emptied the microcentrifuge tube and spread the spores within the unit. The unit fan was turned on for 2–3 s to pull the spores into the interior chamber and then turned off. The unit was left for 4 h or longer without the fan to allow spores to travel and settle upon surfaces. A removable plastic sheet blocked access to the interior and prevented spores and UVC escaping from the unit while the test was proceeding.

Preliminary tests were performed to ensure universal dispersal of spores within the unit's interior and that the spores were not remaining only within the release chamber. This was accomplished using multiple open TSA plates placed within the interior of the unit and in the spore release chamber. The TSA plates within the unit covered the interior's bottom, top and sides. Laboratory tape was used to hold the media plates in place. Two TSA plates were also positioned behind the HEPA filter to ensure its integrity. Spores were released and after 24–48 h settling time, the open plates were covered and incubated at 30°C or 35°C overnight and viewed at 1, 4 and 7 days. The numbers of colonies counted over the surface of the plate were averaged to give a CFU per cm^2 of surface. The open plate tests were repeated with the fan set at low speed ($47\text{--}70 \text{ l s}^{-1}$) for 8 h, medium speed (141 l s^{-1}) for 2 h and high speed (235 l s^{-1}) for 1 h. After the open plates were exposed to the dynamic air flow, the fan was turned off to prevent drying of the media and the plates were left undisturbed for 18–24 h before incubating as above. Subsequent tests had open plates that were placed only on the bottom of the unit. The open plates were later abandoned as even dispersal within the unit was consistently observed. After all tests, the inner surfaces were wiped down with 10% bleach, followed by a wash with 5% sodium thiosulphate and a final rinse with 70% EtOH. The UVC lamp and maximum fan speed were then left on for 24 h before the next test. The bleach solution and sodium thiosulphate washes were not used on the TiO_2 coated surfaces of the second UVC/HEPA unit.

For the remaining tests, no open plates were used when the spores were released and allowed to settle. However, the need to test the reproducibility of the capture method using swabs was performed. At four different trials, dry spores (60–75 mg) were released into the UVC-HEPA unit and the spores were allowed to settle for 4 h. Without any UVC exposure, 7–14 swabs at each test were used to scour different 30 cm^2 areas of the interior surface. Each sample consisted of a sterile synthetic-tipped swab premoistened with 1x PBS that was used to

scrub a 30 cm^2 area of the interior surface, placed into 1 ml 1x PBS, vortexed at high speed for 5 min and heat shocked for 30 min at 65°C . After 10-fold serial dilutions in 1x PBS were made from the sample, $100 \mu\text{l}$ of each dilution was placed, evenly spread on a fresh plate and incubated at 30°C overnight. In order to get as many spores as possible from the swab, it was then processed through two more elutions, one with H_2O and one with tryptic soy broth (TSB) followed by serial dilutions after each elution similar to previously published methods (Sagripanti and Bonifacino 1999; Sagripanti *et al.* 2007). Plates were incubated at 30°C or 35°C and colonies were counted after 24 h incubation, 4 days and 7 days, and the total CFU were determined. The CFUs calculated from the three elutions were added to obtain the total CFU. From this, the method was determined to be reproducible having a standard error of ± 0.26 . The standard errors for the method with other spores were ± 0.28 , ± 0.24 and ± 1.10 for *B. cereus*, *B. thuringiensis* and *B. megaterium* respectively. Figure 5 is a map showing representative locations and corresponding CFUs that also demonstrates a uniform dispersal of spores within the chamber.

For each test and before UVC exposure, a sterile synthetic-tipped swab premoistened with 1x PBS (pre-UVC sample) was used to scrub a 30 cm^2 area of the interior surface and processed as above. Plates were incubated at 30°C or 35°C and colonies were counted after 24 h incubation, 4 days and 7 days, and the total were CFU determined. After the pre-UVC sample was taken, the unit was turned on and allowed to run for 30 min to 48 h. Two post-UVC swab samples were taken from adjacent 30 cm^2 areas of the internal surface. Both unexposed and exposed samples were taken from the bottom, top and sides of the chamber even though preliminary testing showed that spore dispersal was quite uniform over the interior surface. To show that spores were being trapped into the HEPA filter, additional swabs were plunged 15–20 times into the folds of the HEPA filter at various points. Samples of the outside or 'clean side' surface of the HEPA filter were also tested. A final premoistened swab from the interior surface of the spore release chamber was obtained and acted as a second growth control as the UVC did not extend into the release chamber. All post-UVC samples were treated as the pre-UVC samples. The \log_{10} reduction was determined by comparing the number of CFU obtained from the pre-UVC tests to the averaged CFU obtained from the two post-UVC tests. All plates with no growth were held for 7 days. Tests were repeated using a second HEPA filter unit in which the inner surfaces (metal and fibre) were coated with titanium dioxide (TiO_2 P25) by the manufacturer.

Tests with spores in phosphate-buffered saline

The effect of the UVC/HEPA system was also tested using spores in 1x PBS. Known amounts of spores were vortexed three times at highest speed for 5 min, heat shocked at 65°C for 30 min and placed either directly onto TSA plates or small metal strips (aluminium foil) similar to previously published methods (Sagripanti and Bonifacino 1999; Sagripanti *et al.* 2007). For tests using spores directly inoculated onto media plates, the applied spore numbers were verified by two sets of 10-fold serial dilutions at each test. One serial dilution series was incubated immediately and the second set of dilution plates were covered with aluminium foil and placed into the unit and tested at the same time as the test plates. The inoculated plates to be exposed to the UVC were uncovered to allow the UVC to reach the spores. The unit was turned on without the fan and left on for 30 min to 24 h. After the test time, the plates were incubated at 30°C or 35°C and colonies were counted at 1, 4 and 7 days.

For tests using metal strips, a known quantity of spores (i.e. 10 µl of 10^{13–15} spores per µl) was spread with an inoculating needle to attempt to obtain as thin a coating of spores over the strip surface as performed previously by others (Blatchley *et al.* 2005). The metal strips were allowed to dry for 30 min and then placed into the UVC chamber. The inoculated control strips were placed into foil covered plates and placed into the UVC chamber. Without the fan running, the unit was turned on and run for 30 min, 45 min, 3 h or 24 h. To see if spores escaped from the plates or metal strips, open control plates were placed next to the test plates or metal strips and premoistened swabs were used to scrub the unit's interior surfaces and processed. All plates were incubated overnight at 30°C or 35°C following exposure. Both exposed and unexposed metal strips with the spores were placed directly into 1 ml 1x PBS in microcentrifuge tubes and vortexed hard for 5 min. The metal strip was then placed into 1 ml H₂O and sonicated for 30 min and then placed into 1 ml of TSB. All tubes were then heat shocked for 30 min at 65°C and 10-fold dilutions were made from each elution and plated onto TSA plates. In order to examine the entire volume and avoid missing any rare viable spores, the remainder of solution in the elution tubes that had contained the UV-exposed metal strip was plated onto more media plates. This left the metal strip in the last and now empty tube. Finally, 1 ml of TSB was added to cover the metal strip and also heat shocked. All plates and tubes with metal strips were incubated at appropriate temperature for 1, 4 and 7 days. At the end of the incubation time, the tubes were inspected for growth and colonies on the TSA plates were counted. Log reductions were determined by subtracting the CFU

obtained from UVC-exposed tests to the CFU obtained from the unexposed tests. The standard error for the method with the different spores were ±0.23, ±0.27 and ±0.31 for *B. atrophaeus*, *B. stearothermophilus* and *B. thuringiensis* respectively.

Statistical tests

All comparisons of the different results were analysed with ANOVA statistical tests. The null hypotheses were that there were no differences in log reduction results between the different conditions or bacterial spores.

Results

After preliminary tests demonstrated that the spores could be distributed evenly within the UVC test chamber, the tests with the prepared dried spores were performed. Although there appeared to be no real differences in log₁₀ reduction of CFU at different fan speeds, analysis with the ANOVA statistical test yielded a $P < 0.0001$. Tests at 0 l s⁻¹ produced a mean log₁₀ reduction of 12.5 with a SD of 2.26 ($n = 58$). Tests performed with 47–70 l s⁻¹ had a log₁₀ reduction means of 8.34 ± 2.28 ($n = 129$), while the tests at 141 l s⁻¹ and 235 l s⁻¹ produced a log₁₀ reduction means of 6.6 ± 0.85 and 8.9 ± 1.92 respectively. The unit is designed to work continuously so that the UVC fluence rate is over 3000 µW cm⁻². During CBD testing, the UVC fluence rate averaged 3700 µW cm⁻² ± 15.5 µW cm⁻² after the light source was on for 24 continuous hours compared with an average of 2000 µW cm⁻² ± 100 µW cm⁻² when the unit was on for less time (15 min to 6 h). For 30 min, 45 min, 3 h, 24 h and 48 h, the mean log₁₀ reduction with SD were 8.83 ± 6.45 ($n = 118$), 11.19 ± 1.18 ($n = 32$), 12.13 ± 1.59 ($n = 17$), 10.47 ± 2.26 ($n = 127$) and 12.18 ± 1.92 ($n = 40$) respectively. There was no significant difference in the log₁₀ reduction between the short and long exposure intervals ($P > 0.004$).

In order to examine the potential of reducing viable spores on the interior surfaces and not the capture of spores within the HEPA filter folds, further testing was performed with the fan in the off (0 l s⁻¹) position (UVC fluence of 1870 µW cm⁻²). In the tests with airborne spores of *B. cereus*, *B. megaterium* or *B. thuringiensis*, none of the samples from the UVC exposed inner surfaces produced any bacterial growth while the samples not exposed to UVC yielded 10⁶–10¹⁶ CFU depending on spore concentration (log₁₀ reduction of 6–16) ($P < 0.001$) (Table 1). Although the airborne *B. atrophaeus* spores were prepared differently than the other preparations, these tests again resulted in a reduction of viable CFU (range 6–16 logs₁₀ reduction) ($P < 0.001$) for *B. atro-*

Table 1 Log reduction of CFU obtained from inner surfaces after airborne spores are exposed to UVC within UVC/HEPA unit

Organism	Method variance by species	UV exposure time*	Mean CFU (no UV) \pm SD	Mean CFU (after UV) \pm SD	Log reduction	Number of trials
No titanium dioxide						
<i>Bacillus atrophaeus</i>	± 0.26	3 h	$2.0 \times 10^{13} \pm 1.22$	0	13	5
<i>B. atrophaeus</i>		24 h	1.0×10^7	1.5 ± 0.5	7	2
<i>B. atrophaeus</i>		24 h	1.0×10^7	1	6	10
<i>B. atrophaeus</i>		24 h	$1.5 \times 10^6 \pm 0.5$	0	6	2
<i>B. atrophaeus</i>		24 h	$2.2 \times 10^6 \pm 1.3$	1.5 ± 0.5	6	2
<i>B. atrophaeus</i>		24 h	1.0×10^{13}	2 ± 2	13	1
<i>B. atrophaeus</i>		24 h	$2.4 \times 10^{11} \pm 1.14$	0	11	6
<i>B. atrophaeus</i>		24 h	$2.4 \times 10^{13} \pm 0.54$	0	13	5
<i>B. atrophaeus</i>		24 h	$1.3 \times 10^{13} \pm 0.57$	0	13	3
<i>B. atrophaeus</i>		48 h	$2.1 \times 10^{11} \pm 1.6$	0	11	6
<i>B. atrophaeus</i>		48 h	1.0×10^{11}	50 ± 50	10	1
<i>B. atrophaeus</i>		48 h	1.0×10^{11}	10 ± 10	10	1
<i>B. cereus</i>	± 0.28	30 min	$1.33 \times 10^8 \pm 0.57$	0	8	3
<i>B. megaterium</i>	± 1.10	30 min	$2.0 \times 10^7 \pm 1.0$	0	7	3
<i>B. megaterium</i>		30 min	$1.6 \times 10^8 \pm 1.15$	0	8	3
<i>B. thuringiensis</i>	± 0.24	24 h	$1.22 \times 10^6 \pm 0.58$	0	6	4
With titanium dioxide						
<i>B. atrophaeus</i>	± 0.26	24 h	1.0×10^{12}	20 ± 0.5	10	1
<i>B. atrophaeus</i>		24 h	$2.33 \times 10^{11} \pm 0.57$	98.7 ± 0.5	9	3
<i>B. atrophaeus</i>		24 h	$1.5 \times 10^{16} \pm 0.5$	18.5 ± 2.65	15	2
<i>B. atrophaeus</i>		24 h	1.0×10^{16}	297 ± 0.5	14	1
<i>B. atrophaeus</i>		24 h	1.0×10^{16}	950 ± 50	13	1
<i>B. atrophaeus</i>		24 h	$2.25 \times 10^{17} \pm 0.5$	875 ± 125	14	4
<i>B. atrophaeus</i>		24 h	$1.5 \times 10^{17} \pm 0.5$	30.5 ± 0.5	16	2
<i>B. atrophaeus</i>		24 h	$2.66 \times 10^{17} \pm 0.57$	900 ± 100	14	3
<i>B. atrophaeus</i>		24 h	1.0×10^{17}	99.5 ± 0.5	15	1
<i>B. atrophaeus</i>		48 h	$3.33 \times 10^{17} \pm 1.15$	883.3 ± 75	14	3
<i>B. atrophaeus</i>		48 h	1.0×10^{17}	50 ± 50	16	1
<i>B. atrophaeus</i>		48 h	1.0×10^{17}	0	17	1
<i>B. cereus</i>	± 0.28	24 h	$3.0 \times 10^{16} \pm 0.70$	0	16	5
<i>B. thuringiensis</i>	± 0.24	24 h	$1.33 \times 10^{13} \pm 0.57$	0	13	3

*Tests were performed using finite measures of time although the instrument is designed to work 24 h a day, 365 days a year. CFU, colony forming units; UVC, UV radiation in the 'C' band; HEPA, high efficient particulate air.

phaeus. There was a significant difference between the log₁₀ reductions seen with the tests of the *B. atrophaeus* spores performed with the TiO₂-coated filter and uncoated filter ($P < 0.001$). In order for the TiO₂ to act as a photocatalyst and increase the UVC inactivation of the spores, the spores needed to be in contact with it. This precluded any tests with the spores in 1x PBS because these spores were placed on either metal strips or on agar plates. The tests with swabs that were plunged into the HEPA fold yielded viable colonies indicating that the UVC did not deactivate all of the spores before they were entrapped by the HEPA filter and shielded from the UVC radiation. These swab samples produced 1–1000 CFU (data not shown). None of the open plates down stream of the HEPA filter demonstrated bacterial growth.

Using only the uncoated UVC/HEPA filter unit, the tests with the previously wetted spores demonstrated a

large and significant reduction of CFU (range of 5–12 log₁₀ reduction) ($P < 0.0001$) after exposure to the UVC (Table 2). Again, there was no significant difference between exposure times ($P > 0.004$). There were significant differences in the susceptibility to UVC of the different *Bacillus* spp. no matter if the spores were dried and airborne or previously wetted and dried just prior to testing. The *B. atrophaeus* appeared to be more resistant than the other *Bacillus* spp. ($P < 0.001$). None of the post-UV cultures from the swabs of the interior surfaces or HEPA folds and none of the open plates downstream of the HEPA filter during any of these tests yielded any growth.

Discussion

The UVC/HEPA air-cleaning system unit Model UVC/HEPA-500HR was examined utilizing only dry *Bacillus* spp. spores that were either aerosolized or dried upon a

Table 2 Results of tests with spores dried upon surfaces within UVC/HEPA unit

Organism tested	Time of UV exposure*	Method variance by species	Mean CFU/no UV	Mean CFU/after UV	Log reduction	Number of trials
<i>Bacillus atrophaeus</i>	30 min	±0.23	$1.25 \times 10^{13} \pm 0.5$	56.6 ± 20.1	12	4
<i>B. atrophaeus</i>	30 min		$1.33 \times 10^{13} \pm 0.57$	164.25 ± 9.6	11	3
<i>B. atrophaeus</i>	30 min		$1.25 \times 10^{14} \pm 0.5$	1178 ± 9.2	11	4
<i>B. atrophaeus</i>	45 min		1.0×10^{10}	32.5 ± 0.5	9	1
<i>B. atrophaeus</i>	45 min		1.0×10^{11}	290 ± 2.5	9	1
<i>B. atrophaeus</i>	45 min		$1.33 \times 10^{12} \pm 0.57$	442.2 ± 84.6	10	3
<i>B. atrophaeus</i>	45 min		$2.4 \times 10^{12} \pm 1.6$	0	12	5
<i>B. atrophaeus</i>	45 min		$1.2 \times 10^{13} \pm 0.44$	58.9 ± 10	12	5
<i>B. atrophaeus</i>	3 h		1.0×10^{13}	172 ± 0.5	11	1
<i>B. atrophaeus</i>	3 h		1.0×10^{13}	10.5 ± 0.5	12	1
<i>B. stearothermophilus</i>	30 min	0.27	$1.4 \times 10^6 \pm 0.47$	0	6	5
<i>B. stearothermophilus</i>	30 min		$1.18 \times 10^7 \pm 0.46$	4.5 ± 1.77	7	5
<i>B. stearothermophilus</i>	30 min		$9.7 \times 10^6 \pm 0.03$	1.2 ± 0.42	7	5
<i>B. stearothermophilus</i>	30 min		$1.5 \times 10^6 \pm 0.58$	1.7 ± 1.0	6	4
<i>B. stearothermophilus</i>	30 min		1.0×10^6	19.5 ± 0.5	5	1
<i>B. thuringiensis</i>	30 min	0.31	$1.79 \times 10^9 \pm 1.0$	1.5 ± 0.2	9	10
<i>B. thuringiensis</i>	30 min		1.0×10^9	31.5 ± 0.5	8	1
<i>B. thuringiensis</i>	30 min		$1.67 \times 10^9 \pm 0.87$	0	9	9
<i>B. thuringiensis</i>	24 h		$2.17 \times 10^9 \pm 1.17$	1.4 ± 0.9	9	6
<i>B. thuringiensis</i>	24 h		$1.6 \times 10^9 \pm 0.54$	1.3 ± 1.3	8	5
<i>B. thuringiensis</i>	24 h		$1.5 \times 10^9 \pm 0.5$	35.5 ± 4.5	7	2
<i>B. thuringiensis</i>	24 h		1.0×10^9	25.5 ± 0.5	8	2

*Tests were performed using finite measures of time although the instrument is designed to work continuously. CFU, colony forming units; UVC, UV radiation in the 'C' band; HEPA, high efficient particulate air.

metal strip. This would be the expected condition of spores travelling within a building's air system. Wet spores would tend to fall to the bottom surface and to remain there until they dried and were able to be airborne.

The unit is designed to work continuously, resulting in the optimal output of UVC emissions. The peak UVC output and constant prolonged exposure is expected by the manufacturer to more effectively inactivate spores than is shown with the exposure times in this report. We did not discern any significant difference in results of the different exposure times tested in this study. The fact remains that the UVC did inactivate bacterial spores that were exposed to the light emissions even for 30 min.

Overall, the tests utilizing both airborne and previously wetted spores (placed upon a metal strip or agar plate) resulted in inactivation of the majority of spores. When 10^{11} – 10^{13} CFU from the unexposed tests (no UVC) were compared with 10^0 – 10^3 CFU from the exposed tests (with UVC), it is clear that most of the *B. atrophaeus* spores exposed to the UVC did not grow. This pattern continued with the more susceptible *B. stearothermophilus* and *B. thuringiensis* spores although fewer cells in general grew in any of these tests. This might be due to a lower number of spores initially applied to the test surface. The very few CFU that did grow after UVC exposure in these tests

may indicate resistant spores or could be the result of aggregated spores that clumped in the saline as it was difficult to break up tiny aggregates. This clumping probably precluded an even dispersal of spores on the test surface such that the top layer of spores were a barrier to UVC, preventing penetration to lower spore levels. Thus some spores were shielded from the UVC and were able to grow. This problem has been addressed recently and might be resolved by using UVA with the UVC in order to penetrate deeper layers as proposed by others (Kuhn *et al.* 2003). These tests were all performed within an air-conditioned building with controlled humidity. Moisture in the air could cause the spores to aggregate so it is important to note that not all of the spores were inactivated under the test conditions. It remains to be seen how different conditions of relative humidity and temperature will affect the efficacy of the UVC/HEPA unit.

Although there appeared to be a large log₁₀ reduction in the tests of swabs that were inserted into the HEPA folds (data not shown), this is probably due to spores being trapped within the filter fibres. Tests with the fan at low to high speeds (47 – 235 l s⁻¹) pulling air through the filter were hard to interpret because it was unknown what percentage of spores were trapped inside the filter inner folds and what percentage stuck to an interior hard surface before the fan was turned on. Therefore, most of

the tests were performed at the zero setting even though the manufacturer stated that the optimal UVC fluence rate occurred when the fan was running. The filters did not appear to degrade after the continuous UVC exposure because none of the plates or swab tests downstream of the filter produced any bacterial growth during the time of testing. This issue could be addressed in the future.

The addition of a TiO₂ coating on the inner surface of the HEPA filter increased the inactivation of *Bacillus* spores. The added advantage of coupling UVC with a TiO₂-coated HEPA filter unit is that the UVC and photocatalyst on the filter appears to not only inactivate, but also to destroy micro-organisms (Goswami 1995; Goswami *et al.* 1997). It has been suggested that the service life of the HEPA filter may be lengthened by the use of both UVC and TiO₂ because the bacteria and organic compounds that normally build up and clog the filter pores are degraded on the TiO₂ surface (Goswami 1995; Block *et al.* 1997; Goswami *et al.* 1997). However, this point would have to be explored in the future. Control over the number of the dried *B. atrophaeus* spores to be delivered and dispersed by our method was extremely difficult as the measured weight of the spores in the tubes did not always seem to correspond with the number of spores that grew in the control UVC-unexposed tested. This was especially true with the tests with TiO₂ as the expected number of spores (measured) by the weight noted for that tube was always lower than what was realized in the tests. This increase in the log₁₀ of *B. atrophaeus* spores in the TiO₂ tests made comparison with the noncoated tests difficult. However, the fact remains that there was a large reduction of viable spores after exposure to the UVC. The TiO₂-coated HEPA filter tests after 48 h exposure to UVC also produced higher number of spores that were viable compared with the much shorter exposure times. It is possible that when the swabs were rubbed on the metal adjacent to the HEPA folds, they accidentally dislodged spores from within the folds. This could account for one swab yielding 20 or 100 CFU while its partner swab produced no CFU. This can be seen with the large SD. But this cannot explain the three tests with the TiO₂-coated surface that produced almost 10³ CFU from six different swabs. Therefore contamination seems unlikely in this case. Another explanation is more probable. These tests were performed at the end of the study and an examination of the interior of the unit's metallic surface revealed scratches in the TiO₂ surface. It is possible that after repeated scrubbing, scratches were formed in the soft TiO₂ surface. These scratches provided sites where the spores were shielded from the UVC light.

This study has shown that the UVC/HEPA unit tested can effectively inactivate high numbers of *Bacillus* spores before they are trapped in the HEPA filter. The dual use

of UVC and HEPA filters in an air duct of a building air system would inactivate and trap particulates and micro-organisms allowing only clean, decontaminated air to flow inside the building. This would be most important during possible release of bacteria or bacterial spores within a building or at a building's air intake. In addition, the unique shape and ability to segregate the contaminants from personnel who would service the air-duct systems after such a release would preclude any accidental secondary infection. The UVC/HEPA unit could be modified and used in other applications such as in airplane air systems, hospital isolation wards and in other spaces where air quality is of the utmost importance. The unit could be made smaller and mobile to expand its use as a part of a powered air purifying respirator or motorized vehicle air intake.

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