In-Place Testing of In-Duct Ultraviolet Germicidal Irradiation

Stephen N. Rudnick, DSc, CIH **Melvin W. First, DSc, PE, CIH**
Richard L. Vincent **Philip W. Brickner, MD**

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This paper reports results from a field study designed to demonstrate a method for evaluating a building's in-duct ultraviolet germicidal irradiation (UVGI) system using naturally occurring airborne microorganisms and to evaluate its potential effectiveness against selected airborne microorganisms. Forty-eight 64 in. (1.63 m) long ultraviolet germicidal lamps were installed perpendicular to airflow and upstream of the cooling coils in a heating, ventilating, and air-conditioning (HVAC) system for a retail space in a large New York City office building. A portable in-duct UVGI apparatus was used to benchmark ultraviolet susceptibility of naturally occurring airborne microorganisms. Benchmarking was accomplished by measuring the portable apparatus's efficiency for inactivating UVGI-resistant microorganisms in the laboratory at various operating conditions. Inactivation efficiency for both the in-duct UVGI-equipped HVAC system and portable in-duct UVGI apparatus were then measured simultaneously using naturally occurring microorganisms. If the UVGI-equipped HVAC system had higher inactivation efficiency than the portable in-duct UVGI apparatus, then the UVGI-equipped HVAC system's efficiency for inactivating the UVGI-resistant microorganisms used in the laboratory would be expected to be higher than the efficiency of the portable in-duct UVGI apparatus. Based on the results for the tested UVGI-equipped HVAC system, the expected efficiency was at least 88% for Bacillus subtilis *var.* niger *spores, a common surrogate for* Bacillus anthracis *spores.*

INTRODUCTION

Ultraviolet germicidal irradiation (UVGI) uses 254 nm ultraviolet radiation from low-pressure mercury discharge lamps to reduce the indoor concentration of airborne microorganisms in order to reduce the risk of disease transmission. Germicdal UV radiation damages the genetic material of microorganisms so that they are unable to replicate. UVGI can be applied utilizing a number of different approaches, but generally one of the following three methods is used for sanitizing air in public buildings: 1) upper-room UVGI, 2) cooling-coil UVGI, and 3) in-duct UVGI.

With upper-room UVGI, the air in the upper portion of a room is irradiated while UV radiation in the occupied portion of the room is kept below levels that would be harmful to occupants; sufficient vertical air circulation is essential for upper-room UVGI to be effective. The primary objective of upper-room UVGI is to disinfect air in the room where both the infected and exposed occupants are located so that airborne transmission of disease from the infected to the exposed does not take place. Secondarily, microorganisms present in recirculated air may also

Stephen N. Rudnick is a lecturer on industrial hygiene engineering and **Melvin W. First** is professor emeritus of environmental health engineering in the Department of Environmental Health, Harvard School of Public Health, Boston, MA. **Richard L. Vincent** is a research manager and lightning specialist and **Philip W. Brickner** is director of tuberculosis studies in the Department of Community Medicine (Tuberculosis Ultraviolet Shelter Study), Saint Vincent's Hospital and New York Medical College, Manhattan, NY.

be inactivated. Beginning in the 1930s (Wells 1955; Riley and O'Grady 1961) and continuing to the present day (Miller et al. 2002; Xu et al. 2003; First et al. 2007), numerous experimental studies have demonstrated the efficacy of upper-room UVGI. For specific applications, such as hospital surgical rooms where occupants wear protective equipment and unoccupied rooms, whole-room UVGI is also used.

With cooling-coil UVGI, the cooling coils, drip pans, and nearby surfaces are continuously irradiated to control microbial growth. Cooling-coil UVGI is generally concerned with the control of environmental microorganisms. The three primary objectives of cooling-coil UVGI are to 1) minimize aerosolization of microorganisms and allergens, 2) maintain cooling-coil heat transfer and pressure drop, and 3) reduce objectionable odors. Levetin et al. (2001) showed that the use of cooling-coil UVGI resulted in a significant reduction in fungi within insulation near cooling coils. Menzies et al. (2003) measured a 99% reduction in viable microorganisms and endotoxins on irradiated metal surfaces located on cooling coils and drip pans, as well as a large decline in worker-reported symptoms. Other studies have also shown significant reduction in microbial growth on cooling coils (Menzies et al. 1999; RLW 2006). UVGI cooling-coil disinfection is widely used and its application is now a specification for all new U.S. General Services Administration buildings (GSA 2003).

For in-duct UVGI, the air in ductwork or plenums of a heating, ventilating, and air-conditioning (HVAC) system is irradiated with high levels of UV radiation. In-duct UVGI is generally concerned with the control of infectious microorganisms. The objective is to disinfect air that is supplied to an occupied space. Unlike cooling-coil UVGI, which occurs continuously, in-duct UVGI has only a brief period of time to irradiate the air as it flows through the system and, thus, much higher levels of UVGI are required. If in-duct UVGI can be installed in close proximity to the downstream side of the cooling coils and drip pans, they can be irradiated simultaneously. Although studies on in-duct UVGI have been done in the laboratory (VanOsdell and Foarde 2002; RTI 2005), we know of only two previous studies that were done in the field, and these were done long ago (Rentschler and Nagy 1940; Nagy et al. 1954). Portable room-size air-cleaning devices based on UVGI can also be categorized as in-duct UVGI because in these devices room air is passed through a chamber containing UVGI and then returned to the room.

The goals of this study were to develop a methodology for in-place testing of in-duct UVGI and then to apply this methodology to the HVAC system of an occupied building. Kowalski and Bahnfleth (2004) discussed testing and commissioning of in-duct UVGI systems. They suggested three possible methods to ensure that a system performs as designed: 1) air sampling upstream and downstream of the UV lamps while a challenge microorganism is injected upstream, 2) air sampling simultaneously upstream and downstream of the UV lamps for naturally occurring microorganisms, and 3) air sampling downstream of UV lamps for naturally occurring microorganisms before and about a week or so after UV lamps have been activated. A fourth option is air sampling for natural ambient microorganisms from two identical ventilation systems, one downstream of the UV lamps and the other in a comparable location, but without UVGI. Kowalski and Bahnfleth pointed out that the first option, injection of a challenge microorganism, would not likely be permitted. For the third option, downstream air sampling before and about a week or so after the UV lamps are turned on, the outcome cannot be attributed solely to in-duct UVGI because other factors can modify the mix of naturally occurring ambient microorganisms over time. For our work, we chose the second option, air sampling simultaneously upstream and downstream of the UV lamps using naturally occurring airborne microorganisms as the challenge aerosol. We included a methodology to benchmark the UV susceptibility of the naturally occurring microorganisms used during the testing.

Benchmarking was accomplished by using a portable in-duct UVGI apparatus whose efficiency for inactivating a UVGI-resistant microorganism was characterized in this research in the

laboratory under various operating conditions. If the UVGI-equipped HVAC system had a higher inactivation efficiency for naturally occurring microorganisms than the portable in-duct UVGI apparatus operating at a specific set of conditions, then the efficiency of the UVGI-equipped HVAC system for inactivating the UVGI-resistant microorganisms used in the laboratory would be expected to be higher than the efficiency of the portable in-duct UVGI apparatus.

MATERIALS AND METHODS

Laboratory Tests

Portable In-Duct UVGI Apparatus. The portable in-duct UVGI apparatus that was evaluated in the laboratory contains four identical in-duct UV modules; each module has a 5/8 in. (15.9 mm) diameter, 8.3 in. (211 mm) long, 10 W cylindrical low-pressure mercury germicidal lamp suspended on the centerline of a 6 in. (152 mm) diameter, 12 in. (305 mm) long galvanized steel duct. The four modules are separately switched and designed so that they fit together like standard ventilation ductwork. Each module contains a small glass view port on the surface of the duct to allow the UV lamp to be observed safely during operation. In addition, specially designed holders in which the sensor of a UV meter fits allow irradiance to be monitored through a hole in the duct of each module. This single measurement was not representative of the mean irradiance in the module; its only purpose was to verify that the UV output of the lamp in each module remained constant throughout the course of our tests.

A schematic diagram of the portable in-duct UVGI apparatus, including the four UV modules, is shown in Figure 1. Air filtered with a high efficiency particulate air (HEPA) filter and the output of an aerosol nebulizer entered the apparatus through a 6 in. (152 mm) diameter elbow and flowed around a Stairmand disk (Stairmand 1951), which induced radial mixing. The airflow then passed though the four UV modules and around a second Stairmand disk. Although all four UV lamps were switched on for most of the laboratory tests, for a few of the tests only one or two UV lamps were on. The airflow was exhausted by an axial fan; its rate was adjusted

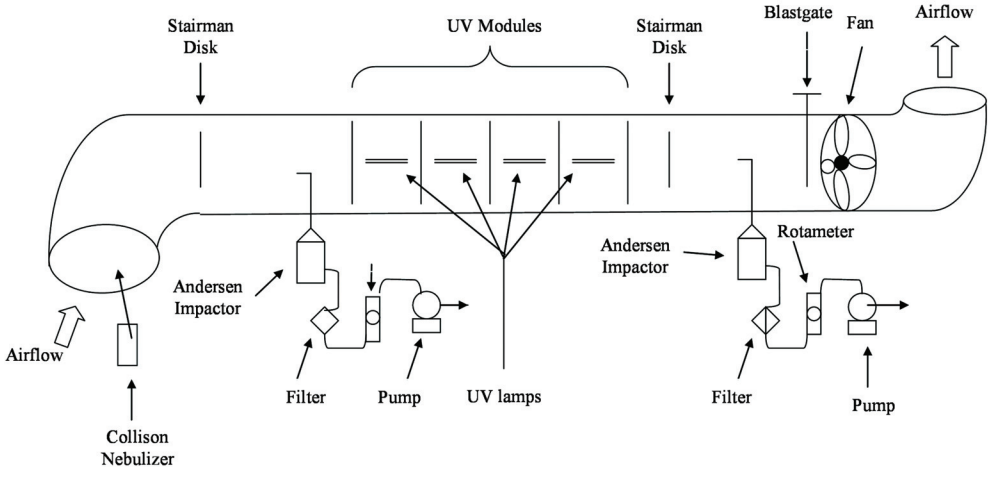

Figure 1. Portable in-duct UVGI apparatus.

with a speed controller or blastgate based on thermal anemometer measurements. Most tests were done at 50 cfm (0.024 m^3/s), although a few were made at 100 cfm (0.047 m^3/s).

Microbiological Aerosol. Microorganisms were aerosolized from aqueous suspensions using a six-jet Collison nebulizer (Hinds 1999). We wanted to benchmark the portable in-duct UVGI apparatus's capability to kill or inactivate microorganisms over a wide range of UV susceptibilities, so two different bacteria were used as test agents in the laboratory: 1) spores of *Bacillus subtilis* var. *niger*, also known as *Bacillus atrophaeus* Nukamura (ATCC 9372), which is very insensitive to UVGI, and 2) *Serratia marcescens* (ATCC 8195), which is very sensitive to UVGI. The suspension of *B. subtilis* var. *niger* spores was diluted to about 10^8 colony-forming units per milliliter with cell-culture grade water prior to being aerosolized. *S. marcescens* was prepared by adding a loopful of two-day-old cells grown on nutrient agar into 100 mL of nutrient broth and culturing the mixture at 77°F (25°C) for 24 hours at 200 rpm. About 1 mL of the resulting culture was pipetted into Eppendorf tubes, washed twice with 1 mL of phosphate-buffered saline solution, harvested by centrifugation at 2300 xg for 15 min., and then stored at –94°F (–70°C). Prior to being aerosolized, aliquots of these bacterial cells were suspended in isotonic saline plus 10% fetal calf serum, which is widely used as a synthetic saliva (Theunissen et al. 1993).

Sampling and Analysis for Laboratory Tests. As shown in Figure 1, air samples were collected at 1 cfm (28.3 L/min) upstream and downstream of the four in-duct UV modules with six-stage Andersen microbial impactors (Andersen 1958) for a 1–5 min. sampling period, depending on concentration. In order to obtain representative samples, both the upstream and downstream samples were taken about five duct diameters downstream of the Stairmand disks. The sampling train consisted of the impactor followed by a HEPA filter, calibrated rotameter, and pump. Most of the cultureable organisms were collected on stages 5 and 6, which have a 50% cutpoint of 1.1 and 0.65 μm, respectively. Analysis of the impactor samples involved incubation at 98.6°F (37°C) for two days and counting colony-forming units in the impactor's Petri dishes, which contained nutrient agar. Positive-hole corrections were made for the colony counts (Andersen 1958). The fraction of microorganisms surviving UVGI (f_s) was calculated from the following equation:

$$f_s = \frac{C_{down}}{C_{up}} \tag{1}$$

where C_{down} and C_{up} are the downstream and upstream microorganism concentrations in colony-forming units per unit volume. The efficiency of inactivation—that is, the fraction of microorganisms inactivated—is equal to $1 - f_s$.

Field Tests

HVAC System. The HVAC system that we used for all field tests supplies 11,000 cfm (5.2 m^3/s) of conditioned air to a ground-level commercial space located in a large New York City office building (Merendino 1992). Outside air (OA) from a large underground OA plenum shared by multiple HVAC systems flows horizontally through a 5.5 ft. (1.7 m) high, 7.8 ft. (2.4 m) wide grille and dampers into a mixing plenum. Return air (RA) from the commercial space is discharged by the RA fan and then divides into two streams: one stream flows through dampers and is exhausted outdoors, and the other stream flows vertically downward through dampers into the mixing plenum where it mixes with OA. This mixed air (MA) can consist of up to 100% OA, when no conditioning is required, to roughly 20% OA when OA is too cold or too hot. MA exits the mixing plenum horizontally through prefilters and final filters and then enters a second plenum. Prefilters and final filters are mounted on the upstream and downstream side of the frame that separates the mixing and

second plenums. During our tests, the walls and floor of the second plenum were covered with aluminum sheet metal to promote reflection of UV radiation, and both prefilters and final filters were removed; thus, for our tests, the mixing and second plenums were combined to form a single plenum, which was 6 ft. (1.8 m) high and 11.5 ft. (3.5 m) wide perpendicular to the direction of airflow and 9.2 ft. (2.8 m) long in the direction of airflow. MA exits the lower section of this plenum horizontally and passes through heating and cooling coils, which are 3.4 ft. (1.0 m) high and 11.5 ft. (3.5 m) wide, where it is heated or cooled by hot or chilled water, as required, before entering horizontally into a supply air (SA) plenum. The SA plenum takes a 90º turn and is drawn horizontally into the SA fan, located inside the plenum. SA exits the SA fan flowing vertically upward through ductwork, eventually discharging through ceiling diffusers into the commercial space.

UVGI System. Twelve units, each having four 64 in. (1.6 m) long 65 W low-pressure mercury germicidal UV lamps, were placed in the 9.2 ft. (2.8 m) long plenum directly upstream of and close to the coils. Each lamp was housed in a closely fitting concentric, cylindrical quartz sleeve to minimize the cooling effect of the airflow, which is known to adversely affect the UV power output of the lamps (Nagy et al. 1954). As shown in Figure 2, the axes of the lamps were vertical; the lamps were arranged in four rows, each of which was perpendicular to the direction of airflow. Each row contained 12 lamps, which were a distance of 11 in. (280 mm) from each other. Lamps in adjacent rows were aligned in the direction of airflow and separated by 4 in. (100 mm). Because the lamps and their sheaths were made of quartz, blockage of the UV rays by nearby lamps did not significantly reduce the UV levels. A UV sensor placed inside the second plenum at a fixed location was used to monitor irradiance during tests. This single measurement was not representative of the mean irradiance in the plenum; its sole function was to ensure that the UV output of the lamps remained constant throughout the testing period.

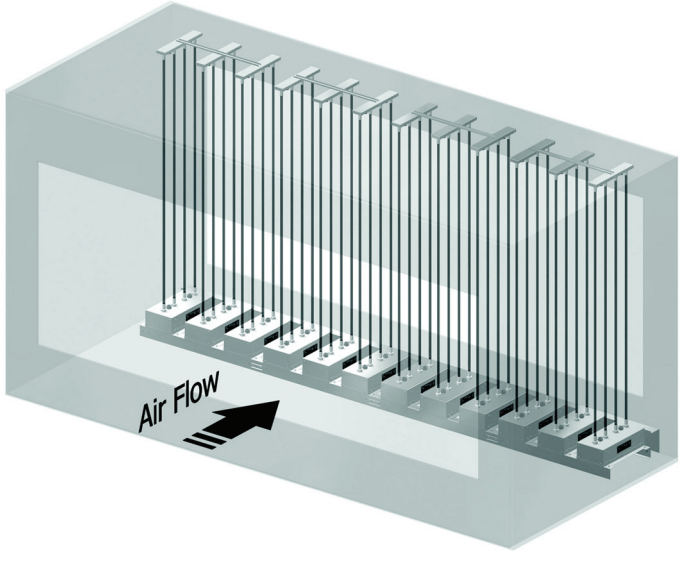

Figure 2. UV fixture inside HVAC plenum.

Portable In-Duct UVGI Apparatus. The portable in-duct UVGI apparatus was configured essentially the same way for the field tests as it was for the laboratory tests except the collison nebulizer was not used. During every test using the portable in-duct UVGI apparatus, all four UV lamps were turned on and airflow was maintained at 50 cfm (0.024 m^3/s).

Testing Protocol. Two types of experimental field tests were performed: 1) tests in the in-duct UVGI-equipped HVAC system and 2) tests in the portable in-duct UVGI apparatus. For both of these tests, naturally occurring airborne microorganisms upstream and downstream of the UVGI were sampled for either 15 or 20 min. and the fraction of microorganisms surviving was calculated from Equation 1. For the in-duct UVGI apparatus, when using 100% OA, air entered from the OA plenum and was exhausted back into this plenum. When using 100% RA, air entered the portable in-duct UVGI apparatus from the RA duct of the HVAC system. Air was exhausted from the portable in-duct UVGI apparatus into the SA plenum through a flexible 6 in. (152 mm) diameter duct attached to the exit of the portable apparatus. If the air had been exhausted instead into the mechanical room where the pressure was higher than in the RA duct, the fan in the portable in-duct UVGI apparatus would have been overpowered, causing the airflow in the portable apparatus to reverse direction. For the UVGI-equipped HVAC system, the upstream sample was taken from the OA plenum when using 100% OA and from the RA duct when using 100% RA. In order to be sure that no microorganisms were shed from the cooling coils into the impactor, the downstream sample was taken as far downstream of the UV lamps as possible but upstream of the coils. To prevent UV radiation from impinging on the downstream sample, a small barrier was placed between the UV lamps and the impactor.

Except for test numbers 1 and 2 (see Table 2), air samples upstream and downstream of the UVGI were collected simultaneously with a single-stage Andersen microbial impactor, which is identical to the sixth stage of the six-stage Andersen microbial sampler (Andersen 1958), for both the UVGI-equipped HVAC system and the portable in-duct UVGI apparatus. For test numbers 1 and 2, six-stage Andersen microbial impactors were used and sampling in the HVAC system and the portable apparatus were done sequentially; that is, a test was done in the HVAC system and immediately after completion of this test, the test was performed with the portable in-duct UVGI apparatus or vice versa. Because the sixth stage of the six-stage Andersen impactor is identical to the single-stage Andersen impactor and because we were only interested in the number of bacteria and fungi, regardless of particle size, the single-stage and six-stage impactors should give equivalent results. We did not use the same impactors in the same upstream and downstream locations in back-to-back tests; the impactors were, however, disinfected using ethanol wipes between tests.

The Petri dishes used in the Andersen microbial impactors contained R2A agar. Four Petri dishes that were taken into the field, but not used, served as controls. All of the Petri dishes were taken back to the laboratory and placed in an incubator at 86°F (30°C) for two days. None of the controls developed colonies. When counting colony-forming units, fungi were differentiated from bacteria through dissimilarities visible with the naked eye. For confirmation, the distinction between filamentous fungi and smooth, shiny bacteria was observed through a stereoscopic microscope.

We chose to use a microbial impactor rather than an impinger for the field tests because we were concerned that a single airborne particle could contain multiple naturally occurring microorganisms. Using an impactor, this particle would be counted only once, whereas using an impinger, each microorganism contained within the particle would be counted separately. If many microorganisms were contained in a single particle, some of these microorganisms might be shielded from the UV rays. Thus, using an impinger, a few large particles could have a large influence on the measurement of the fraction of microorganisms surviving UVGI, whereas using an impactor, they wouldn't.

RESULTS

Laboratory Tests

In order to characterize experimental variability, the portable in-duct UVGI apparatus with all four lamps turned on was tested in the laboratory 12 times at an airflow rate of 50 cfm (0.024 m^3/s) using *B. subtilis* var. *niger* spores as a challenge aerosol. Experimental results are shown in Table 1. On average, the fraction surviving passage through the portable in-duct UVGI apparatus was 0.12 (95% confidence interval: 0.06 to 0.18).

When the portable in-duct UVGI apparatus was tested using *S. marcescens* as a challenge aerosol at an airflow rate of 100 cfm (0.047 m^3/s) with the lamps in only one or two modules powered, no bacteria were detected after passage through the apparatus. Additional tests with one module powered and 90% of the lamp covered were done nine times. On average, the fractions surviving passage through the portable in-duct UVGI apparatus at 50 cfm (0.024 m^3/s) and 100 cfm (0.047 m^3/s) were 0.0257 and 0.0741, respectively. The corresponding 95% confidence intervals were 0.0164 to 0.0350 and 0.0227 to 0.126, respectively.

Field Tests

A total of 15 tests were conducted in a New York City office building during three time periods: test numbers 1 and 2 on September 11–12, test numbers 3 through 8 on November 6–7, and test numbers 9 through 13 on November 21–22 (see Table 2). In each of these tests, the fraction of bacteria and fungi in either 100% OA or 100% RA surviving passage through the UVGI-equipped HVAC system was compared to the fraction surviving passage through the portable in-duct UVGI apparatus. Because each of the 15 tests yielded results for both bacteria and

Table 1. Laboratory Tests with *B. subtilis* var. *niger* Spores*

Test Number	Fraction Surviving UVGI (f_s)
1	0.38
2	0.16
3	0.12
4	0.16
5	0.081
6	0.14
7	0.14
8	0.15
9	0.040
10	0.016
11	0.031
12	0.015
Standard Deviation	0.10
Mean	0.12
95% Confidence Interval	0.06 to 0.18

* Four UV lamps were turned on; airflow was 50 cfm (1.42 m^3/s).

fungi, a total of 30 comparisons could be made. For one-third of these comparisons—three for bacteria and seven for fungi—the fraction surviving UVGI was greater than 1 for either the UVGI-equipped HVAC system or the portable in-duct UVGI apparatus or both. Because this is not possible, these 10 comparisons were discarded, leaving a total of 20 comparisons, which are shown in Table 2. When no distinction is made between bacteria and fungi or between 100% outdoor and 100% RA, the mean fraction of naturally occurring airborne microorganisms surviving passage through the UVGI-equipped HVAC system was 0.57 as compared to 0.69 for the portable in-duct UVGI apparatus; therefore, on average, the portable in-duct UVGI apparatus was less effective at inactivating naturally occurring airborne microorganisms than the UVGI-equipped HVAC system.

Table 2. Field Tests in NYC Office Building*

Test Number	Fraction Surviving UVGI (f_s)		Difference
	HVAC	Portable	HVAC–Portable
Bacteria			
1	0.71	0.91	0.20
3	0.69	0.82	0.13
4	0.57	0.84	0.27
5	0.54	0.54	0.00
6	0.56	0.68	0.12
7	0.48	0.85	0.37
8	0.36	0.76	0.40
9	0.52	0.44	–0.08
10	0.57	0.48	–0.09
11	0.54	0.54	0.00
12	0.30	0.38	0.08
13	0.68	0.40	–0.28
Fungi			
1	0.91	0.48	–0.43
2	0.65	0.81	0.16
3	0.81	0.77	–0.04
4	0.74	0.79	0.05
5	0.43	0.87	0.44
6	0.75	0.76	0.01
7	0.50	0.96	0.46
8	0.13	0.63	0.50
Standard Deviation	0.18	0.18	0.25
Mean	0.57	0.69	–0.57

*Test numbers 1–8 used 100% outside air, and test numbers 9–12 used 100% return air.

In order to determine whether this difference is statistically significant, a paired t-test was used to compare the fraction surviving UVGI for the 20 comparisons of the portable in-duct UVGI apparatus and the UVGI-equipped HVAC system. The difference of 0.12 with the portable in-duct UVGI apparatus having the higher fraction surviving was found to be significant at exactly the 95% confidence level.

DISCUSSION

Laboratory Tests

As shown in Table 1, the mean fraction of *B. subtilis* var. *niger* surviving UVGI for the laboratory tests was 0.12 and the standard deviation was 0.10. Thus, the variability in terms of the coefficient of variation is 83%. Although we were concerned about this degree of variability between ostensibly identical tests, the coefficient of variation for this application can be misleading. We could have chosen to present our results in terms of the fraction not surviving UVGI—that is, the fraction of bacteria killed or inactivated by UVGI. If we had reported our data in this way, then the mean fraction inactivated during the laboratory tests would be 1–0.12, or 0.88; the standard deviation would remain the same (0.10); and the coefficient of variation would be equal to 0.10/0.88, or 11%. One reason that we repeated the same test 12 times was to obtain a reasonable measure of the mean fraction of bacteria surviving UVGI. The 95% confidence interval for the fraction of bacteria killed or inactivated by UVGI is 82% to 94%. Although this confidence interval is still not as small as we would like, for the purposes of benchmarking the portable in-duct apparatus, it is reasonable.

Field Tests

At the 95% confidence level, we conclude that the fraction of naturally occurring airborne microorganisms surviving passage though the portable in-duct UVGI apparatus was greater than the fraction surviving passage through the UVGI-equipped HVAC system. We would expect that the UVGI-equipped HVAC system would also inactivate a greater fraction of *B. subtilis* var. *niger* spores than the portable in-duct UVGI apparatus; that is, the fraction of *B. subtilis* var. *niger* spores surviving passage through the UVGI-equipped HVAC system would be expected to be <0.12. Determination that the fraction of naturally occurring airborne microorganisms surviving passage through the portable in-duct UVGI apparatus was greater than the fraction surviving passage through the UVGI-equipped HVAC system is an important result because *B. subtilis* var. *niger* spores are extremely difficult to inactivate using UVGI; microorganisms that commonly cause disease are generally much easier to inactivate with UVGI. In addition, because *B. subtilis* var. *niger* spores are commonly used as a surrogate for *Bacillus anthracis* spores, the UVGI-equipped HVAC system would also be expected to inactivate at least 88% of *B. anthracis* spores.

We do not know why we detected more microorganisms downstream than upstream for a third of our field tests. We were careful to minimize the possibility of contamination occurring, but it is possible that contamination did occur for some of the samples. Because we did not use the same impactors in upstream and downstream locations in back-to-back tests, it is possible that contamination remained on an impactor being used downstream from an upstream sample taken in a previous test. This is unlikely, however, for the following three reasons: 1) deposited microorganisms would probably not get re-aerosolized, 2) a fresh Petri dish with fresh media was used for every sample, and 3) we cleaned impactors with ethanol using wipes between samples. It's possible, however, that this latter step could also introduce contamination in some of the samples.

Although it could be argued that all of the other tests were suspect because a third of them were discarded, there was nevertheless a statistically significant difference between the fraction surviving UVGI for the portable in-duct UGVI apparatus and the UVGI-equipped HVAC

system for the remaining tests. This difference in favor of the UVGI-equipped HVAC system suggests that it was better at killing or inactivating microorganisms than the portable apparatus. This greater effectiveness of the UVGI-equipped HVAC system is really what is important in that the effectiveness of the portable UVGI in-duct apparatus for killing or inactivating *B. subtilis* var. *niger* spores was determined in the laboratory.

CONCLUSION

It has been demonstrated that the effectiveness of an in-duct UVGI-equipped HVAC system for an occupied building can be tested in the field using a portable in-duct UVGI apparatus without generating or exposing occupants to a challenge aerosol containing test microorganisms. Naturally occurring airborne microorganisms that are already present in the ventilation system from either outdoor or indoor sources can be used without concern for adverse health effects.

For tests on any UVGI-equipped HVAC system, the portable in-duct UVGI apparatus should be designed so that the UV dose to microorganisms can be easily varied. This can be achieved using multiple, separately switched lamps or by controlling exposure time by changing airflow rate through the apparatus. The fraction of *B. subtilis* var. *niger* spores or other difficult-to-kill microorganisms should be measured in the laboratory for a range of UV doses.

In order to make a definitive statement about the effectiveness of the in-duct UVGI-equipped HVAC system, the fraction of naturally occurring airborne microorganisms surviving passage through the portable in-duct UVGI apparatus should be higher than through the UVGI-equipped HVAC system at a statistically significant level. The fraction of *B. subtilis* var. *niger* spores surviving passage through the UVGI-equipped HVAC system can then be expected to be less than the fraction that survived passage through the portable in-duct UVGI apparatus. Similarly, the fraction surviving UVGI in the portable in-duct apparatus can also be measured for other microorganisms. For example, if bacillus Calmette-Guérin (BCG), which is commonly used as a surrogate for *Mycobacterium tuberculosis*, was used as the test microorganism, then the fraction of BCG surviving passage through the UVGI-equipped HVAC system can then be expected to be less than the fraction that survived passage through the portable in-duct UVGI apparatus. For an optimal test, the difference between the fraction of naturally occurring airborne microorganisms surviving passage through the portable in-duct UVGI apparatus and through the in-duct UVGI-equipped HVAC system needs to be no larger than is necessary for statistical significance. The fraction of *B. subtilis* var. *niger* spores or other microorganisms surviving passage through the portable in-duct UVGI apparatus will then be as low as possible; thus, it follows that the expected maximum value of the fraction surviving passage through the UVGI-equipped HVAC system will also be as low as possible. If the fraction of naturally occurring airborne microorganisms surviving passage through the portable in-duct UVGI apparatus turns out to be smaller than through the UVGI-equipped HVAC system, the operating parameters for the portable in-duct apparatus would need to be modified so as to reduce its efficiency (i.e., higher airflow rate or fewer modules). The field test would then be repeated.

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