The Characterization of Upper-Room Ultraviolet Germicidal Irradiation in Inactivating Airborne Microorganisms

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In this study, we explored the efficacy of upper-room ultraviolet germicidal irradiation (UVGI) in reducing the concentration of *Serratia marcescens* and *Mycobacterium bovis* bacille Calmette-Guérin (BCG) aerosols in an enclosed space. We constructed a facility (4.5 m × 3 m × 2.9 m) in which both ceiling- and wall-mounted UV fixtures (UV output: 10W and 5W respectively) were installed. The use of ceiling- and wall-mounted UV fixtures (total UV output: 15W) without mixing fan reduced the concentration of *S. marcescens* aerosols by 46% (range: 22–80%) at 2 air changes per hour (ACH) and 53% (range: 40–68%) at 6 ACH. The use of ceiling- and wall-mounted UV fixtures with mixing fan increased the UV effectiveness in inactivating *S. marcescens* aerosols to 62% (range: 50–78%) at 2 ACH and to 86% (81–89%) at 6 ACH. For BCG aerosols, UV effectiveness in inactivating BCG aerosols at 6 ACH were 52% (range: 11–69%) by ceiling-mounted UV fixture only (total UV output: 10W) and 64% (51–83%) by both ceiling- and wall-mounted UV fixtures (total UV output: 15W). Our results indicated that the equivalent ventilation rate attributable to upper-room UVGI for BCG aerosols ranged from 1 ACH to 22 ACH for ceiling-mounted UV fixtures and from 6.4 ACH to 28.5 ACH for ceiling- and wall-mounted UV fixtures. Both generalized linear and generalized additive models were fitted to all our data. The regression results indicated that the number of UV fixtures, use of mixing fan, and air exchange rate significantly affected UV effectiveness ($p < 0.01$, 0.01, 0.01 respectively). However, the strain difference (*S. marcescens* vs. BCG) appeared less important in UV effectiveness ($p = 0.26$). Our results also indicated that UV effectiveness increased at higher temperature ($p < 0.01$), lower dry-bulb temperature ($p = 0.21$), and colder air from a supply grill located near the ceiling ($p = 0.22$). *Key words*: aerosols, BCG, *Mycobacterium tuberculosis*, *Serratia marcescens*, TB, UV. *Environ Health Perspect* 110:95–101 (2002). [Online 19 December 2001]
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The recent resurgence and current epidemics of tuberculosis (TB) in many developed countries have focused attention on transmission in high-risk settings (*1–3*). The prevalence of human immunodeficiency virus infection, the high case fatality rate of multidrug-resistant TB especially among AIDS patients, and transmission from unsuspected TB patients support the importance of environmental control measures in high-risk settings (*4–6*). Ultraviolet germicidal irradiation (UVGI) that occurs in the upper portion of air in a room has been considered an environmental control measure that could economically reduce exposure to *Mycobacterium tuberculosis* (MTB) droplet nuclei (*7,8*). For this approach, air above people's heads (usually higher than 2.1 m) is subject to 254 nm germicidal ultraviolet C (UVC), whereas lower-room air, where people actually stay and breathe, is not irradiated. A large volume of air can be disinfected without overexposing people to UVC. Currently, upper-room UVGI is recommended by the Centers for Disease Control and Prevention as a supplemental approach for preventing transmission of TB (*9*).

Three factors are important in the efficacy of upper-room UVGI: the upper-room disinfection rate, air volume ratio for the irradiated upper room and the nonirradiated lower room, and the air mixing rate between the upper and lower room (*10*). The disinfection rate in the upper room depends on UV dose and UV susceptibility of the microorganism. UV dose is the product of UV irradiance and exposure time (*11*). Susceptibility of microorganisms to UV depends on the complexity of the microorganism's structure, its repairability, and its general sensitivity (*12*). As the UV dose becomes higher and microbial susceptibility to UVGI increases, the efficacy of upper-room UVGI increases. The desirable scenario is to maintain the maximum amount of UV irradiance in the upper part of the room while minimizing people's exposure to UVC in the lower part of the room. The American Conference of Governmental Industrial Hygienists currently recommends that measured UV irradiance in the lower room should be ≤ 6 mJ/cm² (0.2 μW/cm²) for 8 hr exposure (*13*). The current criterion for installing upper-room UVGI is one 30-W (input) lamp or two 15-W lamps for each 200 square feet (19 m²) of floor area (*14–16*). A 30-W lamp for every seven occupants has been recommended for crowded conditions.

The mixing rate between air in the upper and the lower part of the room is also important in the efficacy of upper-room UVGI (*17*). This air mixing occurs mainly by convection caused by a vertical temperature gradient in nonmechanically ventilated rooms. When a room has a mechanical ventilation system, ventilation type and locations of supply and exhaust also play important roles in vertical air mixing. The presence of a mixing fan (or ceiling fan) also affects vertical air mixing. The volume of upper-room irradiated air and lower room nonirradiated air depends on the room dimensions (especially ceiling height), the type and number of UV fixtures, and reflection characteristics of room surfaces (*18,19*).

Several different types (ceiling-mounted, wall-mounted, and corner-mounted) of UV fixtures are commercially available for upper-room air irradiation (*10,19*). Typical ceiling-mounted fixtures are vertical tubes with disk-shaped louvers that confine emission to a horizontal direction and irradiate throughout all 360 degrees. Another type of ceiling-mounted fixture is the open-tube fixture with upward-facing flanges that block downward radiation. Because this type of UV fixture radiates upward through 180 degrees, reflection from the ceiling should be considered. Wall-mounted fixtures have parallel louvers that are usually coated with a nonreflective material (*19*). These parallel louvers can block downward radiation and confine emission to a horizontal direction. Louvered wall fixtures are equipped with a rear-mounted, polished aluminum parabolic reflector to focus lamp emissions and to maximize irradiation.

Although several studies have reported the efficacy of upper-room UVGI in inactivating airborne microorganisms (*17,20–23*), data with *Mycobacterium* are very limited (*14,24*). Only one study has characterized the currently available louvered UV fixtures using *Mycobacterium parafortuitum* (*24*). One of limitations in that study is that the susceptibility of *M. parafortuitum* to UV has not been

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compared to that of virulent MTB, so extrapolation to efficiency in controlling MTB aerosols is problematic. In fact, there is broad variability among *Mycobacterium* species with respect to UV susceptibility. For example, *M. phlei* is apparently 6–10 times more resistant to UV than is MTB (*14*). Another important limitation is that *Mycobacterium* aerosols were suspended in distilled water (*24*), a very different condition from that of bacterial aerosols released from the human respiratory tract. Suspending medium for aerosols greatly affects both bacterial survival in air and UV susceptibility of airborne bacteria (*25,26*). Therefore, suspending media for studying microbial aerosols must simulate the real-world aerosols as closely as possible. In this study, we used commercially available UV units. We characterized the effect of air exchange rate and air mixing on the efficacy of upper room UVGI with *Serratia marcescens* aerosols in simulated saliva [10% fetal calf serum (FCS)] with and without upper-room UVGI. We used *M. bovis* bacilli Calmette-Guérin (BCG) as a surrogate for virulent MTB. BCG has been extensively compared to MTB and is reported to have the same UV susceptibility as virulent MTB (*14*). Finally, we used 10% FCS as a surrogate for saliva.

Materials and Methods

Bacterial culture methods and preparation of cell stocks. We obtained *S. marcescens* and BCG from American Type Culture Collection (ATCC 8195 and ATCC 35737, respectively; ATCC, Manassas, VA).We maintained *S. marcescens* on nutrient agar slants (DIFCO, Detroit, MI) at room temperature. A loopful of cells was inoculated into 100 mL nutrient broth and cultured at 25°C for 24 hr with agitation (200 rpm). We placed each milliliter of cultured cells in a 1.5 mL Eppendorf tube (Fisher Scientific, Pittsburgh, PA), washed it with 1 mL phosphate-buffered saline (PBS) (pH = 7.4) twice, and harvested it by centrifugation at 1,500 rpm for 10 min. Harvested cells were stored at –70°C for later experiments. Several of these cell aliquots were sampled, serially diluted, and cultured on nutrient agar to appropriate dilutions for aerosolization. We suspended two cell pellets in 150 mL PBS to a cell concentration of approximately 10^6 colony-forming units (CFU) per milliliter. We added FCS (DIFCO) to a final concentration of 10% to simulate the protein concentration of saliva (3.5 mg/mL) (*27*).

We maintained the BCG strain on Lowenstein-Jensen agar (Remel, Lenexa, KS) plates at 37°C. A loopful of cells was inoculated into 500 mL of Middlebrook broth (DIFCO) containing 0.02% Tween 80, and cultured at 37°C for 3–4 weeks with gentle agitation (100 rpm). We harvested cultured cells by centrifugation at 1,500 rpm for 10 min, suspended them in 50 mL PBS, and homogenized them using a tissue grinder to break up large clumps. We filtered homogenates using 5 μm cellulose nitrate membrane filters (Whatman, Maidstone, England) to remove remaining cell clumps. We harvested filtered cell suspensions and resuspended them in 30 mL PBS. Each milliliter of 30-mL cell suspension was allocated into a 1.5 mL Eppendorf tube, washed with 1 mL PBS twice, and harvested by centrifugation at 1,500 rpm for 10 min. We stored harvested cells at –70°C for later experiments. For aerosolization, we prepared cell suspensions in the same way as described above for *S. marcescens*, and we adjusted cell concentration to approximately 105 CFU/mL with 10% FCS. The 6-jet Collison nebulizer (BGI, Waltham, MA) was initially loaded with about 70 mL of cell suspension, and the remaining cell suspension was stored at 4°C during the experiment. We added suspension to the nebulizer every 45 min to maintain a constant aerosolization rate.

We loaded either nutrient agar or Middlebook agar plates into a six-stage Andersen culture plate impactor (Andersen Sampler Inc., Atlanta, GA) for the experiments with *S. marcescens* or BCG respectively. We counted colonies after incubation for 48 hr at room temperature for *S. marcescens* and for 3 weeks at 37°C for BCG.

Description of the experimental facility. The facility was built on the roof of a four-story building in an urban setting in Boston at an elevation of about 19 m from the ground (Figure 1). The size of the facility is 15.6 m^2 (5.2 m × 3 m) with a ceiling height of 2.9 m. It contains an experimental chamber (4.5 m × 3 m) and an anteroom (0.7 m × 3 m) separated by a partition and an interior door. Interior wall and ceiling surfaces are finished with vinyl-covered sheetrock, and the floor is a composite. The facility has an exterior door to the anteroom and three double insulated glazed windows. The interior door separating the anteroom from the chamber was sealed with vinyl tape during the experiments. The chamber is ventilated through an upper-room supply grill and a lower-room air exhaust grill, both fitted with HEPA filters to prevent microbiologic contamination of the supply air and to prevent release of microbiologic agents into the exhaust air. The mechanical ventilation rate was adjusted by a damper located in the exhaust duct to approximately 49 ft^3/m for 2 air changes per hour (ACH) or 147 ft^3/m for 6 ACH. We measured the air exchange rate in the exhaust duct using a hot wire anemometer (Model 8360; TSI Inc., St. Paul, MN) and checked it using the decay rate of a tracer gas (sulfur hexafluoride).

The chamber is essentially airtight, and the air exchange rate estimated by mechanical ventilation was almost identical to the decay of tracer gas. The room is maintained under negative pressure compared with the anteroom and outdoors using a balance of supply and exhaust air, and was checked by smoke test. The negative pressure was always monitored during experiments by manometer. During experiments, temperature and relative humidity (RH) in the room chamber were continuously monitored in front of the exhaust duct by an electrical sensor (Model Humeter 50Y; VAISALA, Woburn, MA). We did not measure the temperature or the ratio of the partial pressure of the water vapor to the saturation vapor pressure for the same RH as the supply air during the experiments. Because untreated outdoor air was introduced directly into the room through the HEPA filter, air temperatures inside reflected those outdoors. We estimated the temperature and RH of supply air with both hourly local climatologic data from the National Oceanic and Atmospheric Administration (*28*) and Boston center urban data from Massachusetts Department of Environmental Protection-Div A (*29*). These two data sets were not significantly different. A 20-in box fan (Cyclone model number 3510; Lasko, Franklin, TN) was located in the center of the room chamber.

Aerosol generation and sampling devices. Aerosols were generated by a 6-jet Collison nebulizer (Model CN-38; BGI). The nebulizer was located outside the test room chamber, and the aerosols were introduced to the center of the chamber through a permanently installed stainless steel pipe. A multi-perforated stainless steel hollow sphere was connected to the end of the steel pipe, thereby projecting condensation in nuclei-like particles more or less uniformly throughout the chamber. A diagram of the aerosol generation device appears in Figure 2A. Particle deposition in this aerosol supply line (approximately 1.5 m length) is expected to be negligible compared to the number of cells aerosolized (< 1%) (*30*).

The sampling line through which we collected all samples penetrated an exterior wall and was sealed airtight to the structure at the point of entry and the inner wall surface. A sampling line was permanently installed at the exhaust grill through sampling ports, and all other parts of the sampling devices were located outside the chamber. An outer tube was sealed airtight to the inner and outer facility walls. An inner tube, approximately 1.5 m long, had O-ring seals attached at appropriate intervals to assure that two O-rings were always inside the outer tube, providing a double seal between the facility interior and exterior.

The sampling line was fit with a 6-stage Andersen culture plate impactor (Andersen, Inc., Atlanta, GA) and other sampling devices (BGI) as presented in Figure 2B. The discharge of the sampler was fit with flat HEPA filter material to remove microorganisms not recovered by the Andersen sampler. A high static pressure pump was connected to the sampling device at the downstream end and provided the desired sampling rate, which we monitored by both a venturi meter and a rotameter. Loss of bacterial aerosols during transport through the sampling line is expected to be negligible (*30*).

UV irradiance and fixtures. We installed commercially available (wall-mounted and ceiling-mounted) UVGI fixtures inside the room. The wall-mounted UV fixture (Model 40-1080A; Atlantic Ultraviolet Corp., Bay Shore, NY) contains one 23-W UV lamp (UV output: 5 W). The ceiling-mounted UV fixture (Model PMCUGI-4PL; Lumalier, Memphis, TN) contains four 9-W UV lamps (Total UV output: 10 W). Both types of UV fixtures have low-pressure mercury discharge lamps and louvers producing a narrow vertically collimated beam. The ceiling-mounted UV fixture was suspended from the center of

the chamber with the bottom of the fixture at a height of 2.3 m from the floor. The wall-mounted UV fixture was placed on a shelf located at a height of 2.1 m. Following the manufacturers' recommendations, we operated both UV fixtures for 100 hr to allow an adequate break-in period before experiments began.

We measured the distribution of UV irradiation for the UV fixtures by radiometer (Model SEL240 3660; International Light, Inc., Newburyport, MA). We mapped UV irradiation by measuring at graduated distances and angles from the horizontal and vertical centerlines of the fixtures before the initial experiment. We monitored UV irradiance from the anteroom through a small hole covered with a fused silica plate located between the experimental chamber and the anteroom during the experiments. We assessed offsets between measurements through the peep hole and room UV irradiance in the upper room.

Experimental procedure for measuring the efficacy of upper-room UVGI. The mechanical ventilation rate (ACH) was set up at least one day before the experiments. *S. marcescens* or BCG cells were aerosolized

by 6-jet Collison nebulizer, which generated a 2 μm mass median particle diameter (*31*). Air pressure for the nebulizer was maintained at 138 kPa (20 lb/in^2). Bacterial aerosols were generated at a rate of 1.5 × 10^6 CFU/min and introduced into the room chamber as described above. Air containing the microorganism aerosols was pulled from the chamber into the sampling line at a flow rate of 1 ft^3/m and then into the Andersen culture plate impactor. We determined the concentration of airborne culturable microorganisms from the total volume of sampled air and the number of resulting colonies. Because most sampled cells (> 90%) were recovered on the third to fifth stages of the Andersen sampler, corresponding to a range of 1.1–4.7 μm, and because particles in this size range present the greatest risk for airborne infection, we loaded only the second and fifth stages with agar plates to measure the efficacy of upper-room UVGI; the range of particle size recovered from fifth stage (1.1–4.7 μm) was the focus of our interest.

We tested the efficacy of upper-room UV irradiation in inactivating airborne microorganisms in a steady-state condition. As a pilot study, we aerosolized *S. marcescens* into the room chamber and took a series of 4-min samples (at 10, 20, 30, 40, 50, 60, 75, and 90 min) after aerosol generation began (time = 0) to determine the time required to reach a steady state at 6 ACH. The concentrations of airborne *S. marcescens* rapidly increased until 20 min after aerosol generation began and were relatively constant thereafter. We also determined the time to reach reduced concentrations after turning on the upper-room UVGI lamps. The concentration of airborne *S. marcescens* rapidly decreased until 30 min after the UV exposure began and remained relatively constant thereafter. Estimation based on box models indicated that approximately 30 mins (at 6 ACH) or 90 min (at 2 ACH) would be needed to reach more than 95% of the steady-state concentration. The results from *S. marcescens* experiments reasonably coincided with the box model estimates.

We also tested the effect of air exchange rate and mixing fan on the efficacy of upper-room UVGI with *S. marcescens*. We measured concentrations of airborne microorganisms with and without upper-room UVGI at two air exchange rates (2 ACH and 6 ACH) with both ceiling- and wall-mounted UV fixtures individually or together. We measured concentrations of *S. marcescens* aerosols with and without mixing fans with and without upper-room UVGI (ceiling- and wall-mounted UV fixtures) at 6 ACH. We measured the efficacy of upper-room UVGI in inactivating BCG
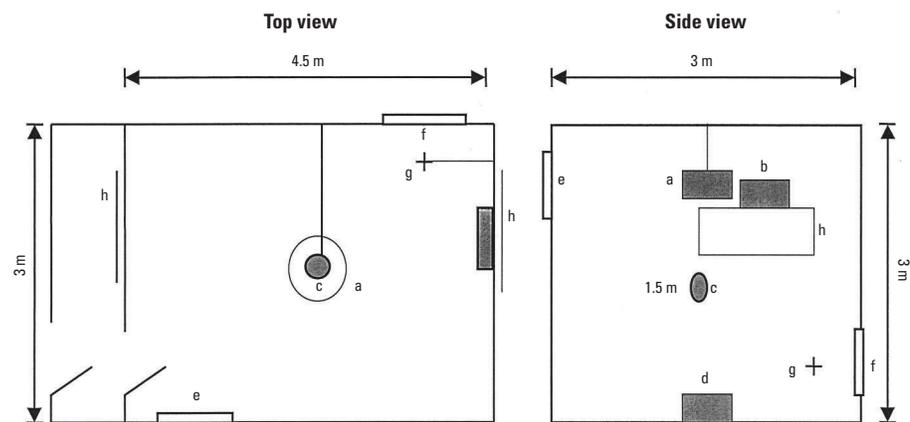

Figure 1. Diagram of experimental room chamber. a, ceiling-mounted UV fixture; b, wall-mounted UV fixture; c, aerosol generator; d, box fan; e, air supply grill; f, air exhaust grill; g, sampling device; h, windows.
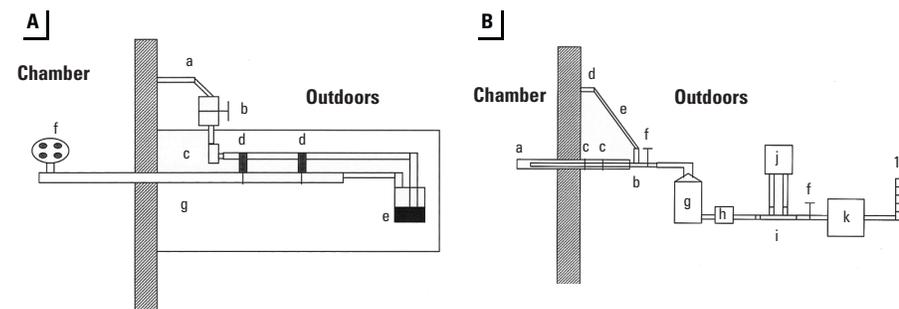

Figure 2. Diagram of aerosol generating and sampling devices. (*A*) Aerosol generating device; a, pressured air line; b, pressure gauge and regulator; c, polycarbonate filter; d, valve; e, collison nebulizer; f, diffuser; g, pipe line. (*B*) Sampling device; a, outer tube; b, inner tube; c, O-ring; d, pressured air; e, purge line; f, valve; g, Andersen sampler; h, flat HEPA filter; i, venturi meter; j, manometer; k, vacuum pump; l, rotameter.

aerosols without the mixing fan at 6 ACH with both ceiling-mounted and combined ceiling- and wall-mounted UV fixtures.

One unshaded window and a lighting system, typical of conditions in patient rooms, were present constantly throughout the experiments. Because visible light may cause photoreactivation of UV-damaged microorganisms, we stored collected samples in a dark box, blocked from visible light. We repeated each experimental condition from once to five times on the same day. We used a positive hole conversion to estimate the probable number of affected microorganisms from recovered CFU (32).

Data analysis. The effect of UV irradiation was expressed either as percent UV effectiveness or equivalent ventilation rate attributable to upper-room UVGI. We calculated percent UV effectiveness as 100 × (1 − C_{uv}/C_o) and calculated equivalent ventilation rates using Equation 1:

$$K_{uv} = K_o\,(C_o/C_{uv} - 1), \qquad [1]$$

where C_{uv} and C_o represent the concentration of airborne culturable microorganisms with and without upper-room UVGI, respectively. K_{uv} represents equivalent ventilation rate (ACH) attributable to upper-room UVGI; K_o represents ventilation rate (ACH) without upper-room UVGI.

We performed two statistical analyses [generalized liner model (GLM) and generalized additive model (GAM)] to find the relation between UV effectiveness and other parameters. Microorganism strain type, use of a fan, ACH, and the number of UV fixtures were categorized and incorporated into both the GLM and GAM by an indicator

variable. In the GLM, we assumed that environmental factors (temperature, RH, and temperature gradient between exhaust and supply air) were linearly related to UV effectiveness. The GAM does not have any assumptions of a linear relationship between environmental factors and UV effectiveness. We used LOWESS—a nonparametric smoothing technique that employs a running regression with a weight declining as the cube of the distance from the center of the neighborhood—to examine UV effectiveness as a function of our predictors (S-plus Version 4.5; Mathsoft Inc., Seattle, WA).

Results

Percent recoveries and particle size distribution. Percent recoveries of total cells aerosolized by nebulizer ranged from 5% to 28% for *S. marcescens* and from 21% to 25% for BCG. Average number of *S. marcescens* colonies counted on stages 1, 2, 3, 4, 5, and 6 were 1, 1, 7, 64, 144, and 3, respectively. For BCG, the numbers were 2, 1, 12, 175, 681, and 85 on stages 1, 2, 3, 4, 5, and 6, respectively. Particle size distributions of recovered CFU from each stage of the Andersen sampler are > 7 μm, 4.7–7 μm, 3.3–4.7 μm, 2.1–3.3 μm, 1.1–2.1 μm, and 0.65–1.1 μm for stages 1, 2, 3, 4, 5, and 6, respectively. More than 90% of recovered cells were from stages 3, 4, and 5 of the Andersen sampler for all experiments, representing an aerodynamic diameter range from 1.1 to 4.7 μm. Count median diameter for *S. marcescens* and BCG aerosols were approximately 1.6 and 1.3 μm, respectively. The RH inside the room chamber was 46–64% for *S. marcescens* and 64–70% for BCG.

The effect of ACH and mixing fan on UV effectiveness (with S. marcescens*).* The number of CFU without upper-room UVGI was 4 ± 6 (mean ± SD) on stage 2 and 207 ± 204 (mean ± SD) on stage 5. With upper-room UVGI on, CFU counts were 2 ± 4 and 94 ± 161 on stages 2 and 5, respectively. We calculated UV effectiveness from stage 5 colony counts only. The experimental results with *S. marcescens* aerosols are summarized in Table 1. Average UV effectiveness in inactivating *S. marcescens* aerosols was 46% (range: 22–80%) at 2 ACH and 53% (range: 40–68%) at 6 ACH with both ceiling- and wall-mounted UV fixtures and without the mixing fan. When the mixing fan was on, the effectiveness of upper-room UVGI increased to 62% (range: 50–78%) at 2 ACH and 86% (range: 81–89%) at 6 ACH (Figure 3).

The efficacy of upper-room UVGI with BCG. The experimental results with BCG aerosols are summarized in Table 2 and Figure 4. The number of CFUs without upper-room UVGI was 1 ± 1 (mean ± SD) on stage 2 and 146 ± 50 (mean ± SD) on stage 5. With upper-room UVGI on, CFU counts were 0 ± 0 and 70 ± 35 on stages 2 and 5, respectively. UV effectiveness calculated from stage 5 colony counts at 6 ACH was 52% (range: 11–69%) with a ceiling-mounted UV fixture only and 64% (range: 51–83%) with both ceiling- and wall-mounted UV fixtures. Equivalent ventilation rates attributable to upper-room UVGI were 9.8 ± 6.4 ACH for the ceiling-mounted UV fixture, and 11.7 ± 7.1 ACH for both ceiling- and wall-mounted UV fixtures.

The regression models. Table 3 shows the GLM results. The number of UV fixtures ($p < 0.01$), ACH ($p = 0.01$), and use of mixing fan ($p < 0.01$) were significantly associated with UV effectiveness. However, the type of microorganism (*S. marcescens* vs. BCG; $p = 0.36$), RH ($p = 0.64$), and temperature gradient (exhaust air–supply air) ($p = 0.27$) are not significantly associated with UV effectiveness. UV effectiveness increased at higher temperature ($p < 0.01$) (Table 3). Multiple R^2 of the GLM is 0.54. Figure 5 shows the nonparametric smoothed curve of UV effectiveness versus temperature gradient (exhaust air–supply air), temperature, and RH from the GAM results. Better UV effectiveness occurred at higher temperatures. RH did not consistently affect UV effectiveness. Higher UV effectiveness occurred when cooler air was introduced into the room from the supply air grill. Nonparametric trends were significant for temperature difference between supply and exhaust air ($p < 0.01$), and RH ($p = 0.05$) but not for temperature ($p = 0.56$) in the GAM. Overall, results were similar.

Table 1. Summary of the experimental data on the efficacy of upper-room UVGI for inactivating *S. marcescens* aerosols.

Type of UV fixture	Mechanical ventilation (ACH)	Mixing fan	Temperature (°C)[a]	RH (%)	UV Effectiveness[b]	Equivalent ventilation rate[c]
C (n = 4)	6.1	No	17–18 (13–15)	27–30 (35–37)	65 ± 11	12.7 ± 6.9
C and W (n = 4)	6.0	No	4–8 (3–6)	30–41 (35–41)	53 ± 15	7.6 ± 4.3
C and W (n = 1)	6.0	Yes	4–8 (3–6)	30–41 (35–41)	88	45.4
C and W (n = 5)	6.0	Yes	8–9 (12–17)	31–52 (31–40)	84 ± 3	36.6 ± 9.8
C and W (n = 4)	2.1	No	20 (21–25)	64 (48–61)	55 ± 30	4.1 ± 3.8
C and W (n = 2)	2.0	No	6–12 (5–11)	44–52 (47–51)	29 ± 2	0.8 ± 0.0
C and W (n = 4)	2.0	Yes	6–12 (5–11)	44–52 (47–51)	62 ± 12	3.8 ± 2.3

Abbreviations: C, Ceiling-mounted UV fixture containing four 9-W UV lamps; W, Wall-mounted UV fixture containing one 23-W UV lamp.
[a]Temperature or RH at the exhaust grill (temperature or RH at the supply grill). [b]UV effectiveness (%) = 100 × (1 −C_{uv}/C_o), where C_{uv} and C_o are concentrations (or recovered CFU) with and without upper-room UVGI, respectively. [c]Additionall sanitary ACH attributable to upper-room UVGI. Equivalent ventilation rate = A × (C_o/C_{uv} −1), where A is ventilation rate without upper-room UVGI (ACH) and C_{uv} and C_o are concentrations (or recovered CFU) with and without upper-room UVGI respectively.

Discussion

We have confirmed that upper-room UVGI can significantly reduce the concentration of culturable *S. marcescens* and BCG aerosols in the lower room. Our findings and past studies with *Mycobacterium* sp. are summarized in Table 4 (*14,24*). First, fixture outputs in Table 4 are less than the total UV output because of luminaire inefficiency (the ratio of luminous flux emitted from the fixture to that emitted by the lamps), which depends on the fixture type (*33*). Our data showed a lower UV effectiveness than those of the two past studies (*14,24*). Some difference in UV effectiveness may be caused by differences in microbiologic factors (species, suspending medium, physiologic condition), UV factors (irradiance level, distribution), room factors (air mixing, volume ratio of irradiated upper-room to nonirradiated lower-room), or environmental factors (temperature and RH) or a combination of these. We used 10% FCS in the suspending medium to simulate saliva conditions, whereas previous studies (*14,24*) used 0.2% bovine serum albumin or distilled water. Past study indicated that UV susceptibility of airborne bacteria depends greatly on the suspending medium (*25*). Proteins coating cell surfaces could protect airborne cells from harmful UV light and other environmental stresses. Therefore, to properly characterize UV sensitivity of *Mycobacterium* aerosols in experimental settings, suspending medium needs to simulate the condition of aerosols released from TB patients.

Second, we did not use a mixing fan for our BCG experiments. Our mixing fan experiments with *S. marcescens* indicated an increased UV efficiency of 63% at 6 ACH. Third, different UV fixture type and ratio of UV output to room size would affect the efficacy of upper-room UVGI. We used louvered UV fixtures, whereas Riley's study (*14*) used open tube UV fixtures without louvers. Given the same UV output, louvered UV fixtures produce 15 times less than those without the louvers (*20*). Thus, this factor alone could account for the observed differences in UV effectiveness. However, unlouvered fixtures are impractical in occupied environments. Finally, we saw a strong correlation with temperature, and the wide range of room temperatures in our experiments might explain some of the differences observed. To estimate the efficacy of upperroom UVGI in reducing TB transmission, all these factors should be considered.

Particles from 1 µm to 5 µm were our main interest because only those particles can reach the lower respiratory tract and cause disease (*34*). In our study, more than 90% of recovered microorganisms ranged from 0.6 µm to 3.0 µm as measured by cascade impaction. Airborne particle size is crucial in the UV sensitivity of microorganism, and airborne microorganisms borne on large particles tend to be more resistant to UV than those associated with smaller particles (*35,36*). The count median diameters (approximately 1.6 µm for *S. marcescens*; 1.4 µm for BCG) in the studies presented here were smaller than those within a similar RH range in a smaller chamber (approximately 2.1 µm for *S. marcescens*; 2.2 µm for BCG) (*36*). The room chamber (46 m^3) in this study is much larger than the bench size chamber (< 0.01 m^3) in the previous study (*36*), and longer residence times may contribute to preferential removal of the larger particles or to more evaporation from individual droplets. Most of aerosolized bacteria (both *S. marcescens* and BCG) would be a singlet given that the size of those microorganisms is 1–2 µm. Some fraction of aerosolized cells in our study could be 2 or 3 multiple organisms. Those multiple organisms ranging from 1 to
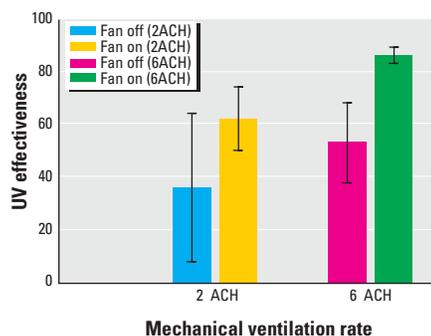
Figure 3. The effect of ACH and mixing fan on UV effectiveness for inactivating *S. marcescens*. Both ceiling- and wall-mounted UV fixtures were used. Each experiment was repeated four times.
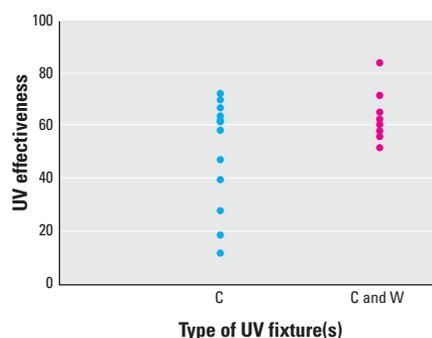
Figure 4. UV effectiveness for inactivating BCG aerosols with either ceiling-mounted (C) or wallmounted (CW) UV fixtures. Ceiling-mounted UV fixture contains four 9-W UV lamps; wall-mounted UV fixture contains one 23-W UV lamp.

Table 2. Summary of the experimental data on the efficacy of upper-room UVGI for inactivating BCG aerosols.

Type of UV fixture	ACH	Temperature (°C)^a	RH (%)	UV effectiveness^b	Equivalent ventilation rate^c
C (n = 4)	8.6	23–26 (20–21)	46–64 (87–90)	66 ± 6	17.4 ± 4.4
C (n = 4)	6.2	22–24 (21–22)	62–70 (73–78)	64 ± 5	11.1 ± 2.4
C (n = 4)	6.1	18–22 (19–22)	46–57 (49–59)	37 ± 18	4.3 ± 3.1
C (n = 2)	6.1	8–9 (4–6)	31–52 (47–60)	25 ± 20	2.3 ± 2.2
C and W (n = 4)	5.7	9–14 (9–12)	33–41 (44–45)	70 ± 10	15.4 ± 9.0
C and W (n = 4)	6.1	11–16 (8–9)	46–50 (59–61)	56 ± 39	8.1 ± 1.6

Abbreviations: C, Ceiling-mounted UV fixture containing four 9-W UV lamps; W, Wall-mounted UV fixture containing one 23-W UV lamp.
^{a}Temperature or RH at the exhaust grill (temperature or RH at the supply grill). ^{b}UV effectiveness (%) = 100 × (1 – C_{uv}/C_o), where C_{uv} and C_o are concentrations (or recovered CFU) with and without upper-room UVGI, respectively. ^{c}Additional sanitary ACH attributable to upper-room UVGI. Equivalent ventilation rate = A × (C_o/C_{uv} – 1), where A is ventilation rate without upper room UVGI (ACH) and C_{uv} and C_o are concentrations (or recovered CFU) with and without upper-room UVGI, respectively.

Table 3. The results of GLM (multiple R^2 = 0.54).

Parameter^a	Value	SE	t-Value	p-Value
Intercept	–7.0	19.9	–0.35	0.58
Microorganism	–6.7	7.3	–0.92	0.26
UV fixture	27.6	7.9	3.5	< 0.01
ACH	24.6	9.5	2.61	0.01
Fan	16.5	6.3	2.60	< 0.01
Temperature	2.4	0.7	3.69	< 0.01
RH	–0.16	0.33	–0.47	0.21
Temperature difference	1.96	1.75	1.12	0.22

^{a}Microorganism (*S. marcescens* = 0, BCG = 1), UV fixture (ceiling-mounted only = 0, ceiling- and wall-mounted = 1), ACH (2 ACH = 0, 6 ACH = 1), fan (no use = 0, use = 1). Temperature, RH, and temperature difference between exhaust and supply air were incorporated into model as continuous variables.

5 µm could be of particular concern because they are small enough to reach the alveolar region in the lung for established infection and likely are more resistant to UV as well. *Mycobacterium* sp. tends to form clumps in culture because of their waxy surface, so multiple organisms of *Mycobacterium* aerosols are very likely released from the infectious TB patient. Therefore, the level of multiple organisms among airborne microorganisms released from the infectious TB patient must be characterized.

We express the efficacy of upper-room UVGI both in terms of the percent of UV effectiveness and as an equivalent ventilation rate. The percent UV effectiveness is defined as $100 \times (1 - C_{uv}/C_o)$ where C_{uv} and C_o are the concentrations with and without upper-room UVGI. The equivalent ventilation rate expresses the UVGI effect as the amount of ventilation with fresh air that would be required to achieve an equivalent reduction in the concentration of airborne microorganisms. Because percent UV effectiveness is related to the concentration ratio, it is more directly applied to the risk ratio between UV on and off. Thus, if UV effectiveness is 50%, given the same exposure time UV would reduce the risk of infection by 50%. The relationship between ventilation rate and particle concentration is nonlinear, so a small reduction in airborne particle concentration may require large increases in ventilation. For virulent infectious agents, even low residual concentrations may be associated with significant risk.

The importance of air mixing in the efficacy of upper-room UVGI has been addressed in past studies (*17,37*) and was confirmed in our studies. The use of a mixing fan increased UV effectiveness on average by 72% at 6 ACH and 63% at 2 ACH. Our results indicated that UV effectiveness is also strongly influenced by the air exchange rate (Figure 3). UV effectiveness in inactivating *S. marcescens* aerosols increased by 46% with an increase in ACH from 2 to 6 ($p = 0.01$). Higher air velocity from the supply grill at 6 ACH might have caused better vertical air mixing, leading to increased UV

effectiveness. Although it is not statistically significant in the model ($p = 0.27$), UV effectiveness also increased with temperature gradient (exhaust–supply air) (Table 3 and Figure 5A). Temperature gradients can have significant effects on internal air mixing.

UV effectiveness significantly increased at higher temperature (Table 3 and Figure 5B). This temperature effect on the efficacy of upper-room UVGI has not been previously reported, possibly because most experiments were performed at room temperature (*14,17,22–24,35,38*) The temperatures in our study ranged widely from near freezing (4°C) to room temperature (25°C). Some of our experimental results at lower temperatures might not be directly applicable to risk settings that are temperature controlled to typical room temperature, and therefore require careful data interpretation. However, our multivariate statistical analyses allow consideration of all the variables individually and interactively. Thus, we can interpret our data for room temperature environments as well

as for colder environments such as might occur in shelters in very cold climates or in artificially controlled cold rooms. The reduced efficacy of upper-room UVGI at lower temperature may be caused by reduced UV output from the lamps, reduced sensitivity of microorganisms at lower temperature, or a combination effect of UV output and microorganism's sensitivity. UV outputs from the low pressure mercury lamps are determined by the pressure of the mercury, which is closely related to the ambient temperature (*19*). It has also been reported that fewer airborne microorganisms survive at high temperatures (*39–41*). Higher susceptibility at higher temperatures might be caused by structural changes in membrane phospholipids, proteins, and DNA (*26,38*). UVC may act synergistically to augment this general temperature sensitivity. Interestingly, a synergistic effect of UVA and elevated temperature on the survival of the convict cichlid fish (*Cichlasoma nigrofasciatum*) has been reported (*42*). Another possible explanation

Table 4. Summary of previous and our upper-room UVGI experiments in inactivating *Mycobacterium* sp. aerosols.

Parameters	Riley (*14*) Setting 1	Riley (*14*) Setting 2	Miller (*24*) Setting 1	Miller (*24*) Setting 2	Our study Setting 1	Our study Setting 2
Microorganism	BCG		*M. parafortuitum*			BCG
Particle size (µm)	0.5–3		0.65–2.1		1.1–4.7[a]	
Suspending medium	0.2% BSA		DW		10% FCS	
Temperature (°C)	NA	NA	15-35		4–26	
RH (%)	25	20, 40	50-90		41–69	
Room size (m³)	61		90		46	
Mechanical ventilation	No		Yes		Yes	
ACH	2	2–4	0	6	6-8	6
Mixing fan	Yes (during aerosolization)		Yes		No	
UV output (W)	17	46	99(28)[b]	99(28)	36(10)	59(15)
UV output/room size (W/m³)	0.28	0.75	1.1	1.1	0.78	1.3
UV fixture type	C1	C₁, W	CN, C₂	CN, C₂	C₂	C₂, W
UV effectiveness[c] (%)	83	88, 89	98	95	52 ± 19 (56 ± 17)[d]	64 ± 10
UV effect[e] (ACH)	10	18–19, 33		120	9.8 ± 6.4 (10.9 ± 6.1)	11.7 ± 7.1

Abbreviations: BSA, bovine serum albumin; C1, open-tube ceiling-mounted fixture; C2, louvered ceiling-mounted fixture; CN, louvered corner-mounted fixture; DW, distilled water; NA, not available; W, louvered wall-mounted fixture. Each study was done under two sets of different conditions.
[a]Particle size distribution of recovered BCG aerosols (80%: 1.1–2.1 µm; 19%: 2.1–3.3 µm; 1%: 3.3–4.7 µm). [b]UV lamp power (UV output). [c]Percent UV effectiveness = 100 (1 - Cuv/Co), where C_{uv} and C_o are concentration with and without upper-room UVGI. [d]Only data with room temperature (18–25°C). [e]Equivalent ventilation rate attributable to UV = A (Co/Cuv − 1), where A is ACH and C_{uv} and C_o are concentrations with and without upper-room UVGI.
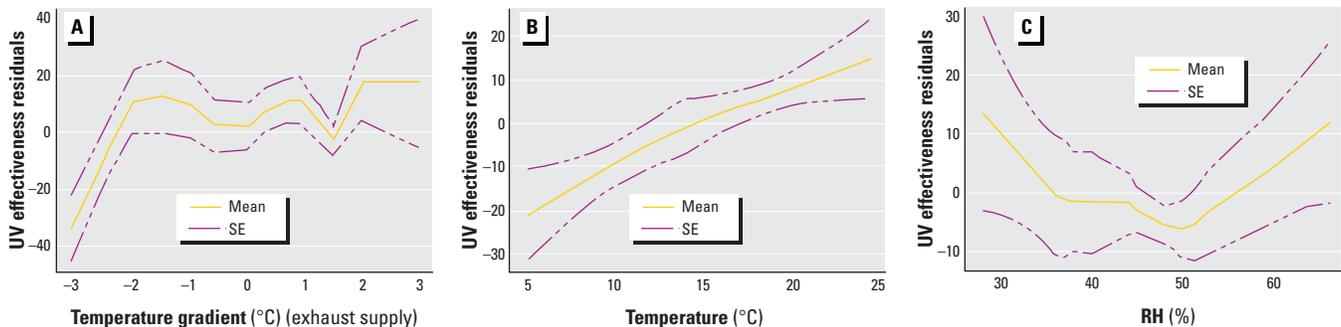

Figure 5. Partial residual plots for the relationship between UV effectiveness and environmental factors from general additive model (GAM).

for higher UV sensitivity at higher temperature is that UV is more likely to cause mutagenic effects on DNA [e.g., *cis-syn* cyclobutane-pyrimidine dimers and pyrimidine (6-4) pyrimidine photoproducts] at higher temperatures. The ability of UVC to damage a given base is determined by the flexibility of the DNA (*43,44*). The DNA of airborne microorganisms becomes more flexible at higher temperature because of active metabolism and a direct temperature effect on DNA.

Previous studies have indicated that UV effectiveness decreases at higher RH, especially > 75% RH.(*36,45*) Decreased UV effectiveness at high RH (> 80%) may be explained partially by increased particle size of airborne microorganisms at high RH. However, the RH effect on UV effectiveness was not significant in our study (Table 3, Figure 5C), probably because the RH range was limited (27–64%). In addition to RH, we used the absolute humidity—the ratio of the mass of water vapor to the total volume of the mixture—to examine the effect of different saturation vapor pressures at different RHs. However, we did not observe any significant relationship between the efficacy of upper-room UVGI and absolute humidity.

In conclusion, we have found that upper-room UVGI can significantly reduce the concentration of airborne microorganisms (*S. marcescens* and BCG) aerosolized in a saliva simulant in a typical mechanically ventilated hospital isolation room. Because upper-room UVGI varied significantly with environmental factors such as temperature, air mixing, and air exchange rate, it is important to optimize environmental conditions to produce the best and most persistent effect in reducing the risk of TB in high-risk settings.

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