

## Design, construction, and evaluation of a chamber for aerobiology

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### Abstract

A chamber was designed and constructed for aeromicrobiology applications. An ultraviolet (UV) radiation source was incorporated to sterilize the chamber between trials. Twelve bacterial species originally isolated from air samples and obtained from the American Type Culture Collection were tested for efficacy of UV radiation disinfection of the chamber, comprising five Gram-positive bacteria, six Gram-negative and one Gram-variable bacterial species. Experiments were designed to determine time needed to sterilize the chamber walls and air within the chamber after an aerosol containing  $\leq 10^8$  bacteria/l of air was introduced or suspensions of the microorganisms were placed on surfaces within the chamber. Exposure of surfaces to UV for 120 min was determined to provide sufficient disinfection for reuse of the chamber for aerobiology studies. © 1997 Elsevier Science Ireland Ltd.

**Keywords:** Chamber design; Sterilization; Survival; Ultraviolet

### 1. Introduction

Microbial aerosols have long been recognized as a potential threat to human health as well as an important factor in assessing indoor air quality (Benard, 1985; Buchanan and Gibbons, 1974; Kreiss, 1992). Microorganisms, e.g., fungi, bacteria and viruses, in an indoor environment can be responsible for building-related illnesses (the Sick Building Syndrome, SBS) (Menzies et al., 1993; Kreiss, 1992; Wallingford and Carpenter, 1986). However, microbiological analysis of air samples poses several difficulties. Airborne organisms may not be actively metabolizing, growing and/or reproducing during transport and, therefore, may not respond to traditional culture techniques (Crawford and Jones, 1979; Kingston, 1971). The most difficult parts of the analysis of air samples are the collection and cultivation of microorganisms in a manner that permits survival and quantitative enumeration of all or most of the microorganisms present in the air samples.

Evaluating collection and cultivation procedures in a reproducible, controllable manner requires the ability to generate standardized bacterial aerosols. The aerosols must be contained and maintained under constant relative humidity (RH) and temperature (Goldberg, 1970, 1971). The ability to alter RH and temperature during an experiment is also necessary, to simulate the natural environment. Many types of chambers have been designed to store aerosols. Some were designed for experiments lasting only a few minutes (Hatch and Dimmick, 1965; Hendersen, 1952) while others were designed for longer storage periods (Goldberg, 1970, 1971; Hood, 1973, 1974). Aerosol chambers are complex structures that allow the aerosol conditions to be altered easily and rapidly. However, such are costly to construct, operate, and maintain.

Prior to initiation of studies to evaluate media for use in monitoring indoor air, we undertook the design, fabrication and testing of equipment to permit generation and maintenance of aerosols under controlled conditions (Levin et al., 1994). Two major requirements included ability to contain the aerosol, i.e. to prevent

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contamination of room air and preclude entry of room air into the chamber, and to maintain constant temperature and RH. In addition, provision for easy sampling, sterilization between trials, and a capability of altering RH and temperature was required.

To achieve our goal, a chamber (550 l air capacity) was designed to permit monitoring viability of the introduced microorganisms as a function of aerosolization. Modifications were made to a chamber already in use to render it leakproof. The sampling procedures were carefully designed to assure safety.

Ultraviolet (UV) light has been shown to be effective as a sterilizing agent, depending on time of exposure, humidity, and type of microorganism (General Electric, 1970; Lawrence and Block, 1968). In designing the chamber for the purpose of carrying out aerobiological experiments to evaluate media efficacy, use of UV radiation for chamber sterilization between trials was also evaluated. The specific effects of UV on bacterial aerosols have been studied and the results reviewed elsewhere (Kreiss, 1992; Urbach and Gange, 1986). UV radiation has been employed extensively to sterilize water and air. Sterilization with lamps which emit wavelengths of 2537 Angstroms is recommended, as is the use of *Staphylococcus aureus* as an indicator species for efficacy. Use of UV in hospital operating rooms has resulted in significant decreases in post-operative infection rates (Hart, 1960) and has also had some positive effect on airborne microorganisms associated with public health risk in barracks, but no effect on infection rates in schools has been demonstrated (Lawrence and Block, 1968). More recently, the importance of proper planning and installation of UV devices, RH, intensity, and microbial species selected for assuring efficacy are factors that have been reported (Riley and Nardell, 1989).

In this report, we describe the construction and evaluation of a chamber for aerobiology and give results of initial trials conducted to determine operating characteristics, as well as efficacy of UV for sterilization of the chamber between trials.

## 2. Materials and methods

### 2.1. Chamber design.

The chamber was constructed from an upright freezer with a volume of 500 l. All motors, piping, shelving and other apparatus were removed. All openings through the walls, for piping, shelf supports, etc., were sealed with silicone rubber (Silicone Sealant, General Electric, NY) until the chamber was demonstrated to be fully leakproof. To demonstrate this, the chamber was filled with an aerosol containing  $10^8$  Colony Forming Units (CFU)/ml of *Bacillus subtilis*. Room air was

sampled (2250 l) and swabs of the exterior of the chamber were taken and assayed. Twelve sample ports were prepared, four each in the sides and rear, all of which could be operated simultaneously. The ports were lined with flexible tubing and sealed with silicone rubber. The front of the chamber was sealed with 1/8 inch plexiglass. The plexiglass and all silicone joints were covered with five layers of polyurethane varnish.

The chamber construction permitted continuous monitoring of temperature and RH, using an RH/temperature probe (Cole Parmer, Niles, Ill, model 37950-10). The probe was inserted through a port which matched the diameter of the probe. Similarly, a port for aerosol introduction was installed. Aerosols were generated using either a Collison Nebulizer (BGH, Cambridge, MA) or a CO<sub>2</sub> spray device (R and D Sprayers, Opelousa, LA) which would allow control of particle size in the range of 5–400  $\mu$ . RH was influenced slightly by spray time, but remained constant after spraying was complete. The humidity was pre-adjusted by spraying moisture (aerosolized sterile distilled water) into the chamber. The chamber was equilibrated to room temperature and the chamber temperature was maintained for a minimum of 4 h.

For sterilization purposes, a UV bulb (G30T8, 120 V, 60 Hz, General Electric, 0.5 mW/cm<sup>2</sup> at 1 foot) was installed on the rear wall of the chamber. The bactericidal properties of UV radiation are well established (Zinsser's Microbiology, 1984). The major mechanism of the lethal effect of UV light on bacteria is attributed to absorption by DNA and resultant damage to the DNA. That is, it has been shown that UV radiation leads to the formation of covalent bonds between adjacent pyrimidine residues in the same DNA strand, resulting in the formation of cyclobutane-type pyrimidine dimers. These dimers distort the shape of the DNA and interfere with normal base pairing. The most effective bactericidal wavelength has been reported to be 240–280 nm (Harim, 1980; Toggle, 1983). The lethal dose for non-sporeforming bacteria varied from 1800–6500 mW/cm<sup>2</sup> (Kingston, 1971).

Changes in internal pressure were obviated by use of membrane filters (0.2  $\mu$ ) which permitted room air to enter when sampling and internal air to exit the chamber during operation of the spray device. Passage of air through the filters assured that bacteria did not enter or leave the chamber except as introduced via the experiments.

### 2.2. Sampling.

The All Glass Impinger (AGI, Ace Glass, Vineland, NJ) was used to collect aerosol samples. The impingers, filled with 40 ml of phosphate buffered saline (PBS) (8 g NaCl, 0.2 g KCl, 1.4 g Na<sub>2</sub>HPO<sub>4</sub>, 0.24 g KH<sub>2</sub>PO<sub>4</sub>/l distilled water, adjusted to pH 7.2), sample at a rate of

Table 1  
Selected characteristics of the bacterial species employed in this study<sup>a</sup>

| Species                            | Gram reaction | Pigmentation                             | Isolation                                                              |
|------------------------------------|---------------|------------------------------------------|------------------------------------------------------------------------|
| <i>Staphylococcus aureus</i>       | Gram +        | Orange                                   | Air, soil and fresh water                                              |
| <i>Pseudomonas aureginosa</i>      | Gram –        | Diffusible fluorescent and pyocyanine    | Air, soil, water and clinical specimens                                |
| <i>Klebsiella planticola</i>       | Gram –        | Dark brown                               | Air, plants, and soils                                                 |
| <i>Serratia marcescens</i>         | Gram –        | Pink, red, and magenta                   | Air, water, soil and food.                                             |
| <i>Arthrobacter polycromogenes</i> | Gram variable |                                          | Air, soil                                                              |
| <i>Cytophaga allerginae</i>        | Gram –        | Yellow, orange, red and carotenoid       | Air, soil, fresh water and marine environments                         |
| <i>Rhodococcus globerulus</i>      | Gram +        | Carotenoid                               | Air, plants                                                            |
| <i>Micrococcus leutus</i>          | Gram +        | Carotenoid                               | Air, soil, dust, water and skin of man and other animals               |
| <i>Pseudomonas aureofaciens</i>    | Gram –        | Diffusible fluorescent and orange-yellow | Air, soil and water                                                    |
| <i>Enterococcus faecalis</i>       | Gram +        | Rarely pigmented                         | Air, clinical material                                                 |
| <i>Aerococcus viridans</i>         | Gram +        | White or gray                            | Air, lobsters, human infections of the urinary tract, and endocarditis |
| <i>Bacillus subtilis</i>           | Gram +        | Brown, red, orange, or black             | Air, surgical dressings and canned foods                               |

<sup>a</sup> Bergey's Manual of Determinative Bacteriology, Buchanan and Gibbons, 1974.

12 l/min. Operation required a vacuum of at least 30 cm Hg, obtained using a Gast Oilless vacuum pump equipped with a vacuum gauge (Gast, Benton Harbor, Michigan). Pump contamination was minimized by placing 0.2 micron filters between the aerosol and the pump, downstream of the impinger.

### 2.3. Bacterial species.

The American Type Culture Collection catalogue (ATCC, Rockville MD) lists twelve bacterial species originally isolated from aerosols (American Type Culture Collection, 1992). All 12 (Table 1) were used in trials designed to test for even dispersal of the aerosols within the chamber, leakage, and efficacy of the UV light in sterilizing the chamber between trials. Transport of microorganisms through the impingers into the vacuum pumps was also tested, employing *Bacillus subtilis* in the preliminary trials. *Bacillus subtilis* cultures were freshly prepared to minimize the number of spores. Data (Table 4) show that all cells were inactivated after 30 min exposure to UV.

### 2.4. Culture preparation.

Stock cultures of the microorganisms listed in Table 1 (stored at –83°C) were streaked on Trypticase Soy Agar (TSA) plates and incubated at 37°C. The cultures were transferred to fresh medium after incubation for 24 h and harvested after a second incubation period. Optical density of the cell suspensions was measured, employing a Bausch and Lomb Spectronic 20 (Re-

ichert, Buffalo, NY), set at 600 NM. The optical density (OD) for each culture was adjusted with PBS to the appropriate value, predetermined by relating OD to viable and the Acridine Orange Direct Count (AODC) (see below).

### 2.5. Aerosol trials.

The chamber was filled with an aerosol containing approximately 10<sup>8</sup> colony forming units/ml (CFU/ml). The suspension was aerosolized using a Collison Nebulizer (BGH Instruments, Boston, MA). Samples were collected in AGIs and assayed for CFUs by plating on TSA. CFU/ml of bacteria in the spray suspension was estimated by plating a series of ten fold dilutions on TSA, using three replicate plates per dilution. Samples were collected immediately after spraying and after exposure to UV for varying times.

### 2.6. Chamber surface sterilization.

Trials were conducted before sealing the plexiglass panel to the front of the chamber, as follows:

A total of 30 uncovered TSA plates, preinoculated with 100 µl of each microorganism were taped at pre-set distances from the UV source on all sides of the chamber, with the inoculated sides facing the UV bulb. The bulb was positioned to eliminate shadowed areas and the chamber surface is highly reflective to maximize distribution of the UV light. Replicate plates were prepared so that a set could be removed at the end of

each time period (10 min, 30 min, 1 h, 1.5 h, 2 h). All trials were conducted in triplicate. Plates were incubated at 37°C for 48 h after exposure.

### 3. Results and discussion

#### 3.1. Chamber integrity.

Results of initial trials demonstrated that the chamber was leakproof, based on results of bacteriological counts of swabs of the chamber surfaces taken before and after the chamber was filled with an aerosol containing  $10^8$  CFU *B. subtilis*/l and analysis of 2250 l of room air. The sampling and injection ports, walls and sides, and all joints were swabbed. The swabs were rolled or streaked on TSA agar plates and incubated at 37°C for 48 h. Corrective measures, i.e. additional silicone sealant, replacement of tubing, etc., were taken as necessary, i.e. when *B. subtilis* was detected. The process was repeated until three consecutive trials indicated no leakage or air contamination from aerosol generation or sampling.

#### 3.2. Aerosol sampling and safety considerations

Results obtained using impingers and replicate tests demonstrated that all ports were equivalent, i.e. the aerosol was distributed evenly within the chamber. Samples taken at different ports at the same time yielded approximately the same CFU/ml. All sample mean counts were within the 0.95 confidence limit (CL), based on three replicate trials, and twelve samples per trial (Table 2).

The AGI operates by passing air through fluid in the sampler. The volume of air sampled is controlled by a combination of negative pressure and a limiting orifice. To establish the efficacy of the scrubbing process, three impingers were connected in series, with negative pressure applied to the third impinger. The

Table 2  
Sampling port variation<sup>a</sup>

| Time <sup>b</sup> | CFU/ml            | S.E. | Range |
|-------------------|-------------------|------|-------|
| 0                 | 48.5 <sup>c</sup> | 3.0  | 40–62 |
| 5                 | 41.8              | 3.7  | 34–64 |
| 120               | 32.0              | 6.2  | 26–47 |

<sup>a</sup>Test Microorganism: *Bacillus subtilis*. Three trials were conducted with twelve impingers per trial. Three replicate plates per dilution, as appropriate, were prepared. The aerosols contained  $2.5 \times 10^8$  bacteria/ml.

<sup>b</sup>Length of time (min.) after aerosolization.

<sup>c</sup>Average of 36 samples (12 sampling ports, three trials).

Table 3  
Density of bacteria in impinger exhaust<sup>a</sup>

| Impinger       | Trial 1 | Trial 2 | Trial 3 |
|----------------|---------|---------|---------|
| 1 <sup>b</sup> | 100     | 100     | 100     |
| 2 <sup>c</sup> | 4.8     | 11.0    | 16.0    |
| 3 <sup>d</sup> | 1.50    | 0.41    | 0.43    |

<sup>a</sup>Three trials were conducted to estimate the rate of transfer of bacteria between impingers.

<sup>b</sup>Values represent percent recovery, relative to impinger No. 1, which contained  $2.5 \times 10^8$  CFU/ml *B. Subtilis*.

<sup>c</sup>Percent recovery for impinger No. 2. Average for the three trials was 10.7%.

<sup>d</sup>Percent recovery for impinger No. 3. Average for the three trials was 0.78%.

air sample passes through each impinger consecutively. The first impinger was inoculated with ca.  $10^8$  CFU/ml *B. subtilis*. Organisms were recovered in each impinger. Recoveries in the second and third impingers indicated that 10.6% of the organisms pass through the impinger, when a concentration of ca.  $10^8$  CFU/ml is present in the impinger and 0.78% will pass through if the number of CFU is approximately  $10^7$ /ml (Table 3). These results underscore the need for protection at the pump inlets, especially if a hazardous microorganism is employed. The accuracy of published data, gathered under the assumption that there was retention of all cells collected, is, therefore in doubt, based on the results obtained and reported here.

#### 3.3. Disinfection of surfaces

As shown in Table 1, the bacterial species included in this study represents a range of Gram reaction, pigmentation, and source of isolation. All were airborne isolates. Since pigmentation has been associated with resistance to the effect of UV light, experiments were conducted to assure the chamber was effectively decontaminated prior to use and to assure safety of the laboratory personnel.

Results of three trials carried out to determine efficacy of the UV source are given in Table 4. In each trial, the initial cell concentration was established by adjusting the optical density of cell suspensions prepared from 24 h cultures, using plate counts on TSA agar as the final determination of CFU/ml.

Table 4 presents results obtained when initial cell concentrations were in the range of  $10^4$ – $10^9$  CFU/ml. Approximately 99.9% reduction in cell concentration was obtained after exposure to UV for 10 min, but a few viable cells were recovered even after exposure for 90 and 120 min.

Table 4  
Effect of ultraviolet light on the bacterial species employed in this study

| Bacterial Species                   | Time (Min) <sup>a</sup> |                 |    |    |    |                 |
|-------------------------------------|-------------------------|-----------------|----|----|----|-----------------|
|                                     | 0 <sup>b</sup>          | 10 <sup>c</sup> | 30 | 60 | 90 | 120             |
| <i>Aerococcus viridans</i>          | $4.6 \times 10^4$       | 20              | 6  | 0  | 0  | 0               |
| <i>Rhodococcus globerulus</i>       | $1.6 \times 10^5$       | 266             | 35 | 7  | 3  | ND              |
| <i>Bacillus subtilis</i>            | $2.4 \times 10^5$       | 8               | 5  | 0  | 0  | 0               |
| <i>Micrococcus leutus</i>           | $1.1 \times 10^5$       | 164             | 18 | 1  | 0  | ND <sup>d</sup> |
| <i>Enterococcus faecalis</i>        | $5.6 \times 10^6$       | 32              | 9  | 0  | 0  | 1               |
| <i>Pseudomonas aureofaciens</i>     | $1.2 \times 10^6$       | 31              | 9  | 2  | 1  | 0               |
| <i>Cytophaga allerginae</i>         | $1.1 \times 10^7$       | 15              | 5  | 1  | 0  | ND              |
| <i>Serratia marcescens</i>          | $8.4 \times 10^8$       | 61              | 0  | 0  | 0  | ND              |
| <i>Arthrobacter polychromogenes</i> | $6.2 \times 10^8$       | 140             | 35 | 4  | 0  | ND              |
| <i>Pseudomonas aeruginosa</i>       | $1.2 \times 10^9$       | 106             | 3  | 1  | 0  | ND              |
| <i>Staphylococcus aureus</i>        | $6.0 \times 10^9$       | 117             | 3  | 1  | 0  | ND              |
| <i>Klebsiella planticola</i>        | $1.0 \times 10^9$       | 3               | 1  | 1  | 1  | ND              |

<sup>a</sup> Exposure to UV.

<sup>b</sup> Average number of organisms/petri dish. Number based on triplicated dilutions from original suspension in each of three replicate trials.

<sup>c</sup> Total number of colonies observed after exposure to UV for indicated time.

<sup>d</sup> No data available.

Similar results were obtained over a broad range of initial cell densities, i.e.  $10^4$ – $10^9$  CFU/ml. Although a significant reduction by UV light after a short exposure time was observed, many viable cells were recovered even after exposure for 90 min. The decrease in viable cells was greatest during the first 10 min of exposure, when ca. 99% of the bacteria were rendered non-culturable. However, die-off during the next 110 min was much less. *P. aeruginosa* decreased 99.99% after the first 10 min of exposure and 96.8% after a subsequent exposure for 110 min. Gram-negative, Gram-positive and Gram variable microorganisms exhibited similar behavior.

Thus, despite a very high initial effect, viable cells were recovered after exposure for 120 min to UV. All bacterial species were present in relatively high numbers after exposure for 30 min. Although the initial effect was significant, the effect observed at 10–90 min was not as great, in terms of decrease in number of viable bacteria recovered. Thus, within 10–30 min the survival rate averaged 19%, ranging from 0–33%.

### 3.4. Disinfection after aerosolization

Aerosol trials were conducted using three of the microorganisms in the test set (*S. marcescens*, *B. subtilis* and *S. aureus*) to estimate time needed to assure that no viable microorganisms remained in the chamber air. A mixture containing ca.  $15 \times 10^8$  *S. marcescens*,  $16 \times 10^8$  *B. subtilis*, and  $12.6 \times 10^8$  *S. aureus* cells was introduced to the chamber and exposed to UV for 3 h. Approximately 40% of the chamber air was sampled at the end of the time period. No viable microorganisms were detected. Thus the aerosols were sterilized by UV, but it is clear that at least a 120 min exposure time for

surface sterilization was necessary to decontaminate the chamber by UV irradiation.

In conclusion, a chamber, designed for aerobiology studies, described here, can be relied upon to contain an aerosol without contaminating room air or endangering the operator. The aerosol can be maintained for at least 3 h, during which time the temperature and RH can be maintained. Use of UV as a disinfectant is effective, but at least 1 h is required for disinfection between trials. Precautions must be taken to prevent contamination of pumps and other vacuum devices during sample collection.

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