ORIGINAL

Suitability of ultraviolet (A)- light emitting diode for air stream disinfection

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Abstract : We previously developed a high powered light-emitting diode device capable of discharging germicidal ultraviolet irradiation (UVA-LED) at an approximate wavelength of 365 nm. This study examined the bactericidal activity of UVA-LED in moving air streams. Aerosols of *Escherichia coli* DH5α were exposed to UVA-LED irradiation using a stable current (0.5 A and 1.2 mW/cm^2) or pulse current (1.0 A and 0.2 mW/cm^2). Settle plate analysis was used for bioaerosol sampling, where results were expressed as Colony Forming Units. A -3 Log inactivation of the *E. coli* population occurred after 75 minutes of constant exposure to stable current. The pulse current produced inactivation within a similar timeframe. Our results might be significant as a basic study for further investigations about the effect of UVA-LED on airborne bacteria and its suitability for air disinfection applications. J. Med. Invest. 56 : 150-156, August, 2009

Keywords **:** *airborne pathogens, air disinfection, ultraviolet germicidal irradiation (UVGI), ultraviolet light emitting diodes (UV-LED)*

INTRODUCTION

Indoor air quality, sick building syndrome and control of infection in healthcare facilities have become major public health concerns over the last decade and into the new millennium. Possible health risks due to poor indoor air quality have been discussed in international and European working groups and projects (California EPA 2005 ; INDEX project 2005 ; SCHER 2007). Among the factors implicated in the pollution of indoor air are the heating, ventilation and air conditioning (HVAC) systems, occupants, sources (indoor, outdoor, and within mechanical systems) and pollutant pathways (EPA 1991). The main technologies used to clean indoor air are ventilation, filtration, ultraviolet germicidal irradiation (UVGI) and carbon adsorption. Other technologies still in development include photo catalytic oxidation (PCO), ionization, pulsed UV light, pulsed electric fields, ozone generators, microwave radiation, plasma fields, gamma irradiation and impregnated filters (1). The microbial killing ability of UVGI on airborne bacteria was first demonstrated by Wells and Wells (2). UVGI, emitted at a wavelength of 245 nm by

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low pressure mercury lamps, has traditionally been used for the reduction of airborne pathogens in laboratories and health care facilities. The evidence of UVGI effectiveness in upper room air disinfection of bacteria and viruses has already been proved (3-5). Disadvantages of mercury lamps such as mercury waste products, short life span of the lamp, lost energy in the form of non-light-producing heat and high cost have led scientists and facility managers to seek a new environmentally friendly technology that overcomes these demerits (6). LED is a promising new technology that has many advantages over incandescent lamps : reliability, miniaturization, low power consumption and a choice of wavelength operation between 200-365 nm (6). We have developed a sterilization system using high power LED equipment that emits ultraviolet irradiation at the UVA band, 365 nm approximate wave length. The germicidal activity of this device has been proven for a wide range of microorganisms (7). Therefore, this study focused on evaluating the germicidal activity of the new UVA-LED on airborne pathogens to determine the utility of integrating the unit into an air disinfection system. Building a system for experimental air disinfection using UV irradiation and airborne aerosols is challenging due to experimental problems including uncontrolled conditions and dependence on the test apparatus. Consequently, sanitary procedures, efficient bioaerosol sampling and procedures expenses should be considered in order to obtain reliable data on the performance of UVGI against airborne microorganisms (8, 9). We designed a primary test apparatus that operates with filtered air stream and under controlled conditions of temperature and air velocity in order to obtain as much accurate and reproducible results as possible. An ultrasonic nebulizer, used for bioaerosol generation, was connected to an aerosol test chamber wherein bioaerosol was mixed and exposed to UV irradiation while in a biological safety cabinet. For sanitary precautions, we used aerosols of non-pathogenic *Escherichia coli* DH5α as a model organism for the experimental procedures. Although *E. coli* is not a true airborne pathogen, it is commonly used as benchmark for UVGI design (8). Settle plate sampling was used to monitor the killing ability of UV irradiation. The results, reported as colony forming units, were used to calculate survival fractions.

MATERIALS AND METHODS

Bacterial sample preparation

Escherichia coli DH5α was purchased from Takara Bio Inc. (Otsu, Japan). Samples were prepared as previously described (10). Briefly, stationary phase *E. coli* DH5α grown on a Luria-Bertani agar plate (LB plate) was used to resist the effect of the ultrasonic wave during aerosol generation using the ultrasonic nebulizer (11, 12). *E. coli* cultured overnight in Luria-Bertani broth, was centrifuged for 3 min at 10,000 rpm and 4°C, washed twice with phosphate buffered saline (PBS, pH 7.4), and adjusted to an optical density of 1.0 at 600 nm for a bacterial concentration of 2×10^8/ml colony forming units (CFUs).

Bioaerosol Generation

An ultrasonic nebulizer (Omron NE-U17, Omron Co. Ltd., Kyoto, Japan) was used to generate a bioaerosol with particle sizes of 1-8 μm in 25-80% relative humidity. The ultrasonic generator created a 1.7 MHz ultrasonic wave through water to the solution to be vaporized, evaporation occurred as the result of cavitations. The procedure was adjusted based on the bacterial count per ml, volume of the aerosol chamber and capacity of the bioaerosol generator. An aerosol of 3 ml of bacterial solution (*E. coli* DH5α in PBS) with overall concentration of 2×10^8/ml made in one minute was introduced into the aerosol chamber at 2 L/min air flow speed at maximum mist volume (to produce averaged 80% RH inside the chamber) as a control measure for 5, 15, 30, 45, 60, and 90 min time intervals. Trials conducted to produce lower RH% conditions inside the chamber, but the survival fraction of *E. coli* under control measures was severely affected.

UV test chamber

UV test chamber is made of Acrylic 1 cm wall thickness, The inner dimensions of the test chamber was 10×10×20 cm (width×depth×height) giving an entire 2 L air volume. A separate cover was made from glass (for UVA) or quartz (for UVC) 3 mm thickness. During the procedures, the cover was held tight to the body of the chamber by adherent material, Fig. 2. The aerosol chamber was connected to the ultrasonic nebulizer via the upper right port (10 mm diameter, 2 cm from the top) and the filtered air outlet on the lower left side (10 mm diameter, 2 cm from the bottom) of the chamber. Bioaerosol entered from the bioaerosol inlet to the inside

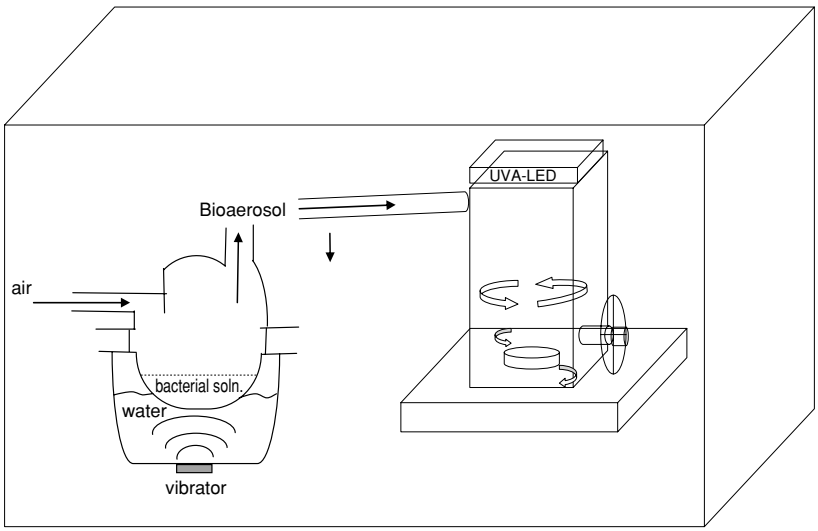

Fig. 1 The process of bioaerosol generation, mixing and UVA-LED exposure in the aerosol chamber.

of the chamber and was circulated freely by the action of the fixed fans at the bottom of the chamber. Bacteria in the air were kept inside the chamber and filtered air passed through the filtered air outlet during aerosol generation. After aerosol generation ceased, the aerosol mixing chamber was completely separated from the system and tightly closed in order to keep the bacteria inside circulating for the length of the indicated exposure time, Fig. 1. During the experiments, the temperature or relative humidity was measured using a RH manager (KN Laboratories, Inc., Osaka, Japan).

UVC or UVA exposure

UVC created by a low pressure mercury lamp system (UVP, Upland, CA) at a wavelength of 245 nm at 4 watts had an intensity 2.5 mW/cm^2. UVC intensity inside the UV test chamber was adjusted by increasing the distance from the lamp and measured with an ultraviolet radiometer (UVD-S254, Ushio Inc, Tokyo, Japan). The measured UVC intensity that passed through the quartz cover was 0.13 mW/cm^2 ; this intensity was used in calculating the utilized and wasted energy during the experiment on *E. coli* (refer to the bioaerosol sampling section). The UVC intensity measured at the bottom of the chamber, 10 ± 6 μW/cm^2, was used as an indicator of concurrent UVC intensity during exposures, Fig. 2. The high powered UVA-LED was composed of nine LEDs (Nichia NCSU033E, Nichia Corp., Tokushima, Japan) connected in series to a single power source in a metal chassis (Takagi Mfg. Co. Ltd., Japan), Fig. 3. Water cooling coils within the metal chassis were connected to the cooling system (Eyela

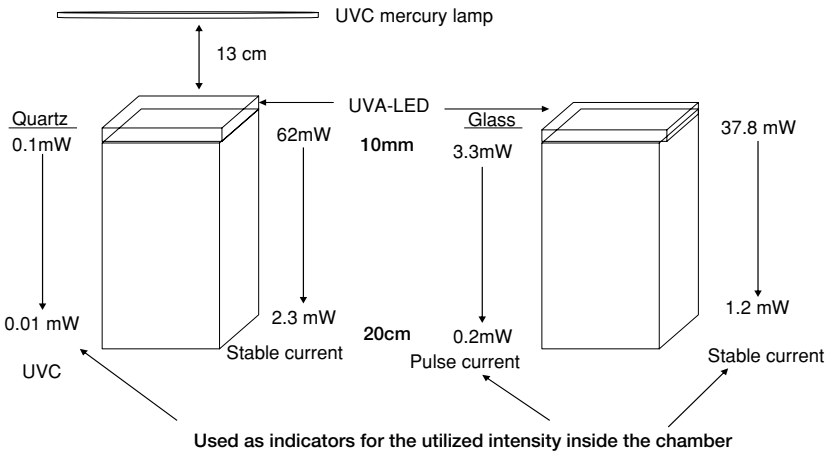

Fig. 2 The measured intensities of the UVA-LED (stable and pulse current) and UVC mercury lamp inside the test chamber using both quartz and glass covers.

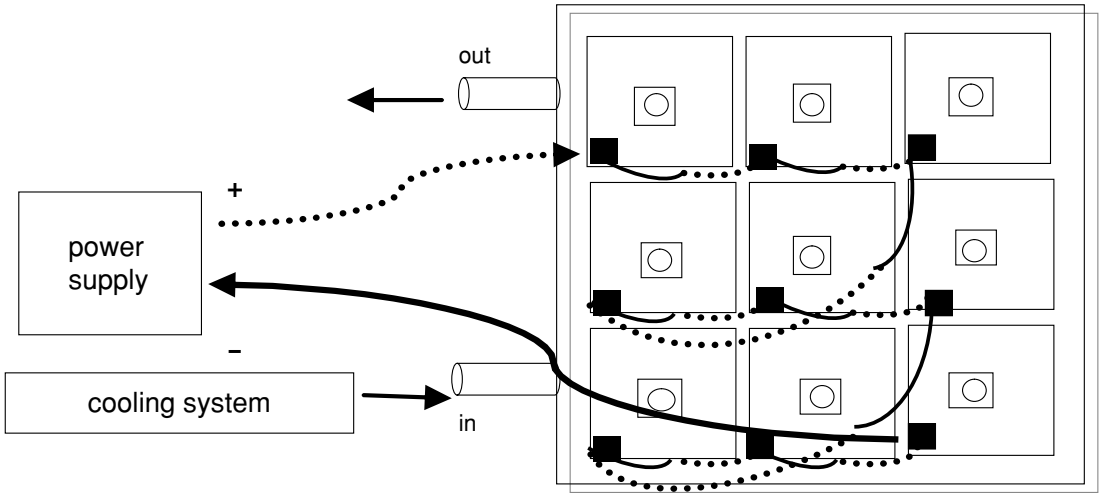

Fig. 3 Diagram of the UVA-LED showing the dimensions of each LED, the electrical circuit and the cooling system.

CCA-1111, Tokyo Rikakikai Co. Ltd., Japan). UVA intensity was measured with the UVD-S365 radiometer. Two types of UVA irradiation protocols were used for this study. In the stable current irradiation protocol, the UVA-LED was powered at 0.5 A with 150 mW/cm^2 UVA output intensity. The pulse current irradiation protocol powered the UVA-LED at 1.0 A current intensity, duty time tenth of stable current (10 ms on and 100 ms off) with a theoretical UVA output intensity of 300 mW/cm^2. The measured UVA intensity that passed through the glass cover inside the chamber was 37.8 mW/cm^2 for each LED under stable current and 3.3 mW/cm^2 for those under pulse current. These intensities were used to calculate the utilized and wasted energy for UVA-LED during the experiment on *E. coli* (refer to the bioaerosol sampling section) The measured UVA intensity at the bottom of the chamber was 1.2± 0.4 mW/cm^2 (stable current) and 0.2± 0.06 mW/cm^2 (pulse protocol), which were used as indicators of concurrent UVA intensity during the exposure procedures.

Bioaerosol sampling

The advantages of passive aerosol sampling or settle plate sampling as a control measure for the sampling of *E. coli* aerosols have been described (13). Five-centimeter LB plates were inserted into the bottom of the test chamber before bioaerosol generation. The plates were kept covered during the exposure time, and then exposed for 2 min of sampling. The survival fraction after time S_t was calculated from the following equation :

$$S_t = \text{Log}\ (N_t/N_0) \quad (1)$$

Where N_t is the number of colonies after time t and N_0 is the number of colonies at the start. Survival curves were generated to depict the effectiveness of UVA-LED or UVC irradiation on the *E coli* population. The total utilized energy (E_{ut}) of UVA-LED or UVC used to perform -3 Log reductions of *E. coli* could be measured from the following equations in the form of Watt/cm^2 :

$$E_{ut\ (UVC)} = I_s \times T_s \quad (2)$$

$$E_{ut\ (UVA\text{-}LED)} = \frac{9 I_s \times T_s}{A} \quad (3)$$

Equation (2) calculates the total utilized energy of UVC needed to perform a -3 Log inactivation of *E. coli*. (I_s) represents the measured UVC intensity that passes from the quartz cover in mW/cm^2 and (T_s) is the exposure time in seconds. Equation (3) calculates the total utilized energy of UVA-LED stable or pulse current needed to perform a -3 Log inactivation of *E. coli*. (I_s) represents the measured UVA intensity that passes from the glass cover for a single LED in mW/cm^2 multiplied by 9 (the total number of LEDs), (T_s) is the exposure time in seconds and (A) represents the surface area of the glass cover. The percentage of wasted energy (E_w) was determined using the following equation :

$$E_w\% = \frac{E_{ut}}{E_{out}} \times 100 \quad (4)$$

E_{out} represents the energy output (Watt/cm^2) of the UVC lamp or the UVA-LED used to perform a -3 Log inactivation of *E. coli*. The output energy (E_{out}) for the UVA-LED was obtained from the following equation :

$$E_{out\ (UVA\text{-}LED)} = \frac{9 I_s \times T_s}{A} \quad (5)$$

Where (I_s) represents the intensity of the UVA light output (mW/cm^2) for each LED.

RESULTS AND DISCUSSION

A -3 Log inactivation of the *E. coli* population was recorded after a 75 min exposure to both the UVA-LED stable current (0.5 A, 1.2 mW/cm^2) and pulse current (1.0 A, 0.2 mW/cm^2) treatments, producing biphasic decay curves, Fig. 4. A similar biphasic

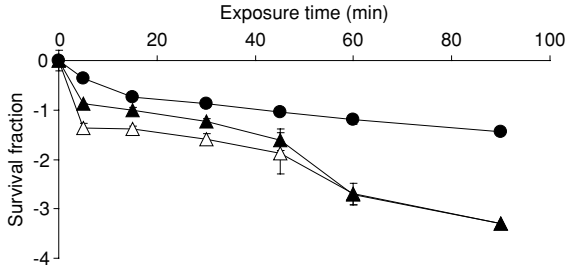

Fig. 4 The survival curve of *E. coli* DH5α after exposure to the UVA-LED stable or pulse protocol. Closed circle : control ; closed triangle : pulsed UVA exposure ; open triangle : stable UVA exposure. Data are expressed as means ± SD of three independent experiments.

decay curve was obtained after the 5 min exposure to UVC irradiation (0.01 mW/cm^2) that produced -3 Log reductions, Fig. 5. The measured temperature

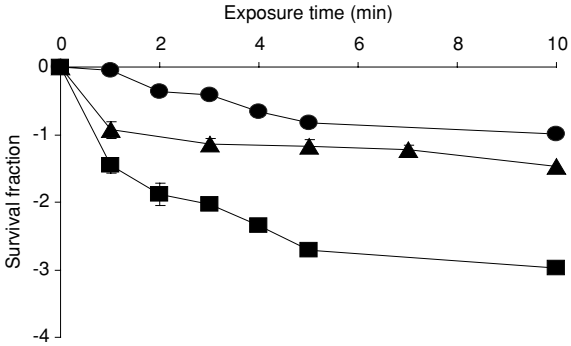

Fig. 5 Survival curve of *E. coli* DH5α after exposure to UVC (0.01 mW/cm^2) Circle : control ; triangle : stable UVA exposure ; square : UVC exposure. Data are expressed as means ± SD of three independent experiments.

and RH concurrently with UVA-LED exposure revealed an average RH of 80% and an average temperature of 26°C ; Fig. 6a and 6b respectively. The total utilized energy of the UVA-LED stable current exposure that led to 99.9% inactivation of the *E. coli* population was 15.3 W/cm^2 (25% of the total output energy) in 75 min, 1.3 W/cm^2 for the UVA-LED pulse current (11% of the output energy) in 75 min

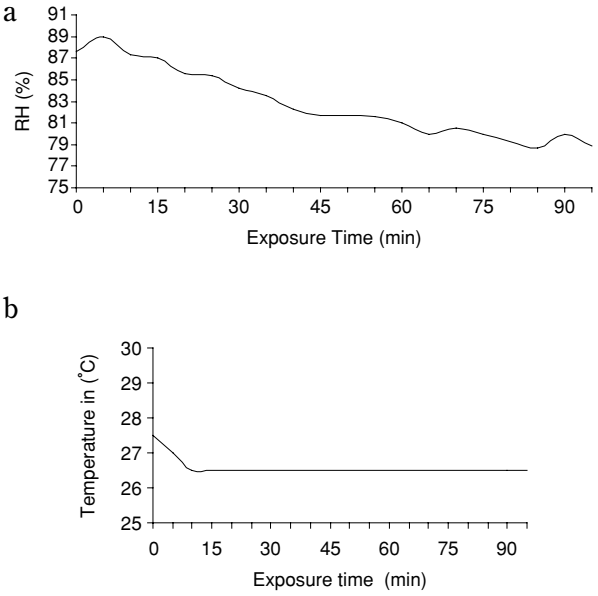

Fig. 6 Fig. 6a ; Changes in relative humidity (RH%) and Fig. 6b ; changes in temperature both measured during stable UVA exposure in the test chamber.

and 87 mW/cm^2 of UVC irradiation, which represented 5% of the total output energy ; as shown in Table 1.

Table 1 The percentage of the wasted to the utilized energy with the use of UVA-LED or UVC mercury lamp.

	Time consumed to perform -3 Log reductions (min)	Total utilized energy (W/cm^2)	% of the utilized to the output energy
UVA-LED Stable current			
(37.8 mW/cm^2)	75	15.3	25%
UVA-LED Pulse current			
(3.3 mW/cm^2)	75	1.3	11%
UVC			
(0.1 mW/cm^2)	10	0.087	5%

UVA-LED and UVC exposures produced biphasic decay curves for an *E. coli* population ; Fig. 4, 5. It has been reported that many microbial decay curves exhibit a delay curve shoulder when exposed to relatively low levels of UV irradiation. Some studies on the effect of RH on the *E. coli* decay curve showed

increased resistance with increased RH while others could not definitively establish any relationship with RH (14). Others claim that airborne bacteria are ten times more resistant to radiation at high relative humidity as when floating in lower air humidity (15). Former studies have found that 0.5-5 mW/cm^2 is needed to sterilize *E. coli* in air using germicidal UVC emitted from low pressure mercury lamps (16-18). In the current study, as a result of the use of relatively low UVC intensity in the exposure procedures (0.01 mW/cm^2), 87 mW/cm^2 was needed to reach a -3 Log reduction of an *E. coli* population with a biphasic decay curve. Inactivation by UVA exposure showed that stationary phase *E. coli* are more resistant to UV exposure and demonstrated the presence of a time delayed response, while *E. coli* are more vulnerable to UVA exposure during exponential growth (19). Therefore, we presume that three factors are needed to obtain biphasic decay curves for UVA-LED or UVC exposure to *E. coli* aerosols : relatively high RH percentage, relatively low UV intensity, and the use of *E. coli* in the stationary phase of the cell cycle. The effect on *E. coli* to long ultraviolet showed that the energy needed to produce 50% killing was 50 W/cm^2 with a threshold type survival curve. The amount of energy needed for a 50% reduction in the population using short ultraviolet irradiation was 5 mW/cm^2 with a linear survival curve. Therefore, 10^4 to 10^5 times more energy was required for long ultraviolet irradiation (16). In the present study, the energy needed to produce -3 Log reduction of *E. coli* by UVA-LED stable current was 175 times that utilized by UVC mercury lamb. Meanwhile, for UVA-LED pulse current ; the total utilized energy was 14 times that for UVC lamb. Furthermore, our results indicate that the utilized energy of the UVA-LED stable current that produced -3 Log bacterial reductions was more than ten times that used to produce the same effect on *E. coli* with the UVA-LED pulse current. This phenomenon might be related to the higher power of the pulse current 1.0 A vs. 0.5 A for stable current. Accordingly, we suggest the initial use of the UVA-LED pulse protocol for these studies on air disinfection rather than the stable current protocol due to lower energy consumption. Advantages of UVA-LED over the UVC mercury lamp were a lower level of wasted to utilized energy, safety exposure procedures and the possibility of exposure to certain small air volumes. Some disadvantages were explicit, such as the relatively low microbial killing ability and time wasting, which might be related to factors such as the use of acrylic, which is a poor reflective material for UV light ; the small size of the air chamber, which fasten the speed of the airborne bacteria and renders them resistant to UVA irradiation and the higher distance of vertical exposure (20 cm) as we previously reported higher bactericidal activity of UVA-LED at an exposure distance of 2 cm. Accordingly, we recommend the use of higher UV reflective material and shorter exposure distance for further studies on UVA-LED and air disinfection. In our previous study on the effect of UVA-LED exposure to *E. coli* DH5α in PBS in 6-well plates, 315 W/cm^2 of UVA irradiation was required to inactivate 10^4-10^6 CFU/well of *E. coli*. In this study, only 15.3 W/cm^2 of UVA stable current and 1.3 W/cm^2 of pulse current irradiation were required to inactivate 3 ml of 2×10^8/ml of aerosols of *E. coli* in PBS, indicating that bacteria are more vulnerable to UVA irradiation in air than in solution. Oxygen toxicity in *E. coli* results when the degree of oxidative stress related to ROS production exceeds the capacity of the cellular defense system, leading to damage of DNA, RNA, proteins and lipids (20), this might explain the mechanism of cell death due to UVA-LED exposure ; previously our research group has reported that the bactericidal activity of UVA-LED might be related to the oxidative effect of UVA mediated (ROS) as (OH^-) and (H_2O_2) radicals, a mechanism which differs from UVC exposure ; others reported, the bactericidal mechanism of UVC irradiation is linked to DNA damage in bacterial cells (21). In conclusion, we suggest that the UVA-LED could be used in prospective studies of airstream disinfection and have great possibility by improvement in the future.

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REFERENCES

1. Kowalski W : Immune Building Systems Technology. McGraw-Hill, New York, 2002, pp.165-166
2. Wells W, Wells M : Air-borne infections. J Am Med Assoc 107 : 1698-17, 1936
3. Jensen M : Inactivation of Airborne Viruses by Ultraviolet Irradiation. Appl Microbiol 12 : 418-420, 1964

4. Riley R, Nardell E : Clearing the Air. The Theory and Application of Ultraviolet Air Disinfection. Am Rev Resp Dis 139 : 1286-1294, 1989
5. Riley R, Knight M, Middlebrook G : Ultraviolet Susceptibility of BCG and Virulent Tubercle Bacilli. Am Rev Respir Dis 113 : 413-418, 1976
6. Newell P, Brates N, Wyner E : Mercury-free Metal Halide Arc Lamps. U.S. Patent 6,731,060, 2005
7. Hamamoto A, Mori M, Takahashi A, Nakano M, Wakikawa N, Akutagawa M, Ikehara T, Nakaya Y, Kinochi Y : New Water Disinfection System Using UVA Light-Emitting Diodes. J Appl Microbiol 103 : 2291-2298, 2007
8. Kowalski W, Bahnfleth W : Effective UVGI System Design through Improved Modeling. ASHRAE Transcriptions 106 : Pt 2, 2000
9. Kowalski W, Bahnfleth W : Proposed Standards and Guidelines for UVGI Air Disinfection. IUVA News 6 : 20-25, 2004
10. Bhaskar S, Upadhyay P : Design and Evaluation of an Aerosol Infection Chamber for Small Animals. Int J Pharm 255 : 43-48, 2003
11. Allison G, Emanuele D, Eginton P, Williams R : The Effect of ultrasound on *Escherichia coli* Viability. J Basic Microbiol 36 : 3-11, 1995
12. Vollmer A, Kwakye S, Halpern M, Everbach E : Bacterial Stress Responses to 1-Megahertz Pulsed Ultrasound in the Presence of Microbubbles. Appl Environ Microbiol 64 : 3927-3931, 1998
13. Pasquarella C, Pitzurra O, Savino A : The Index of Microbial Air Contamination. J Hosp Infect 46 : 241-256, 2000
14. Kowalski W, Bahnfleth W, Witham D, Severin B, Whittam T : Mathematical Modeling of Ultraviolet Germicidal Irradiation for Air Disinfection. Quant Microbiol 2 : 249-270, 2000
15. Rentschler H, Nagy R, Mouromseff G : Bactericidal Effect of Ultraviolet Radiation. J Bacteriol 41 : 745-774, 1940
16. Hollaender A : Effect of Long Ultraviolet and Short Visible Radiation (3500 to 4900 A°) on *Escherichia coli*. J Bacteriol 46 : 531-541, 1943
17. Sharp D : The Effect of Ultraviolet Light on Bacteria Suspended in Air J Bacteriol 39 : 535-547, 1940
18. Sharp D : The lethal Action of Short Ultraviolet Rays on Several Common Pathogenic Bacteria. J Bacteriol 37 : 447-460, 1939
19. Berney M, Weilenmann U, Ihssen J, Bassin C, Egli T : Specific Growth Rate Determines the Sensitivity of *Escherichia coli* to Thermal, UVA, and Solar Disinfection. Appl Environ Microbiol 72 : 2586-2593, 2006
20. Farr B, Kagoma T : Oxidative Stress Responses in *Escherichia coli* and *Salmonella typhimurium*. Microbiol Rev 55 : 561-585, 1991
21. Friedberg C, Walker C, Siega W : DNA repair and Mutagenesis. ASM Press, Washington D C, 1995