

Crystalline–UVC deactivation of airborne microorganisms: Clinical and laboratory analysis of a novel germicidal device

David Kirschman, M.D.

Objective. To perform an hospital-environment and laboratory evaluation of a novel crystalline-UVC (C-UVC) in-room air germicidal device for the inactivation of air pathogens.

Summary of Background Data. With emerging concerns regarding airborne environmental pathogens, including those leading to hospital-acquired infections (HAI), there is increased interest in new germicidal technologies.

Methods. Using standardized bacterial air sampling techniques, air bacterial concentrations were obtained before and after air treatment with the in-room C-UVC device in hospital operating suite and central supply settings. Additionally, the C-UVC device was tested using established single pass microorganism methods for inactivation efficiency of bacteria, viruses and spores.

Results. In single-pass testing the C-UVC device inactivated 99.97% of bacteria, 99.91% of spores, and 100% of viruses. In a hospital Operating room setting, the device reduced mean airborne bacteria levels from 39 to 7 CFU/m³. In a hospital clean supply room setting, the device had no effect on levels of 11 CFU/m³.

Conclusion. The C-UVC device, when employed as an in-room recirculation unit, provides significant reduction in airborne bacterial levels in a hospital environmental setting. These results are further supported by laboratory evaluation demonstrating high microorganism inactivation efficiency.

Aerobiotix, LLC, 717 Congress Park Dr., Dayton, OH 45459 USA

© 2013 Aerobiotix, LLC. All rights reserved.

INTRODUCTION

Airborne pathogen levels in healthcare settings are a significant, yet under-appreciated cause of hospital acquired infections and surgical site infections. Infections acquired at hospitals are the number four cause of death in the United States, exceeding the combined mortality of breast cancer, AIDS and traffic accidents at an annual cost estimated at \$40 billion (McCaughey, 2008; Mitka 2008). Increasingly, the microorganisms causing these infections have mutated into antibiotic resistant strains, making the resulting morbidity/mortality of a healthcare associated infection greater than ever. Surprisingly, there is no minimum U.S. standard for the number of bacteria, viruses, or fungi in hospital air, including critical areas of surgery suites, immunocompromised patient areas, or intensive care units.

Hospital airborne pathogen levels and hospital acquired infection

The World Health Organization (WHO) has established that airborne microbial levels in an operating suite should be below 10 colony forming units (CFU)/m³ (Wilson 2001, Gruendemann, 2001). However, recent studies have found significant airborne microbial contamination in modern medical centers with measured bacterial concentrations in intensive care units and operating suites settings of 50 to 250 CFU/m³. Commonly isolated airborne microorganisms are *Staphylococcus epidermidis*, *Staphylococcus aureus* (including MRSA), and *Pseudomonas* species (Holcatova, 1993). Airborne particles containing influenza virus were found in 84% of hospital emergency ward air samples. 22 different types of fungal spores were isolated in hospital wards with concentrations from 175-1396 CFU/m³. The WHO recommends fungal levels below 50 CFU/m³ for healthcare facilities (Tormo, 2002). Published data on airborne levels in operating rooms indicates the air is far from sterile, and is often no cleaner than the air in general wards (Kowalski, 2012).

Surgical site infection and airborne pathogen levels

Infection rates in joint replacement surgery are correlated with airborne concentrations of bacteria near the wound (Table 1, Lidwell, 1983). Up to 90% of bacterial contaminants found in operative wounds gain access to the wound by the airborne route (Howorth, 1985). Most of the bacteria in surgical site infections are shed from the skin or are attached to skin particles or particulate matter less than 5 microns in size. These particles become transiently airborne and float on air currents before implanting in the wound (Hardin and Nicols, 1995). More than 90% of bacteria contaminating clean wounds come from the ambient air, and a substantial part of these bacteria contaminate the wound directly during clean-wound surgery (Whyte, 1982). Most of the bacteria in surgical site infections are shed from the skin or are attached to squames or particulate matter less than 5 microns in size. These particles become transiently airborne and float on air currents before implanting in the wound (Hardin and Nicols 1995).

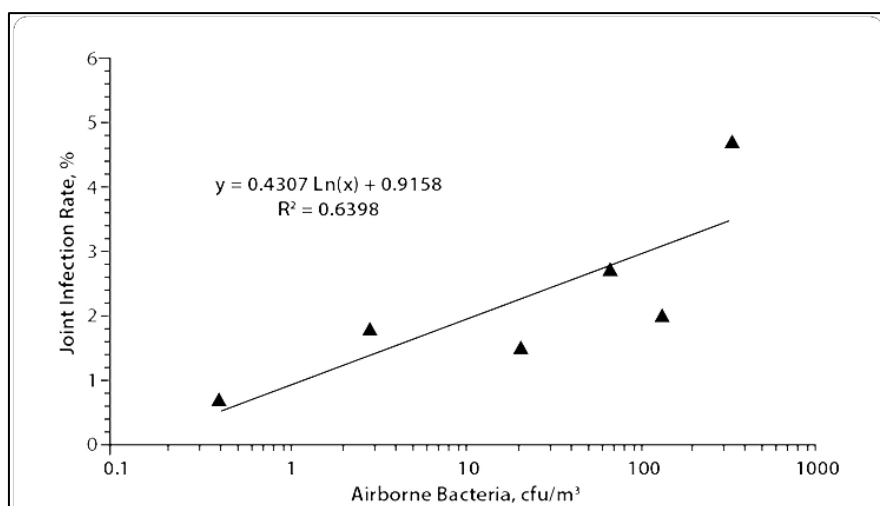


TABLE 1. The infection rate in joint replacement surgery is directly proportional to the concentrations of airborne bacteria of the operating room. *Adapted from Lidwell, 1983.*

Current air-germicidal technologies

Ultraviolet light, particularly the 254nm C-band wavelength (UVC), is effectively biocidal via DNA absorption, intercalation and the formation of D-dimers. Employment of UV light in health care settings, particularly in tuberculosis care, has been documented since the pre-antibiotic era. The biocidal effect of UVC depends on multiple variables, particularly UVC intensity and duration of exposure.

Upper room UV units, which consist of shielded UV lamps mounted near a room ceiling, have been in sporadic use since the 1930s. Although there is data supporting their effectiveness in inactivating pathogens, there are significant drawbacks. These are passive devices which depend on variable room air currents to circulate microbes. This passive operation limits the number of pathogens effectively exposed. Additionally, since the lamps are open to the environment, reflected rays may be visible in the room, and finally, these units require a fixed installation and physical plant modification to install.

In response to the shortcomings of upper-room UV systems, permanently-placed in-duct UV systems have been developed. These systems are either placed at the time of hospital construction or require modifications to the existing ventilation system. Installation of fixed systems in an existing facility requires modification of the existing infrastructure, which can be expensive and have the counterproductive effect of releasing pathogens via the renovation activity itself. Access to the units for critical maintenance is difficult and the devices cannot be relocated once installed.

Most importantly, in-duct units do not actively disinfect within the room itself. Studies have indicated that the source for pathogens in critical healthcare settings (such as operating rooms) is the occupants of the room, not the HVAC system (Kowalski, 2012). Bacteria and viruses are continually released by patients and healthcare workers. Introduction of clean air via the duct system does not serve to directly inactivate or sequester these pathogens as they are being released. Therefore, the performance of in-duct systems is ultimately limited.

The design of a UVC system must be optimized to maximize dosage while maintaining adequate air flow volumes. At a given UVC radiation output, air flow volume and UVC dosage are inversely related, resulting in necessary compromise in the design of such systems. The crystalline UVC (C-UVC) system was developed to maximize UVC dosage and inactivation efficiency while maintaining high air flow volumes.

MATERIALS AND METHODS

A. The Crystalline-UVC (C-UVC) Air Disinfection Device



FIGURE 1. The crystalline UVC (C-UVC) air disinfection-recirculation device.

The C-UVC device (Aerobiotix, Dayton, OH) is a novel in-room air disinfection-recirculation unit. (Fig. 1) It utilizes a hybrid of biological and physical systems to remove bacteria, fungi and viruses from the air. Its key biocidal technology is a reactor system which provides simultaneous physical filtration and irradiation of high-volume air flow with minimal resistance. The reactor system utilizes C-band ultraviolet light (UVC) focused on a reaction chamber filled with a multitude of clear cylindrical silicate crystals. The silicate crystals function as a solid media filter, slowing and trapping organisms as they pass via the air stream. A unique feature of silicate crystal is that it can be efficiently penetrated by UVC irradiation. Therefore, while organisms are slowed or trapped in the solid crystalline matrix, they are inactivated by the penetrating UVC dosage. This has the effect of increasing the inactivation efficiency over prior UV technologies.

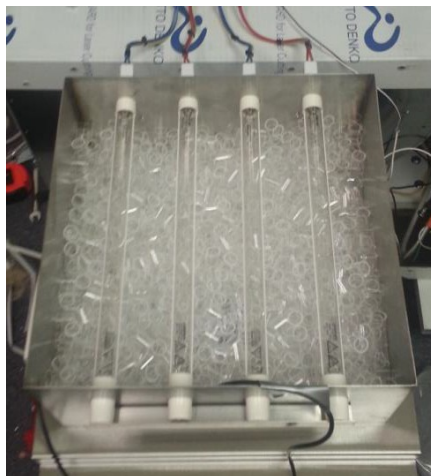


FIGURE 2. The C-UVC crystalline matrix consisting of multiple packed silicate cylinders and adjacent UVC lamps.

The reactor system is augmented by a standard HEPA-type filter and prefilter to add additional physical filtration and prevent particulate contamination of the reactor system. Additionally, the HEPA system serves to trap inactivated particles, which though they are not infectious, can still cause allergic responses. UVC is additionally projected at the intake side of the HEPA filter to prevent potential filter contamination.

B. Laboratory analysis for single-pass inactivation of airborne bacteria, virus and spores.

An independent, accredited laboratory (RTI, Raleigh, NC) RTI conducted a series of tests to determine the efficiency of microorganism inactivation by the C-UVC device. The unit provided by Aerobiotix was installed in RTI's high-flow test rig (Figures 3 and 4). A total of three forms of organisms were tested: bacterial spores, virus and vegetative bacterial cells. The airflow was a constant 450 cubic feet per minute (cfm).

- *Bacillus atrophaeus* (*B. atrophaeus*), is a spore-forming bacterium with spore size ranging from 0.7-0.8 x 1-1.5 μ m. The organism is a ubiquitous environmental bacterium, found at high levels in soil and highly associated with indoor dust. *B. atrophaeus* is generally difficult to inactivate and has value as a stimulant historically used for many applications and permits comparison to past testing and studies of inactivation efficiency of air cleaning devices.
- *Staphylococcus epidermidis* (*S. epidermidis*), is a common gram-positive organism shed by humans, and was used as the representative vegetative bacterium.
- The MS2 virus is an *Escherichia coli* (*E. coli*) bacteriophage that roughly approximates the aerosol-related physical characteristics of human viruses. Individual virus particles of health-threatening agents may be somewhat smaller or larger than MS2.

The testing was performed in a test duct as illustrated in Figure 3. The test duct provides steady air flow and aerosol drying and neutralization along with sampling ports for bioaerosol sample collection. The cross section of the duct is 0.61 m by 0.61 m (24 in. by 24 in.). The locations of the major components, including the sampling probes, device section, the bioaerosol generator (site of aerosol injection) and samplers are shown.

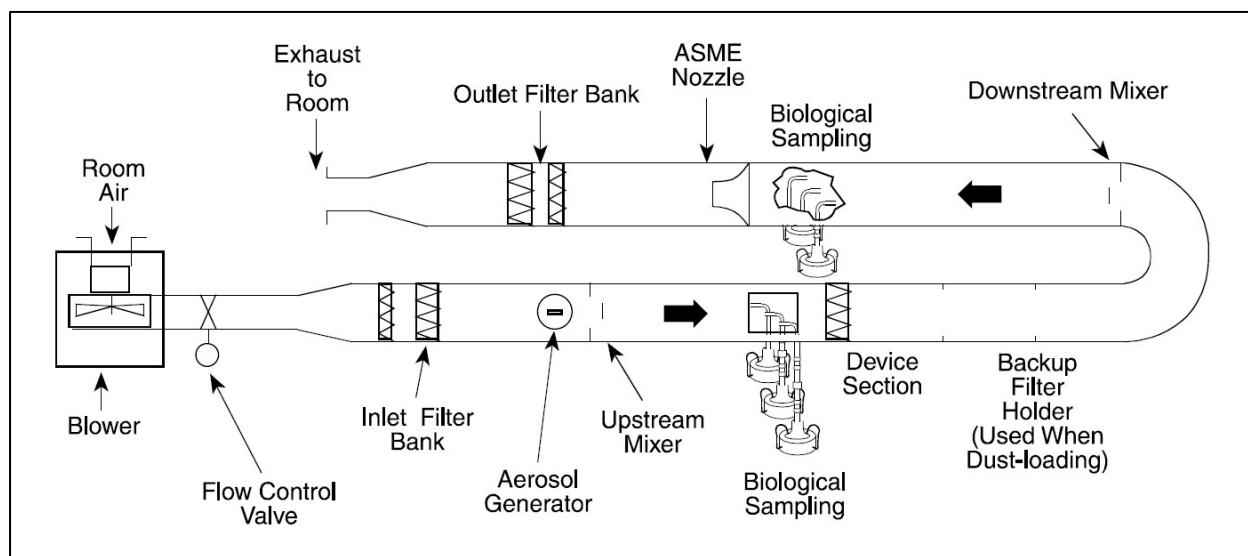


FIGURE 3. Laboratory setup for single-pass biocidal testing of the C-UVC device.

The unit was installed as shown in Figure 4. There is a 24 x 18 inch (61 x 46 cm) air intake at the lower front of the unit and a 24 x 12 inch (61 x 30.5 cm) clean air exhaust at the top of the unit. Transitions were manufactured to connect the air intake and clean air exhaust to the rig. The unit was mounted with the air intake facing upstream so that all the incoming challenge bioaerosol was required to pass through the air intake of the unit and exit through the clean air exhaust.



FIGURE 4. The C-UVC test setup for single-pass testing (introduction and collection of bioaerosols).

Fresh cultures of each microorganism were prepared and checked for quality before each run. The challenge bioaerosol suspensions were aerosolized using a Collison modified MRE-type six-jet nebulizer (BGI, Waltham, MA). The output of the nebulizer was mixed with clean, dry air prior to its entry into the test duct to create the dry bioaerosol challenge. The organisms were aerosolized in water and tests were conducted at room temperature.

The unit was challenged with one microorganism at a time. For each round of sampling, six upstream and six downstream bioaerosol samples were taken. A standard sampling time was determined and used for all organisms to facilitate direct comparisons. Bioaerosol samples were collected from the air stream with sampling probes positioned within the test duct at both the upstream and downstream sampling sites.

B. atrophaeus and *S. epidermidis* were aerosolized in water, and sampling was done using Andersen one-stage viable bioaerosol samplers loaded with Petri dishes containing appropriate growth media. The one-stage sampler is a multiple-jet impactor. A positive-hole correction was used to adjust colony counts from the Andersen multiple-hole impactor for the possibility of collecting multiple colonies through a hole.¹ After sampling, the Petri dishes were removed from the sampler and incubated overnight at 37° C. Organism identity was confirmed by microscopy, and Colony Forming Units (CFUs) were enumerated. The MS2 virus was aerosolized in water and sampling was accomplished using all-glass impingers (AGI's) containing impinger fluid. After sampling, the impinger fluid was analyzed for viable virions by a standard plaque assay.

For each run, the challenge bioaerosol was injected upstream of the device. A “device off” transmission test was also performed for each organism, to determine the microorganism loss that would occur simply as a result of deposition or inactivation in the test duct. For the “device off” test, the unit was completely shut off, filters and crystals were removed and step 3 was omitted.

B. Airborne bacterial levels in a hospital setting

Air samples were taken in two locations in an urban hospital setting in Dayton, Ohio, USA. Two locations were chosen: An 5 x 6m active operating room and an 8 x 10m clean instrument supply room. The sampling locations were immediately behind the sterile instrument table in the operating room, and adjacent to the instrument tray assembly area of the instrument supply room (Figure 5). Samples were taken with the C-UVC device in place, but turned off, and then repeated after four hours of the C-UVC device running. In the supply area, normal workday activity proceeded as usual. In the operating room, three urological procedures were performed with standard room turnovers and setups during the four hour test period.

The NIOSH method 0800 was followed, using an Anderson N-6 type sampler and calibrated vacuum pump. The sampling pump was set to 28.3L/minute for a five minute sampling time. The sampler was cleaned with 70% isopropanol between samples. Tryptic soy agar (TSA) plates were used for the selection of aerobic environmental bacteria. The samples were sent to an independent environmental laboratory (EMLab, Marlton, NJ) for incubation, morphology and gram stain.

Samples were taken in the following groups: unexposed control, operating room baseline, operating room after 4 hour use of C-UVC unit, supply room baseline and supply room after 4 hour use of C-UVC unit.



FIGURE 5. C-UVC device in clean instrument supply room (*left*). In operating room (*right*).

RESULTS

A. Laboratory analysis for single-pass inactivation of airborne bacteria, virus and spores.

Single Pass In-duct Test Results

Table 2 presents the efficiency results for the air cleaner. The inactivation efficiencies were calculated as shown in the equation2 below:

$$\text{Airborne Inactivation Efficiency (\%)} = 100 (1 - \text{Corrected Survival Rate})$$

The calculation of the test organism survival rate (culturable transmission) was based on the ratio of the downstream to upstream culturable organism counts. To remove system bias, the Survival Rate was corrected by the results of the “device off” transmission test. The “device off” transmission rate was calculated in the same manner as the survival rate. The “device off” value was 1.07 for MS2, 0.71 for *S. epidermidis*, and 0.81 for *B. atrophaeus*.

Test Organism	Inactivation Efficiency (%)
<i>B. atrophaeus</i>	99.91
<i>S. epidermidis</i>	99.97
MS2 Virus	100.0

TABLE 2: Single-pass organism inactivation rates

All samples were collected for 10 seconds upstream and 1 minute downstream. Three samples were collected simultaneously from up and down stream ports followed by another three within 5 minutes of the first set. The difference indicates the time needed to change out sampling plates or impingers and the device and rig continue to run during this time. The average of the 6 upstream samples and the average of the 6 downstream samples for each run was used in the calculations.

B. Airborne bacterial levels in a hospital setting

After appropriate incubation of the TSA plates (n=10), bacterial count, morphology and Gram stain were performed. Table 3 shows the results.

Test Condition	Mean bacterial count (CFU/m ³)	Bacterial Morphology
Unexposed Control	0	NA
Operating Room baseline	39	G(+) Cocci, G(-) rods
Supply Room baseline	11	G(+) Cocci
Operating Room C-UVC	7	G(+) Cocci
Supply Room C-UVC	11	G(+) Cocci

TABLE 3a. Bacterial air concentrations and morphology in each test modality.

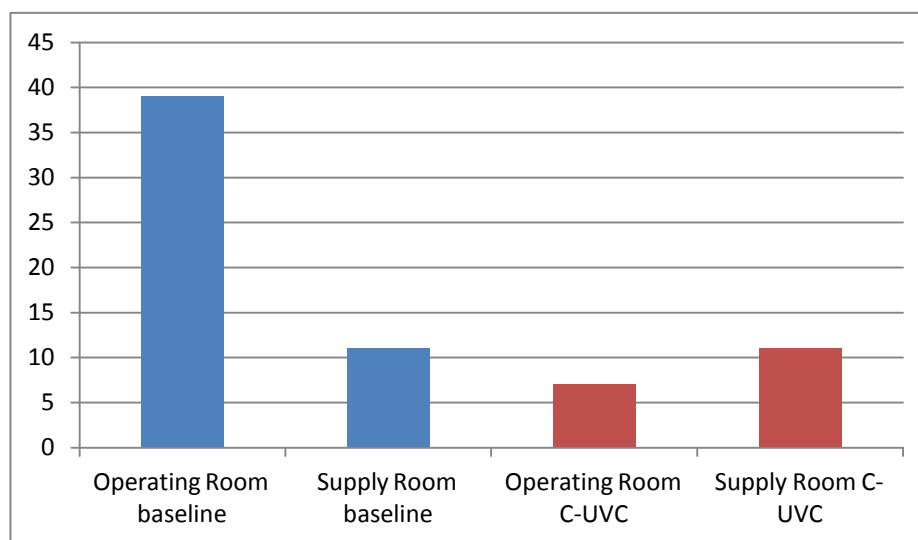


TABLE 3 b. Baseline bacterial concentrations (blue) and levels after C-UVC treatment (red). Units are CFU/m³.

DISCUSSION

Recently, in the United States, there has been an increased awareness and scrutiny of hospital acquired infections and surgical site infections. However, relatively little attention has been given to the role of airborne bacterium, virus and spore levels in healthcare facilities, and even less effort has been given to the remediation of such organisms. Although a number of technologies have been developed for active inactivation of airborne pathogens, systems rarely see significant community utilization. This is despite the fact that UV-based germicidal technologies have an established record of effectiveness in health care facilities. Indeed, the US Centers for Disease Control and Prevention, in its Guidelines for Environmental Infection Control in Health-Care Facilities stipulates: “As a supplemental air-cleaning measure, ultraviolet germicidal irradiation is effective in reducing the transmission of airborne bacterial and viral infections in hospitals.”

In the laboratory setting, the C-UVC device demonstrated broad-spectrum inactivation of microorganisms, including bacteria, virus and spore forms. The device demonstrated greater than 99.9% inactivation efficiency during a single pass through the device. Since the device also performs air recirculation, it can be presumed that even higher levels of inactivation are achieved in real-world settings due to multiple device passes. The device was also able to achieve high inactivation rates while achieving the high airflow rate of 12.7 cubic meters per minute (450 cfm). High air flow rates are required for adequate air exchange in a typical operating room.

In the clinical operating room setting, the C-UVC device reduced airborne bacteria concentrations from 39 to 7 CFU/m³. This represents a major improvement, including bringing the operating room in compliance with the WHO recommendation for operating rooms of 10 CFU/m³. In the supply room setting, the device did not improve the airborne bacteria concentration. However, levels were already quite low at 11 CFU/m³. It is likely that, in areas which are already highly clean, additional cleaning brings reducing returns.

CONCLUSION

The in-room C-UVC system represents a novel and effective means to reduce airborne microorganisms in both laboratory and clinical settings. The device achieves high inactivation efficiencies over a broad spectrum of potential pathogens, including bacteria, viruses and spores. The C-UVC system represents a safe, practical and effective method for reducing airborne pathogens in health care settings, and its adoption should be considered in facilities with elevated airborne pathogen levels.

REFERENCES

- Gruendemann BJ, and Stonehocker S. (2001). *Infection Prevention in Surgical Settings*. WB Saunders, Philadelphia.
- Hardin W. and Nichols R. (1995). Aseptic Technique in the operating room. In *Surgical Infections*. D.E. Fry, Ed., Little Brown and Company Boston 109-117.
- Holcatova I, Bencko V, Binek B (1993) Indoor air microbial contamination in the operating theatre and intensive care units of the surgery clinic. *Indoor Air* 93, 375-378.
- Howorth FH (1985) Prevention of airborne infection during surgery. *The Lancet* 1, Feb 16, 386-388.
- Kowalski, W. (2012) *Hospital Airborne Infection Control* CRC Press, Boca Raton.
- Lidwell, O. (1983) Airborne contamination of wounds in joint replacement operations: the relationship to sepsis rates. *J Hosp Infect* 4, 111-131.
- McCaughey, B. (2008). *Unnecessary Deaths: The Human and Financial Costs of Hospital Infections*. (p. 70). Committee to Reduce Infection Deaths.
- Mitka, M. (2008). Public, Private Insurers Refusing to Pay Hospitals for Costs of Avoidable Errors. *JAMA*, 299(21), 2495-2496. doi: 10.1001/jama.299.21.2495.
- Tormo MR, Gonzalo GM, Munoz RA, Sinva PI. (2002). Pollen and spores in the air of a hospital out-patient ward. *Allergol Immunopath* 30(4) 232-238.
- Whyte W, Hodgson R, Tinkler J. (1982). The importance of airborne bacterial contamination of wounds. *J Hosp Infect* 3, 123-135.
- Wilson, J (2001) *Infection Control in Clinical Practice*. Balliere Tindall, Edinburgh.