

Crystalline UV-C Inactivation of Airborne Microorganisms: Clinical and Laboratory Analysis of a Novel Germicidal Air Recirculation Technology

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Introduction: Hospital acquired infections (HAI) are the number four cause of death in the United States at an annual cost estimated at \$40 billion. With emerging scientific data regarding the contribution of air contamination to surgical site infections (SSI), there is increased interest in considering innovative adjunctive technologies to support best air quality in operating rooms (ORs). **Objective:** The study was designed to test the effectiveness of the novel AEROBIOTIX (ABX) crystalline ultraviolet (C-UVC) germicidal irradiation in-room air disinfection and filtration technology on reducing airborne particle contamination in the OR. **Methods:** Single-pass laboratory-based tests were performed to evaluate inactivation efficiency of ABX C-UVC reaction chamber on aerosolized bacteria, viruses and spores. Clinical field tests were carried out to assess the efficacy of C-UVC air recirculation/filtration device on reduction of airborne colony forming units (CFU) and viable particle counts (VPC) in the OR. **Results:** In single-pass laboratory-based testing the C-UVC device inactivated 99.97% of bacteria, 99.91% of spores, and 100% of viruses. In the clinical OR setting, the deployment of the ABX C-UVC air recirculation/filtration unit substantially reduced average airborne bacteria levels and 5µm airborne particles by 82 and 84 % respectively. Over 95% reduction was seen in particles 1 µm, 3 µm and 10 µm in size. **Conclusion:** The ABX C-UVC device is effective in reducing airborne contamination and improving air quality in the OR, supporting reduced SSI risk for patients and improved safety for the surgical team.

HAI 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 billion¹. Airborne bacteria-laden particles in healthcare settings are an under-appreciated, contributing cause of HAI, specifically SSI². Current methods of ensuring good air quality in most ORs include only engineering controls, namely positive air pressure and increased air changes per hour (ACH/h). High OR traffic and increased OR occupancy can circumvent engineered control measures and disrupt/resettle circulating airborne particulates, 30% of which can be bacteria laden³⁻⁹. A positive correlation between SSI and airborne bacterial levels in operating rooms has been shown.² Similarly, strong associations between SSI risk and OR traffic, number of OR personnel, door opening during cases as well as uncovered hair and skin of surgical team members have been reported in literature¹⁰⁻¹⁶. Yet, no standards for indoor air microbiological or particulate counts have been promulgated for ORs in the US⁹. Strict guidelines are in place as established by USP 797 for Pharmacy Clean Rooms in the US. In some European countries, including Switzerland, air quality standards for ORs are firmly established. According to Swiss regulations, ORs performing procedures including solid organ transplant, orthopedic cases and cardiac

surgery would be categorized as class I with a bacteria limit of ≤ 10 CFU/m³ in circulating air. A category of class II with an airborne bacteria limit of ≤ 50 CFU/m³ would encompass emergency wards, premature infant and delivery rooms, intensive care units and burn units. A category of class III with an airborne bacteria limit of ≤ 200 CFU/m³ would include sterile processing departments, radiology departments, and pediatrics⁹. The World Health Organization (WHO) guideline is in alignment with this Swiss standard, recommending that airborne microbial levels in the OR should be <10 CFU/m³. Review of peer reviewed studies reflects a wide range of reported airborne bacteria counts (CFU/m³) in the OR environment^{4, 10, 15, 17}. The continuing emergence of antibiotic resistant microbial strains further supports the imperative to reduce the burden of environmental and airborne contamination in ORs.

Ultraviolet germicidal irradiation (UVGI) is a widely employed technology used as an adjunct to manual environmental cleaning and disinfection. The UVGI disinfection method utilizes short-wavelength C-band ultraviolet light (UV-C) to completely eradicate a broad-range of microorganisms. Prolonged exposure of bacteria, spores and viruses to UV-C causes substantial

DNA damage interfering with a wide range of vital functions inducing microorganism inactivation/killing.

UVGI-based technologies can similarly serve as powerful adjunctive modalities for improving indoor air quality in the OR. Several UVGI “air-scrubbing” systems are currently utilized for various applications in hospitals and healthcare facilities^{18, 19}. Unfortunately, due to their compact size and large airflow, the UV-C dose delivered to microorganisms is not sufficient to meet minimum exposure time of 0.25 sec to ensure inactivation of microorganisms¹⁸.

AEROBIOTIX has developed a portable crystalline-UVC-enabled HEPA filtration device for use as an adjunct to current engineering controls to improve air quality in ORs. This is especially critical for surgical procedures involving implants. The key biocidal technology is a reactor system with UV-C light focused over the reaction chamber filled with clear cylindrical silicate crystals. The silicate crystals provide a solid UV-permeable media filter to increase the exposure of air bioburden to the UV-C light (>0.25 sec). A unique feature of silicate crystal is that it can be efficiently penetrated by UV-C irradiation (C-UVC). Therefore, while organisms are slowed or trapped in the solid crystalline matrix, they are inactivated by the penetrating UV-C dosage. This has the effect of immensely increasing the antimicrobial efficiency over prior UV “air-scrubbing” technologies. In addition, the C-UVC component is further augmented by two filter systems which physically remove air particulates.

In this study, ABX C-UVC unit was tested both in laboratory-based and clinical settings to confirm its efficacy in inactivating airborne microorganisms and reducing particle counts in the high risk OR environment.

MATERIALS AND METHODS

STUDY LOCATIONS

An independent, accredited laboratory (RTI, Raleigh, NC) conducted single-pass tests to determine the efficiency of microorganism inactivation by the C-UVC/HEPA device. For field testing, airborne bacterial

and particulate levels were sampled in two locations in an urban hospital setting in Dayton, Ohio, USA.

TECHNOLOGY

The C-UVC/HEPA device was designed by AEROBIOTIX, Dayton, OH (Figure 1). The unit was developed to accommodate the 450 ft³/min (CFM) airflow in a standard OR environment. The 24x18 inch (61x46cm) air intake is located at the bottom of the unit, adjacent to the motor. The 24x12 inch (61x30.5 cm) clean air exhaust is positioned at the top of the device. Both air ports are supplemented with filtration systems: an inlet air filter cartridge (performance level 1=PL1) and a HEPA air outlet filter respectively. The C-UVC (254nm) reactor is placed in the path of the airflow (Figure 1), at the center of the unit between the two filtration systems. The silicate crystals within the reactor are designed to form a solid UV-C permeable media to slow down the airstream and prolong C-UVC exposure of airborne microorganisms.

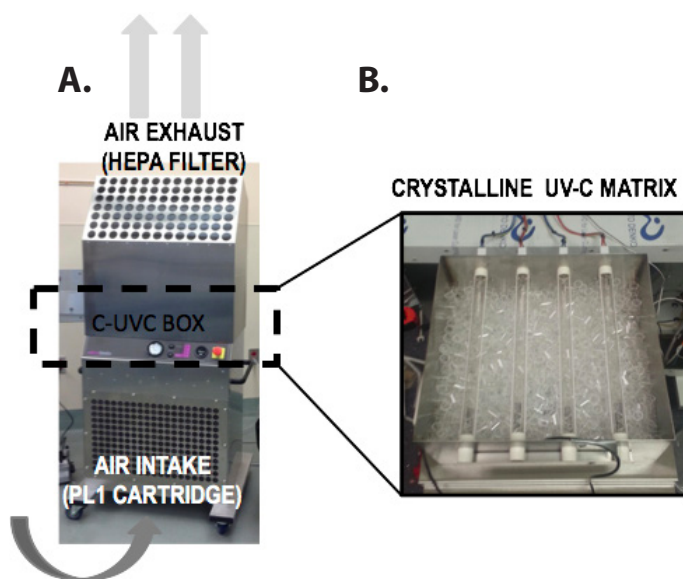


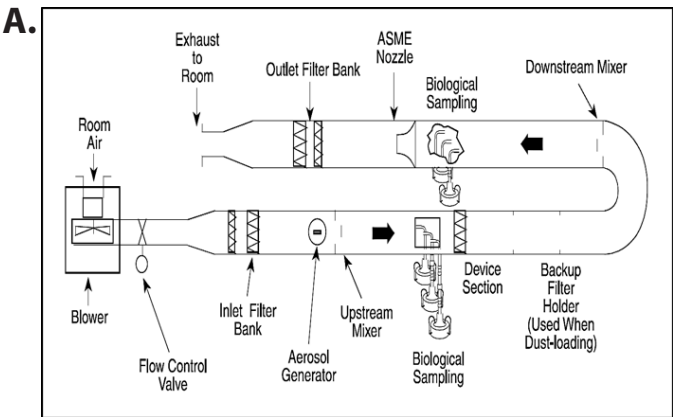
Figure 1. The Crystalline-UVC (C-UVC)/HEPA Air Disinfection Technology Overview. (A) Air recirculation device: Air stream flows from the bottom (via performance level 1 (PL1) filtration cartridge) and exits via HEPA filter air exhaust assembly at the top of the unit; (B) The C-UVC crystalline matrix is positioned on the air path and consists of multiple packed silicate cylinders with adjacent UV-C lamps for most efficient germicidal irradiation.

LABORATORY TESTING: HIGH-FLOW TEST RIG WITH BIOAEROSOLS

The microbiocidal testing was performed in a test duct. The duct (24x24in diameter) was designed to provide a steady airflow to ensure aerosol drying and neutralization for efficient sample collection. The components of the duct set-up including sampling probes, device section, bio-aerosol generator and samplers are depicted in Figure 2A. Unit transitions were manufactured to connect the air intake and the clean air exhaust to the rig (Figure 2B). The unit was mounted with the air intake facing upstream to enable the incoming challenge bio-aerosol to pass through the air intake and exit via the clean air exhaust (Figure 2B).

Bacillus atrophaeus (*B. atrophaeus*), *Staphylococcus epidermidis* (*S. epidermidis*) and MS2 bacteriophage were maintained under standard culture conditions, checked for quality and prepared into water-bioaerosol suspensions using a Collison modified MRE-type six-jet nebulizer (BGI, Waltham, MA). Aerosolized output was mixed with clean, dry air prior to entry into the test duct at room temperature. The unit was challenged with one type of microorganism at a time. Sampling was done using Andersen one-stage viable bioaerosol samplers loaded with growth media-containing Petri dishes (n=6 upstream; n=6 downstream per microorganism). A positive-hole correction was used to adjust colony counts from the Andersen multiple-hole impactor to allow for the possibility of collecting multiple colonies through a hole. 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 sampled using all-glass impingers (AGI's) containing impinger fluid. After sampling, the impinger fluid was analyzed for viable virions by a standard plaque assay. The inactivation efficiencies were calculated as follows: *Airborne Inactivation Efficiency (%) = 100*(1-Corrected Survival Rate)*.

A “device off” transmission test was performed for each microorganism as a control for microorganism loss due to deposition or inactivation in the test duct. The unit was completely shut-off, filters and crystals were removed (Figure 2A).



C.

| Test Organism | Type | Inactivation Efficiency (%) |
|-----------------------|------------------------|-----------------------------|
| <i>B. atrophaeus</i> | Spore-forming bacillus | 99.91 |
| <i>S. epidermidis</i> | Gram (+) Coccus | 99.97 |
| MS2 Virus | Bacteriophage | 100.0 |

Figure 2. Experimental design and results for single-pass laboratory-based test. (A) Schematic diagram and (B) laboratory set-up for biocidal testing of the C-UVC/HEPA device; (C) Single-pass organism inactivation rates (% efficiency). All samples were collected for 10 seconds upstream and 1 minute downstream (n=6 for each location per microorganism tested).

FIELD TESTING

Airborne Bacterial Levels

Two rooms were analyzed for air bioburden with adjunct filtration/recirculation C-UVC/HEPA ABX unit in place (ON/OFF conditions). A 5x6 meter active urology OR and 8x10 meter sterile processing instrument assembly area were chosen as two distinct environmental settings. The sampling locations were immediately behind the sterile instrument table in the OR, and adjacent to the instrument tray assembly area in sterile processing, respectively. 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 sterile processing instrument assembly area, normal workday activity proceeded as usual. In the OR, three urological procedures were performed with standard room turnovers and setups during the 4-hour test period.

The NIOSH method 0800 was followed, using an AndersonN-6 type sampler and calibrated vacuum pump. The sampling pump was set to 28.3L/minute for a 5 minute sampling time. The sampler was cleaned with 70% isopropanol between collections. Tryptic soy agar (TSA) plates were used for the selection of aerobic environmental bacteria, n=10/group. The samples were sent to an independent environmental laboratory (EMLab, Marlton, NJ) for incubation, morphology and gram stain. Groups were analyzed as follows: 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.

Airborne viable particle testing

In the test OR (described above), the laser induced fluorescence sampling unit (BIOTRAK® Real-Time ParticleCounter 9510-BD) was placed 4 meters from the C-UVC device, so that it would not be sampling direct air currents from the C-UVC air output. In each test modality, air samples were taken every 60 seconds, until the detected particle levels reached a stable equilibrium. Data is presented as viable particle counts (VPC) per cubic meter (m³) for particle sizes 1, 3, 5 and 10 micrometers (µm).

RESULTS

C-UVC device is highly efficient in inactivating a broad spectrum of aerosolized microorganisms

The ABX C-UVC system was developed to maximize UV-C dosage required for full inactivation of airborne pathogens while maintaining a high air flow volume of 450 CFM. Laboratory-based in-duct tests were carried out to measure microorganism survival rate after a single-pass exposure to the device C-UVC reaction chamber (Figure 2A/B). The calculation of the test

organism survival rate was based on the ratio of the downstream to upstream organism counts. All samples were collected for 10 seconds upstream and 1 minute downstream (n=6 for each location per microorganism tested). Results showed over 99.9% inactivation efficiency of *B.atrophaeus*, *S. epidermidis* and MS2 virus test microorganisms during a single-pass test (Figure 2C) confirming highly biocidal property of C-UVC chamber design.

C-UVC air recirculation device installed in the active OR setting is highly effective in reducing airborne bioburden

To evaluate the effectiveness of the C-UVC air recirculation device in the clinical operating room setting ABX C-UVC units were installed in an active OR with standard positive pressure ventilation. The supply room in sterile instrument processing area was designated as clean area and utilized as an environmental setting control for comparison purposes.

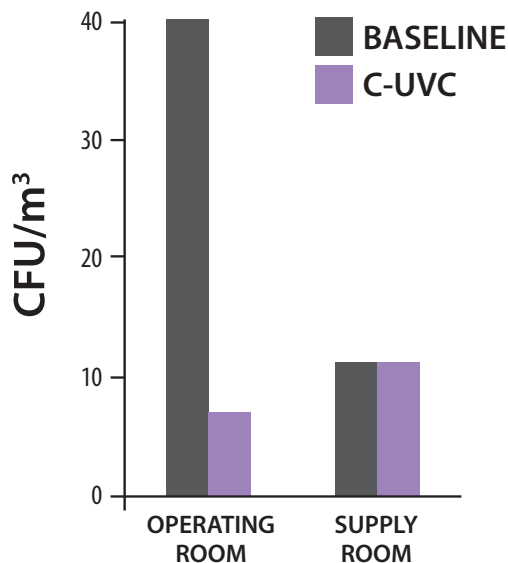
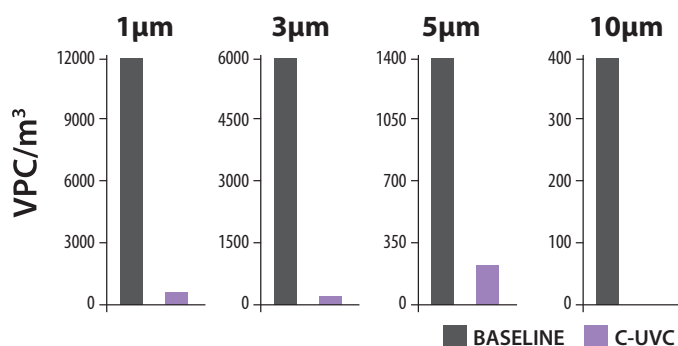


Figure 3. Airborne microbial testing in the OR after CUV/HEPA air recirculation device utilization. Graphical representation of data obtained from microbial sampling experiments. Colony-forming units per cubic meter (CFU/m³), n=10/group.

Quantifications of viable airborne microorganisms were performed with C-UVC device in place. Samples were taken over the 4-hour period with device turned OFF (BASELINE) and ON (C-UVC). Two surrogate measures of microbial air contamination were used to evaluate air quality, the colony-forming units (CFU/m³) and viable particle counts (VPC/m³) respectively. During baseline condition in the OR (device-off; no C-UVC) the average bacterial counts were at 39 CFU/m³. Deployment of the C-UVC device produced significant decrease in air microbial burden as evidenced by sharp reduction in airborne bacteria counts to 7 CFU/m³ (= 82% reduction) (Figure 3). As expected, no improvement in bacterial counts was seen in the sterile processing area supply room due to initially low bacterial levels detected during baseline period (11 CFU/m³).

The reductions in VPC/m³ strongly correlated with CFU data exhibiting 84% reduction in the air content of a 5µm particle, a count considered to reflect microbial contamination due to similarity in size with bacteria (Figure 4)²¹. Overall, over 95% reduction in 1µm, 3µm particles and complete elimination of 10µm particles provide strong evidence for the significant improvements in air quality following C-UVC device deployment in the OR.



| VPC Size | % Baseline level | % Air Reduction |
|----------|------------------|-----------------|
| 1 µm | 4.6 | 95.4 |
| 3 µm | 3.5 | 96.5 |
| 5 µm | 15.8 | 84.1 |
| 10 µm | 0 | 100 |

Figure 4. Airborne viable particle testing in the OR after C-UVC/HEPA air recirculation device utilization. Graphical representation of data obtained from real-time viable particle counting experiments. Data presented as viable particle count per cubic meter (VPC/m³) by particle size. Data displayed in the table reflects percent calculation relative to baseline particle quantity as well as percent of total particle reduction for each particle size.

DISCUSSION

This study is the first report providing evidence of efficacy of AEROBIOTIX patented crystalline UV-C/HEPA microbiocidal filtration technology in dramatically improving air cleanliness. The technology addresses a number of current drawbacks in UV-C-driven “air-scrubbing” systems. Given that biocidal effect of UV-C depends on multiple variables, particularly, UV light intensity and duration of exposure, ABX system maximizes UV-C dosage and inactivation efficiency while maintaining high air volumes. Results of laboratory testing of ABX C-UVC system confirm over 99.9% inactivation efficiency of aerosolized bacteria, spores and viruses at 450 CFM (12.4 m³/min) air flow volume (Fig.2). ABX C-UVC system is supplemented with two filter systems designated to greatly augment C-UVC function by filtering out inactivated bacteria, spores and viruses (Fig.3) as well as viable particulates (Fig. 4)^{4, 17}.

It is well established that contamination of a surgical implant and subsequent development of SSI can occur with a very small bacterial inoculum²¹. Following surgical implantation, host’s immune systems responds to the implanted material rather than its bacterial load. Implant-associated microorganisms slow down their replication and form strong attachment to the implanted material surface²¹. Firm adherence of bacteria to the surface is a crucial prerequisite for biofilm formation and establishment of a chronic, drug-resistant and costly infection. Given the results of the current study, ABX C-UVC technology provides an important innovation in reducing surgical infection risk in procedures involving implants.

The ABX C-UVC device represents a safe, practical and effective method for minimizing the contribution of airborne microorganisms to a surgical infection. The adoption of ABX technology should be strongly considered by hospital decision makers as an adjunct to current engineering controls in ORs performing high risk procedures, in particular, those involving implants²¹.

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