White Paper

Ultraviolet in-room air disinfection and recirculation greatly reduces viable airborne particle concentrations in the operating room setting.
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Objective. To evaluate the effectiveness of a novel in-room air disinfection technology through the use of real-time viable airborne particulate counting.

Methods. Using laser particle fluorescence techniques in real time, airborne viable particle concentrations were obtained before and after air treatment with the in-room C-UVC device in an active hospital operating room setting.

Results. A large number of viable airborne particles were found in the active positive-pressure operating room setting, with an average of 18628 viable particles per cubic meter in the 1.0-10.0 um diameter range. Thirty minutes after activation of the C-UVC device, the number was reduced to 914 viable particles per cubic meter in the same size range. Reductions were consistent across the particle sizes.

Conclusion. The C-UVC device, when employed as an in-room recirculation unit, provides significant reduction in airborne viable particle levels in a hospital environmental setting. The use of laser fluorescence-based techniques to track airborne viable particulate counts is a valuable tool for the rapid assessment of airborne bioload and the evaluation of air germicidal technologies.

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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. A recent report on antimicrobial resistance (CDC, 2014) states, “Without urgent, coordinated action by many stakeholders, the world is headed for a post-antibiotic era, in which common infections and minor injuries which have been treatable for decades can once again kill.” Although several hospital air quality standards have been adopted by various European regulatory bodies (Iudicello 2013), there is currently 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.

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Airborne viable particle measurement

Until recently, the only reliable method for determining airborne microbial concentrations was through direct air sampling and culturing. Air impaction and agar plating devices have been in use for decades for this purpose. Unfortunately, these methods have significant limitations in that they do not permit continuous sampling, use relatively small sample volumes, are expensive/time consuming, and are prone to contamination. Furthermore, standard culturing techniques may not grow fastidious but pathogenic organisms, such as *C. difficile*.

New technologies have been developed which allow for the direct identification of airborne viable particulates. These technologies are based on the principle of laser induced fluorescence (LIF) which utilizes the intrinsic fluorescence of microbial constituents to determine whether a particle is viable. Certain cell metabolites associated with cell viability fluoresce when excited by ultraviolet light. The metabolites most commonly associated with cell viability are tryptophan, NADH, and flavin molecules. In this study, we employed the Biotrak System (TSI, Minneapolis, MN). This device has been cleared by the USFDA and performance validated in accordance with USP Validation of Alternative Microbial Methods. LIF technologies have been rapidly adopted in the clean-room and pharmaceutical industries to maintain assurance of targeted bioload levels.

In this study, we concentrated on viable airborne particles in the 1.0 to 10.0 um size. This particle size range has been most closely identified with pathogenic airborne bacterial populations (Kowalski, 2012). It is important to note that for any given environmental air sample, there will be orders of magnitude higher amounts of viable particles than culturable bacterial colony forming units. Viable particles include a broad population of prokaryotic and eukaryotic cells which cannot be grown in standard culture.

In our previous investigations of the location used for this study, we cultured 39 bacterial CFU/m³ consisting of Gram positive cocci and Gram negative rods. This compares to 18625 viable particles/m³ detected by LIF in the same location under the same conditions. LIF is a much broader measure of overall bioload in the airborne environment than traditional air culturing techniques.

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).
Crystalline–UVC deactivation of airborne microorganisms

**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 and distance, air flow volume and UVC dosage received 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, Miamisburg, 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.

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 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. The Laser Induced Fluorescence (LIF) viable particle counting system

The Laser Induced Fluorescence (LIF) system combines existing particle counting technology with viable detection technology in a single portable instrument package. The BIOTRAK Particle Counter incorporates several technologies enabling it to optically measure total and viable particles.

In the test operating room, the LIF unit 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.
The viability detector incorporates technology based on laser-induced fluorescence (LIF). The basis of viability detection using LIF is the intrinsic fluorescence of microbial constituents. There are certain cellular metabolites associated with cell viability that fluoresce when excited by ultraviolet light. These metabolites include tryptophan, NADH, and the flavin. These metabolites have their own respective excitation and emission curves. The most common excitation source is a 405 nm laser diode. Figure 4 demonstrates the wavelength-dependent excitation and emission bands for riboflavin.

The excitation band for riboflavin is fairly broad, meaning that wavelengths of 300-500 nm will excite the metabolite causing fluorescence. The emission curve shows the emitted fluorescence wavelength. Each of the viability metabolites has its own distinctive LIF excitation and emission curves.
RESULTS

Airborne viable particle levels in operating room setting

Air samples were taken in two locations in an urban midwestern USA hospital setting. The location used was a 5 x 6m active general surgical operating room with standard positive-pressure ventilation. The sampling locations were immediately behind the sterile instrument table. Samples were taken with the C-UVC device in place, but turned off, and then repeated after 30 minutes of the C-UVC device running. In each test modality, air samples were taken every 60 seconds, until the detected particle levels reached a stable equilibrium.

Samples were taken in two groups: operating room active baseline, operating room active after 30 minutes use of C-UVC unit.

<table>
<thead>
<tr>
<th>Viable Particle Size (um)</th>
<th>Baseline Control VPC/m³</th>
<th>C-UVC VPC/m³</th>
<th>VPC Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>11585</td>
<td>530</td>
<td>-11055 (95.4%)</td>
</tr>
<tr>
<td>3.0</td>
<td>5528</td>
<td>192</td>
<td>-5336 (96.5%)</td>
</tr>
<tr>
<td>5.0</td>
<td>1212</td>
<td>192</td>
<td>-1020 (84.2%)</td>
</tr>
<tr>
<td>10.0</td>
<td>303</td>
<td>0</td>
<td>-303 (100%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>18628</strong></td>
<td><strong>914</strong></td>
<td><strong>-17714 (95.1%)</strong></td>
</tr>
</tbody>
</table>

**TABLE 2**: Viable particle count (VPC) baseline and 30 minutes after C-UVC activation.

![Graph of viable particle count (VPC) baseline and 30 minutes after C-UVC activation.](image)

**TABLE 3**: Graph of viable particle count (VPC) baseline and 30 minutes after C-UVC activation.
**CONCLUSION**

Even with appropriate air exchange and positive pressure ventilation, active operating rooms contain a large number of airborne viable particles and airborne bioload. The in-room C-UVC system represents an effective means to dramatically reduce airborne bioload in an active operating room setting. Viable particle counting using LIF technology represents an efficient and accurate means to measure airborne bioload and evaluate air germicidal technologies. The C-UVC system represents an effective method for reducing airborne pathogens in health care settings, and its adoption should be considered in facilities with elevated airborne pathogen levels.

**REFERENCES**


