Food Safety and Security Laboratory

FINAL REPORT

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Evaluation of ActivTek RCI$_{tm}$ Cell for control of *Clostridium difficile* on Stainless Steel Surfaces

Summary

Stainless steel coupons were inoculated with, *Clostridium difficile,* placed in a controlled environmental chamber and exposed to oxidative gases produced by a Radio Catalytic Ionization Cell (RCI) manufactured by ActivTek. The initial inoculum was 5.1 $\log_{10}$ CFU/cm ^{2} and exposure times were 0, 2, 4, 8, 12 and 24 h. Reductions in *Clostridium difficile* were observed at all exposure times.
The 24 hour exposure to RCI resulted in a 2.7 log CFU/cm^2 reduction. Background/ambient ozone and hydrogen peroxide levels were measured in the chamber prior to and after activating the RCI Cell.

Introduction:

The term nosocomial infection refers to an infection that is acquired in the hospital or a health care facility (Chotani et al., 2004). Environmental contamination has produced devastating consequences in these facilities, resulting in the morbidity and mortality of tens of thousands of patients every year.
Persons who visit hospitals, nursing homes, or health clinics have a risk of acquiring an infection as a result of their stay (Tilton, 2003). It is estimated that approximately one patient in ten acquires an infection as a result of an extended visit in one of these health care facilities (Tilton, 2003). Nosocomial acquired infections are responsible for approximately 100,000 deaths with an annual cost approaching $29 billion (Kohn et al., 1999). One of microbial pathogens responsible for nosocomial infections and of growing concern in health care environments is *Clostridium difficile* (Johnston and Bryce, 2009).

Nosocomial infections have a number of potential causes that promote the spread of disease. Common health care surfaces such as countertops, bedding, bedpans, and medical devices can all be used to transmit and spread disease from one person to another (Hota, 2004). Under hectic and stressful conditions, these surfaces can become easily contaminated, often by overworked employees. Cutbacks in staffing at health care facilities due to budget constraints, has placed a greater burden on health care facilities to find ways to remediate contaminates with limited resources (Chotani et al., 2004). Older and poorly designed buildings may harbor contaminates that are not easily eliminated using conventional disinfection methods. Studies have shown that microorganisms such as *Staphylococcus aureus* and *Candida albicans* survive in environmental reservoirs found in health care facilities (Hota, 2004).

The World Health Organization reported that 40% of all commercial buildings pose a serious health hazard due to indoor air pollution.

Historically, UV light has been used in health care and other indoor air environments to provide continuous decontamination. UV light is a "line of sight" technology and does not provide the most effective means of control. Ideally, a system for continuous decontamination would produce antimicrobials which reduce contamination on surfaces and in the air. The Radio Catalytic Ionization Cell produces oxidative gases that inactivate microorganisms in the air and on surfaces. These gases can reach all surfaces in health care and related environments.

The purpose of this study was to evaluate the efficacy of a Radio Catalytic Ionization Cell (RCI) in reducing populations of *Clostridium difficile* on stainless steel surfaces.

Experimental Design:

Stainless Steel Coupons inoculated with *Clostridium difficile* – ATCC # 17858 were placed into controlled environmental chamber (Terra Universal – Anaheim, CA) and subjected to treatment with a Radio Catalytic Ionization Cell (ActivTek) for periods of 0, 2, 4, 8, 12 and 24 hours. Humidity levels were controlled at 45-50%. In the chamber, humidity levels were controlled at 45 – 50%. A control study was conducted in which inoculated coupons were placed in an identical chamber without the RCI cell. Levels of Ozone and Vaporized Hydrogen Peroxide were measured using Draeger tubes.

Materials and Methods

Preparation of Cultures:

Clostridium difficile (ATCC # 17858) was used for this study. Bacterial cultures were grown in Tryptic Soy Broth (TSB; Difco Laboratories, Detroit, MI) and YM broth (Difco Laboratories, Detroit, MI) respectively to mid-exponential phase followed by a wash and re-suspension in 0.1% peptone water (PW).

Preparation of environmental surfaces:

Environmental surfaces were simulated using coupons made of stainless steel (6.4 x 1.9 cm). Before treatment and inoculation, all coupons were cleaned using Fisherbrand Sparkleen* detergent (pH 9.5 – 10 in solution; Fisher Scientific). Stainless steel coupons were sterilized by autoclaving.

Preparation of Samples and RCI Treatment:

The coupons tested were dipped per microbial inoculum and vortex 15 sec optimizing microbial dispersion. Sterile binder clips were used to hang each coupon from a cooling rack for 1 h until dryness in a laminar flow biohazard air hood. The initial microbial population attached to the stainless steel coupons was targeted at 10^6 CFU/ sq. cm. The actual initial inoculum was 5.1 $\log_{10}$ CFU/cm ^{2}. The inoculated stainless steel coupons were transferred to a controlled airflow Biological Safety Cabinet (Nuaire) at 26°C, 46 % relative humidity (ambient conditions), and exposed to the oxidative gases produced by the RCI cell for periods of 0, 2, 4, 8, 12 and 24 hours. Inoculated controls were prepared and placed in the test cabinet for 0, 2, 4, 8, 12 and 24 hours without RCI treatment. Ozone levels in the test cabinet were monitored throughout the study (Model 500, Aeroqual, New Zealand) Ozone and Hydrogen Peroxide levels were also measured hourly using Draeger Tubes.

Sampling:

At the end of the designated holding time, coupons were placed into 30 ml of 0.1% peptone water and vortexed for 30 sec; samples were serially diluted and plated onto Tryptic Soy Agar (TSA; Difco Laboratories, Detroit, MI) for bacteria recovery. The colony-forming units per square centimeter (CFU/cm^2) were estimated after incubating at 35^oC for 24h.

Results and Discussion:

The exposure to oxidative gases produced by the RCI cell, including ozone and vaporized hydrogen peroxide resulted in reductions in *Clostridium difficile* 1.7 log CFU/cm^2 after two hours, 2.1 log CFU/cm^2 after 4 hours, 2.4 log CFU/cm^2 after 8 hours, 2.5 log CFU/cm^2 after 12 hours and 2.7 log CFU/cm^2 after 24 hours. The reductions in the control samples were less than 0.5 log CFU/cm^2 after 24 hours in the chamber without the RCI cell.

Background levels of ozone were measured at 0.03 ppm in the chamber prior to activating the RCI cell. During the experiment, ozone levels ranged from 0.04 ppm to 0.06 ppm. Levels of vaporized Hydrogen Peroxide in the test chamber ranged from 0.019 to 0.035 PPM.

This experiment demonstrated the effectiveness of the RCI cell at reducing populations of *Clostridium difficile* on stainless steel surfaces.

Based on this initial study, the technology has applications for controlling *Clostridium difficile* on surfaces in health care environments. It could act as an important adjunct to hand sanitation as a means of preventing hospital acquired *Clostridium difficile* infections. Future research will evaluate this technology on other types of surfaces and in simulated room environments.