Target: **Sick Building Syndrome**
Indoor Air Pollution

Test Lab: Biosecurity Laboratory Food Safety Systems LLC

Test: Evaluation of the Efficacy of CIMR® at Reducing Populations of Methicillin Resistant Staphylococcus aureus and Listeria monocytogenes on Stainless Steel Surfaces

Issues:
WHO (https://www.who.int/) reports 40% of all public buildings pose serious health hazards due to indoor pollution This issue, named Sick Building Syndrome is a concern for homes, schools, offices, and health care facilities.

Negatives:
- Appearance
- Odor

Infections Caused by:
- Mycotoxins
- Staphylococcus aureus
- Listeria monocytogenes

Outcome
- Irritation
- Allergic Reactions
- Disease

Benefit
CIMR® generated dry hydrogen peroxide (DHP) reaches all air and surfaces to address lapses in cleaning routines and unreachable surfaces to end Sick Building Syndrome.

Test Outcome Summary:
CIMR® technology is effective in reducing populations of Staphylococcus aureus and Listeria monocytogenes on stainless steel surfaces without producing ozone.

Attached
Evaluation of the Efficacy of a Continuous Infectious Microbial Reduction system at Reducing Populations of Methicillin Resistant Staphylococcus aureus and Listeria monocytogenes on Stainless Steel Surfaces. Biosecurity Laboratory Food Safety Systems, LLC January 6, 2010.

Evaluation of the Efficacy of a Continuous Infectious Microbial Reduction system at Reducing Populations of Methicillin Resistant *Staphylococcus aureus* and *Listeria monocytogenes* on Stainless Steel Surfaces.

Biosecurity Laboratory
Food Safety Systems, LLC
January 6, 2010

SUMMARY

Stainless steel coupons were inoculated with Methicillin resistant *Staphylococcus aureus* and *Listeria monocytogenes*, placed in a controlled environmental chamber and exposed to Vaporized Hydrogen Peroxide by a Continuous Infectious Microbial Reduction (CIMR) System. The initial inoculum was 6.6 log,0 CFU/cm2 for Methicillin resistant *Staphylococcus* and 6.2 log10 CFU/cm2 for *Listeria monocytogenes*. The exposure times were 0, 2, 4, 8, and 24 h. Levels of Hydrogen peroxide and background/ambient ozone levels were measured in the chamber prior to and after activating the CIMR system.

The exposure to the Vaporized Hydrogen peroxide produced by the CIMR system for a 2h period resulted in reductions in Methicillin resistant *Staphylococcus aureus* and *Listeria monocytogenes* of 2.0 Log,0 CFU/cm2. Four hours of exposure resulted in log reductions for Methicillin resistant *Staphylococcus aureus* and *Listeria monocytogenes* > 2.7 Log,0 CFU/cm2.

Eight hours of exposure resulted in reductions of Methicillin resistant *Staphylococcus aureus* and *Listeria monocytogenes* > 3.5 Log,0 CFU/cm2 were observed for both pathogens.

INTRODUCTION

Microbial contamination of indoor air and affected surfaces represents a major public health problem and a potential source for sick-building syndrome. For example, certain species of mold and bacteria may cause health concerns in homes, schools, offices and health care facilities (Hota, 2004). In addition to being unattractive to see and smell, mold also gives off spores and mycotoxins that cause irritation, allergic reactions, or disease in immune-compromised individuals (Bahnfleth et al., 2005).

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 each 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).

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 Continuous Infectious Microbial Reduction (CIMR) system produces low levels of Vaporized Hydrogen peroxide that inactivates microorganisms in the air and on surfaces. This antimicrobial gas can reach all surfaces in health care and related environments.

The purpose of this study was to evaluate the efficacy of the Continuous Infectious Microbial Reduction (CIMR) system which is designed to produce gas phase hydrogen peroxide at reducing populations of Methicillin Resistant *Staphylococcus aureus* and *Listeria monocytogenes* on stainless steel surfaces.

MATERIALS AND METHODS

Preparation of Cultures:

Methicillin-resistant *Staphylococcus aureus* (ATCC # 33591) and *Listeria monocytogens* (KSU #56 & #70) were used for this study. Bacterial species were independently 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). The microbial cultures were combined by specie type to ca. 10^8 CFU/ml.

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 CIMR® 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 were approximately 10^7 Log m CFU/cm^2 for Methicillin Resistant *Staphylococcus aureus* and 6.2 Log m CFU/cm^2 for *Listeria monocytogenes*). 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 controls were prepared and placed in the test cabinet for 2, 4, 8 and 24 hours without CIMR® treatment. Vaporized Hydrogen Peroxide Ozone levels in the test cabinet were measured using Draeger tubes. Ambient ozone levels were monitored throughout the study (Model 500, Aeroqual, new Zealand).

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°C for 24 h.

Vaporized Hydrogen peroxide produced by the CIMR® System for periods of 2, 4, 8 and 24 hours.

Time of Exposure	CIMR® Treated Samples Methicillin Resistant *Staphylococcus aureus* Log0 CFU/cm2	CIMR® Treated Samples – *Listeria monocytogenes* Log0 CFU/cm2	Control Log0 CFU/cm2
0 Time	6.60	6.20	6.70
2 Hours	4.40	4.10	6.65
4 Hours	3.80	3.45	6.70
8 hours	2.80	3.10	6.70
24 Hours	2.50	2.15	6.65

RESULTS AND DISCUSSION

Table 1 shows the log, CFU/sq. cm. reductions of Methicillin-resistant *Staphylococcus aureus* and *Listeria monocytogenes* on stainless steel surfaces associated with the CIMR treatment as compared to controls that received no treatment.

Ozone levels were measured in the test chamber at 0.001 - 0.002 ppm. The ambient level of ozone in the control study was measured at 0.002 ppm. Levels of vaporized Hydrogen peroxide in the chamber ranged from 0.04 -
0.08 ppm. All of these levels are well below OSHA limits for continuous interaction.

Table 1: Population (log10 CFU / sq. cm) of Methicillin resistant *Staphylococcus aureus* and *Listeria monocy- togenes* on Stainless Steel surfaces observed after 0, 2, 4, 8 and 24 h of exposure to the Continuous Infec- tious Microbial Reduction (CIMR®) system.

Based on the results of this study, the Continuous Infectious Microbial Reduction (CIMR®) system has the potential to reduce sources of microbial contamination in health care and other indoor air environments. This technology is effective at reducing populations of Methicillin resistant *Staphylococcus aureus* and *Listeria monocytogenes* on stainless steel surfaces. The active antimicrobial in the CIMR® System is Vapor- ized Hydrogen Peroxide. The system does not produce measurable levels of ozone.

REFERENCES

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