Target: **Swine Flu (H1N1)**
Test Lab: Kansas State University
Food Safety and Security

Test: Evaluation of CIMR® for Inactivation of Swine Flu on Stainless Steel Reducing Populations Swine Flu (H1N1) on Stainless Steel Surfaces

Issue:
HiN1 outbreak was sufficeint to trigger the CDC Emergency Operations Center to coordinate the public health response.

Negatives:
Rapid Spread – Triggered the Pandemic Plan

Infections Caused by:
Virus

Outcome
Flu-Like Symptoms

Benefit
CIMR® effective gas phase hydrogen peroxide reaches all air and surfaces to address lapses in cleaning routines and unreachable surface environmental reservoirs

Food Safety and Security

October 4, 2009

Research Report – Evaluation of Continuous Infectious Microbial Reduction System (CIMR) for the Inactivation of Influenza A H1N1 on Stainless Steel Surfaces

Background

Novel influenza A (H1N1) is a new flu virus of swine origin that first caused illness in Mexico and the United States in March and April, 2009. H1N1 is an acute and highly contagious respiratory virus similar to seasonal flu but affecting a younger age group. Less immunity exists to this novel strain of the flu than to seasonal flu. H1N1 influenza in humans can vary in severity from mild to severe. The H1N1 virus is thought to spread in the same way seasonal flu is spread: from person to person through droplets produced by coughs and sneezes, or from touching contaminated surfaces and then touching your mouth, nose or eyes. Novel H1N1 infection has been reported to cause a wide range of flu-like symptoms, including fever, cough, sore throat, body aches, headache, chills and fatigue. In addition, many people also have reported nausea, vomiting and/or diarrhea. The virus can stay alive on surfaces and your hands and body for at least two hours.

The first novel H1N1 patient in the United States was confirmed by laboratory testing at CDC on April 15, 2009. The second patient was confirmed on April 17, 2009. It was quickly determined that the virus was spreading from person-to-person. On April 22, CDC activated its Emergency Operations Center to better coordinate the public health response. On April 26, 2009, the United States Government declared a public health emergency and has been actively and aggressively **implementing the nation's pandemic response plan**

By June 19, 2009, all 50 states in the United States, the District of Columbia, Puerto Rico, and the U.S. Virgin Islands have reported novel H1N1 infection. While nationwide U.S. influenza

surveillance systems indicate that overall influenza activity is decreasing in the country at this time, novel H1N1 outbreaks are ongoing in parts of the U.S., in some cases with intense activity.

Preliminary Experiment – Inactivation of Influenza A H1N1 on Inoculated Stainless Steel Surfaces using PHI technology

The Continuous Infectious Microbial Reduction System (CIMR) is a UV based technology designed to produce low levels of vaporized Hydrogen Peroxide as an environmental antimicrobial treatment. The system does not produce ozone. The antimicrobial effect is due to the germicidal properties of UV and vaporized H2O2.

The effect of the CIMR system on the inactivation of Influenza A *H1N1* (ATCC # VR-333) was evaluated in this study. The procedures for maintaining the virus culture and enumerating the virus prior to and after treatment were obtained from Dr. Rick Falkenberg – FSPT. The virus culture was maintained on ATCC complete growth medium and minimum **essential medium (ATCC, Manassas, VA., USA) with 2 µM L**-glutamine and Earle's BSS adjusted to contain 1.5 g/L **sodium bicarbonate, 0.1 µM non-essential amino acids, and 1.0 µM** sodium pyruvate, 90%; fetal bovine serum, 10% and cultured in Trypticase Soy Agar with added; sodium bicarbonate, non-essential amino acids, and combination of sodium pyruvate and fetal bovine serum, in aerobic growth conditions at 37.0°C and *Influenza A* at 33-35° C.1 Cells from both of the above (approx. 1x107 CFU/ml) from a 24 hour static culture incubated at 37.0°C and *Influenza A* at 33-35°C were used to inoculate various 5 cm x 3 cm stainless steel coupons. The inoculum suspensions were enumerated by surface plating in duplicate samples on TSA after serial dilution in 0.1% peptone solution. The plates were incubated for 24 hour at 37.0°C.

A 100 µl droplet from the initial inoculum suspension of each of the bacteria/viruses was used to inoculate the external surface (6.3 cm x 1.8 cm) on # 8 stainless steel coupons. This resulted in a final inoculum level of approximately 7.0-log CFU/5 g sample. The inoculated samples were air dried for 1 hour at 22.0°C prior to treatment with the no-ozone producing cell. The 1 hour drying allows the inoculated cells to attach to the surface host and minimize the growth of inoculated cells during drying. Four stainless steel coupons were used for each sampling time.

A biocontainment chamber (BL 2 Enhanced) was equipped with a CIMR Cell and allowed to equilibrate for a period of two hours prior to placement of 12 inoculated coupons inside the chamber. The effect of the no-ozone producing cell treatment was measured at 0, 2, 4, 6, 8, 12 and 24 hours. A control study was conducted in the same chamber without the presence of the CIMR cell. Temperature, relative humidity, and ambient Ozone levels and Hydrogen Peroxide levels were monitored in the chamber.

After treatment, each of the 5 cm x 3 cm coupons were transferred into a 400 ml stomacher bag (Fisher Scientific Inc., PA., USA) combined with 50 ml sterile 0.1% peptone

surveillance systems indicate that overall influenza activity is decreasing in the country at this time, novel H1N1 outbreaks are ongoing in parts of the U.S., in some cases with intense activity.

Preliminary Experiment – Inactivation of Influenza A H1N1 on Inoculated Stainless Steel Surfaces using PHI technology

The Continuous Infectious Microbial Reduction System (CIMR) is a UV based technology designed to produce low levels of vaporized Hydrogen Peroxide as an environmental antimicrobial treatment. The system does not produce ozone. The antimicrobial effect is due to the germicidal properties of UV and vaporized H2O2.

The effect of the CIMR system on the inactivation of Influenza A *H1N1* (ATCC # VR-333) was evaluated in this study. The procedures for maintaining the virus culture and enumerating the virus prior to and after treatment were obtained from Dr. Rick Falkenberg – FSPT. The virus culture was maintained on ATCC complete growth medium and minimum **essential medium (ATCC, Manassas, VA., USA) with 2 µM** L-glutamine and Earle's BSS adjusted to contain 1.5 g/L **sodium bicarbonate, 0.1 µM non-essential amino acids, and 1.0 µM** sodium pyruvate, 90%; fetal bovine serum, 10% and cultured in Trypticase Soy Agar with added; sodium bicarbonate, non-essential amino acids, and combination of sodium pyruvate and fetal bovine serum, in aerobic growth conditions at 37.0°C and *Influenza A* at 33-35° C.1 Cells from both of the above (approx. 1x107 CFU/ml) from a 24 hour static culture incubated at 37.0°C and *Influenza A* at 33-35°C were used to inoculate various 5 cm x 3 cm stainless steel coupons. The inoculum suspensions were enumerated by surface plating in duplicate samples on TSA after serial dilution in 0.1% peptone solution. The plates were incubated for 24 hour at 37.0°C.

A 100 µl droplet from the initial inoculum suspension of each of the bacteria/viruses was used to inoculate the external surface (6.3 cm x 1.8 cm) on # 8 stainless steel coupons. This resulted in a final inoculum level of approximately 7.0-log CFU/5 g sample. The inoculated samples were air dried for 1 hour at 22.0°C prior to treatment with the no-ozone producing cell. The 1 hour drying allows the inoculated cells to attach to the surface host and minimize the growth of inoculated cells during drying. Four stainless steel coupons were used for each sampling time.

A biocontainment chamber (BL 2 Enhanced) was equipped with a CIMR Cell and allowed to equilibrate for a period of two hours prior to placement of 12 inoculated coupons inside the chamber. The effect of the no-ozone producing cell treatment was measured at 0, 2, 4, 6, 8, 12 and 24 hours. A control study was conducted in the same chamber without the presence of the CIMR cell. Temperature, relative humidity, and ambient Ozone levels and Hydrogen Peroxide levels were monitored in the chamber.

After treatment, each of the 5 cm x 3 cm coupons were transferred into a 400 ml stomacher bag (Fisher Scientific Inc., PA., USA) combined with 50 ml sterile 0.1% peptone

Cell ranged from 0.05 – 0.08 PPM. No vaporized H2O2 was measured in the control chamber. The relative humidity ranged from 47 – 59% and the temperature from 70 – 72 degrees F in both the control and treated chambers.

This preliminary study indicates that the CIMR Cell was effective at inactivating *Influenza A* H1N1 virus on inoculated stainless coupons under the conditions of these tests. Additional testing is recommended to evaluate other strains of the virus and other environmental surfaces and application parameters. Studies to better identify the mode of action for this technology are also recommended.

This is a progress report on the evaluation of the CIMR Cell for inactivating Influenza H1N1 on environmental surfaces. Additional research is planned to fully evaluate the effect of this technology on Influenza H1N1 under controlled laboratory conditions.

The results of this study are preliminary and should not be used to position the CIMR Cell as a medical device or as a means of reducing the risk of H1N1 infections.

It is recommended that you meet with FDA officials to obtain guidance on future research and how best to position this technology to consumers.