



October 14, 2009

To: Joseph Urso – Aerus - Electrolux / ActivTek Environmental  
From: James Marsden, Ph.D.  
Subject: Inactivation of *Influenza A H1N1* using Radiant Catalytic Ionization (RCI™)

## **Interim Progress Report**

This is an interim progress report on the evaluation of the RCI™ system for inactivating Influenza H1N1 on environmental surfaces. Additional research is planned to fully evaluate the effect of the reactive oxygen species (ROS) produced by the RCI™ system on Influenza H1N1 under controlled laboratory conditions.

I recommend that Aerus Corporation meet with FDA officials to obtain guidance on future research and how best to position the RCI technology to consumers.

### **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**

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  $\mu$ M L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1  $\mu$ M non-essential amino acids, and 1.0  $\mu$ M 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. Cells from both of the above (approx. 1x10<sup>7</sup> 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  $\mu$ l 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 RCI™ treatment being initiated. 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 was equipped with an RCI™ cell (obtained from Aerus Corporation) and allowed to equilibrate for a period of two hours prior to placement of 12 inoculated coupons inside the chamber. The effect of the RCI™ treatment was measured at 0, 1, 2, 4, 6, 8, 12 and 24 hours. A control study was conducted in the same chamber without the presence of the RCI 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 solution, and then blended with a AES Easy Mix Stomacher (AES Laboratories, Princeton, NJ., USA) for 2 min at normal speed. Wash fluid was serially diluted, followed by surface plating for enumeration. A centrifugation method was used to recover low populations of ROS injured bacteria and viruses. The centrifugation method (Mossel and others 1991) was modified and used to concentrate the bacterial and virus populations in the wash fluid so that less than 250 CFU/ml of bacteria can be enumerated by the surface plating.

## Results and Discussion

**Table 1. Average recoveries (Log CFU/cm<sup>2</sup>) of *Influenza A H1N1* on inoculated stainless steel coupons treated using an RCI™ Cell for periods of 1, 2, 4, 6, 8, 12 and 24 Hours**

Sample	<i>Influenza A H1N1</i> Treated with RCI Cell	<i>Influenza A H1N1</i> Control
Initial – 0 Time	6.8	6.9
1 Hour	4.1	6.7
2 Hours	3.4	6.4
4 Hours	1.2	6.1
6 Hours	BDL	5.5
8 Hours	BDL	5.7
12 Hours	BDL	5.7
24 Hours	BDL	5.7

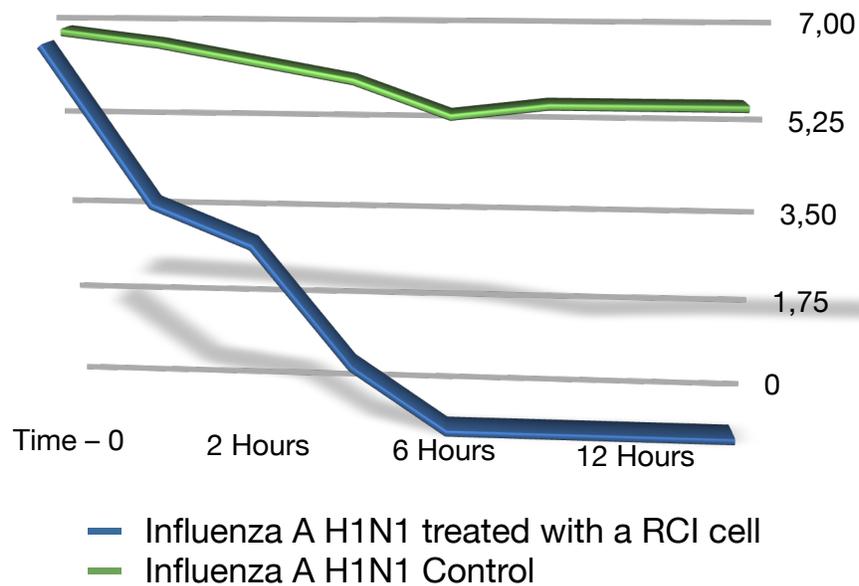


Table 1 summarizes the results of the preliminary study. The study demonstrated the effectiveness of the reactive oxygen species produced by the RCI™ cell in the inactivation of Influenza A – H1N1. After 6 hours of treatment, levels of the H1N1 virus on inoculated stainless steel coupons were below the detection limit. No recovery was observed at 8, 12, or 24 hours. The ambient ozone levels in the chamber ranged from 0.02 – 0.04 PPM. Levels of vaporized Hydrogen Peroxide ranged from 0.06 – 0.09 PPM. The relative humidity ranged from 45 – 57% and the temperature from 70 – 73 degrees F.

Based on the results of this preliminary study, it appears that the reactive oxygen species produced by the RCI™ cell are 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.