Inactivation of Picornaviruses using EcoQuest Radiant Catalytic Ionization

Introduction

The viral family *Picornaviridae*, which includes Hepatitis A virus, is characterized as including viruses which are non-enveloped with single stranded positive sensed RNA genomes known to be very resistant to physical and chemical means of inactivation (1). Hepatitis A virus (HAV) is known to spread predominantly through contaminated drinking water and food sources. During an outbreak, HAV can contaminate and remain infectious on various environmental surfaces. Standard disinfection processes are usually not effective for inactivating this virus due to the high resistance. Stringent disinfection and sanitation procedures of an affected environment in combination with stringent employee hygiene procedures are recommended for preventing HAV outbreaks in the food industry.

The purpose of this study was to validate the complete inactivation of HAV using a closely related enterovirus as a surrogate virus following exposure to the EcoQuest Radiant Catalytic Ionization Cell™ (RCI-Cell™) system. The RCI-Cell™ system is an advanced oxidation tool which combines UV inactivation in the presence of hydroxical radicals so that synergy between two highly effective inactivation technologies occurs. Efficacy of this technology was determined by inoculating stainless steel coupons with virus and allowing inoculum to dry. Control samples were taken at this time and then coupons were exposed to the RCI-Cell™ for various times. Non-treated control samples were also evaluated to compare the reduction in infectious virus titer in a 24 hour period in controlled environments. Efficacy was determined by measuring any reduction in infectious titer using end-point titration in tissue culture for treated and coupons compared to non-treated positive control coupons.

Materials and Methods

Virus and cells. BEV-2 (ATCC VR-754, Manassas, VA) was propagated in Madin Darby Bovine Kidney (MDBK, ATCC CCL-22) cells. MDBK cells were propagated in minimum essential medium (Eagle) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate with 7% fetal bovine serum supplemented with 2.5 mg/L amphotericin B, 0.67 g/L streptomycin, and 0.3 g/L penicillin. MDBK cells were infected with BEV-2 without the addition of 10% FBS. Inoculum titer was assessed using tissue culture infective dose 50, $TCID_{50}$ and calculated by the Reed-Muench method (2).

Virus inactivation. Type 302 stainless steel (McMasterCarr, Altanta, GA) coupons (2 x 10 cm^2, thickness 0.8 mm) were sterilized by autoclaving for 15 min at 121^o C. In a biosafety class II cabinet, 100 µl of BEV-2 was added to each test coupon and spread to cover the entire surface using the pipette tip and allowed to dry completely for approximately 20 minutes. Then, the inoculated coupons were placed into a sterile transport container and transported to the test chamber. The test coupons were then placed within the test chamber and exposed to the RCI-Cell™ system for a 24 hour period. As non-treatment controls, test coupons were also prepared as described above and added to a test chamber which would not be exposed to the RCI-Cell™ system for a 24 hour period. One inoculated coupon was removed initially for both the RCI-Cell™ chamber and the non-treated control chamber to be used as an initial measure of starting virus titer. The RCI-Cell™ device was then turned on and samples were removed from both testing chambers after 2, 4, 8, 12, and 24 hours by removing a test coupon and preparing it for virus recovery as described below.

Virus Recovery. BEV-2 was recovered from the stainless steel surfaces by adding the test coupon to a sterile 50 ml conical vial containing 5 ml of infectivity media. Tubes were then vortexed for 1 minute to release virus from the inoculuated coupon. Samples were titrated by infecting confluent MDBK wells in a 96-well format using $TCID_{50}$ endpoint titration. Plates were incubated at 37° C, 5% CO_2 for 48 hours. Cytopathic effect (CPE) typical for BEV-2 was determined for each well and viral titers were reported as $TCID_{50}$/ml.

Results

The average amount of BEV-2 virus recovered from the control stainless steel coupons in all experiments was 6.00 log_{10} $TCID_{50}$/ml. Following treatment with the RHI-Cell™, the average log reductions of infectious BEV-2 virus were 3.87 and 4.87 log_{10} $TCID_{50}$/ml following 2 and 4 hour treatments (Figure 1). No infectious BEV-2 was recovered following the 8, 12, or 24 hour exposure to the RCI-Cell™ system. Infectious BEV-2 was recovered from all coupons sampled following 2, 4, 8, 12, or 24 hour periods in the chamber not receiving the RCI-Cell™ treatment (Figure 1)

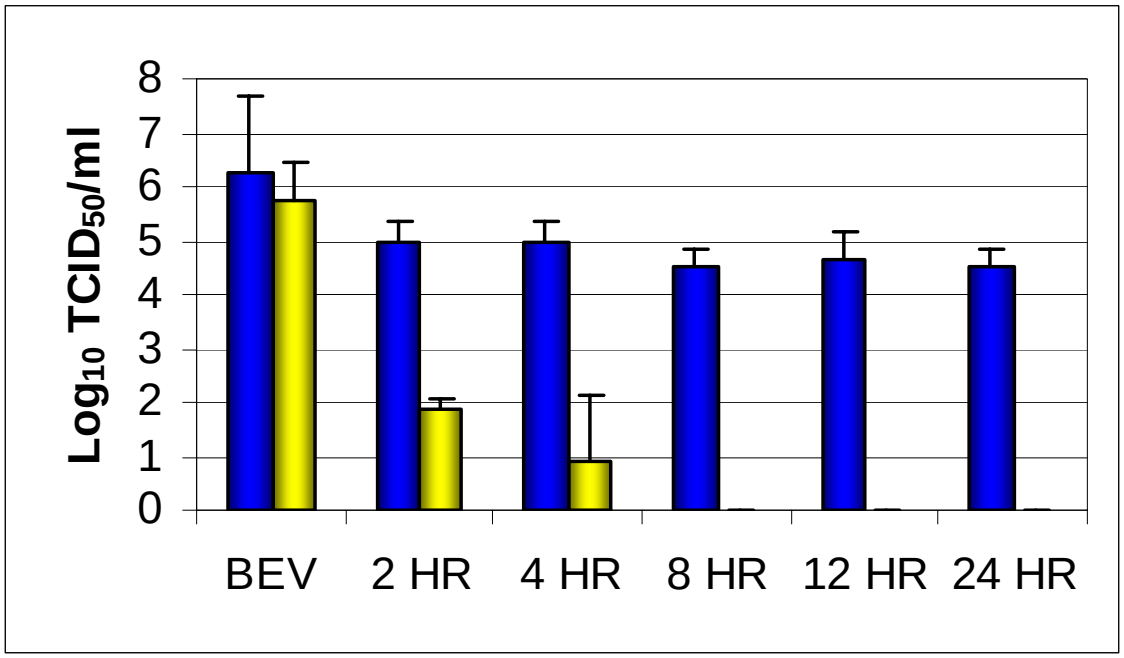

Figure 1: Infectious BEV recovered and reported as $TCID_{50}$/ml in MDBK cells following treatment to RCI-Cell™ (yellow bars) or no treatment to RCI-Cell™ (blue bars) following 2, 4, 8, 12, or 24 hour sampling times.

Reference:

1. **Hollinger, F. B., and S. U. Emerson.** 2003. Hepatitis A Virus, p. 799-840. *In* D. M. Knipe and P. M. Howley (ed.), Fields Virology, Fourth ed, vol. 1. Lippincott Williams & Wilkins, Philadelphia.
2. **Reed, L. J., and H. Muench.** 1932. A simple method for estimating 50% endpoints. Am J Hyg **27:**493-497.