Evaluation of the Efficacy of CIMR® verses Ecoquest's low ozone System in Reducing Murine Norovirus Titers
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Introduction

Members of the genus *Norovirus* are nonenveloped viruses with a linear, positive-sense, single-stranded RNA genome. Noroviruses are in the family *Caliciviridae*, which also includes the genera *Sapovirus*, *Lagovirus*, and *Vesivirus*. Formerly known as "Norwalk-like viruses" or "small round structured viruses," noroviruses cause acute gastroenteritis in humans, typically lasting 24 to 48 h, and infect people of all ages.

Recently, the first murine norovirus, was isolated from mice. This newly described pathogen of mice can be grown in cell culture, providing the first example of a norovirus that can be cultured in vitro. In these studies, the efficacy of CIMR® verses Ecoquest's low ozone platform has been evaluated against Murine norovirus (MNV), as a representative of the *Caliciviridae* family, using an in vitro culture system.

Experimental Design

Virus stock and culture

MNV-4 used in this study was maintained in RAW267.4 cells, a murine macrophage cell line. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. The virus was propagated, concentrated, and purified. Purified viral stocks were titered via plaque titration. Viral stocks were stored in a -80ºC freezer.

Preparation of surfaces

To assess efficacy of the CIMR® and EcoQuest's Low-Oxidation (ozone) decontamination systems for reducing MNV titers, virus-contaminated surfaces were exposed to the decontamination system for various time periods. Decontamination was evaluated on three types of surfaces: Stainless steel, carpet and cloth. Stainless steel cassettes measuring 1.5 inches by 1.25 inches were used as the stainless steel surface. Samples of carpet and cloth were cut to 1 inch squares. Prior to the experiment, all surfaces were sterilized in a steam autoclave. To contaminate the surfaces, 200 µL of MNV viral stock (1×10^7 PFU/ml) was pipetted onto the center of each surface, covering ~ 1-2 cm. The surfaces were allowed to air dry in a type II biosafety cabinet. At the end of the hour, the zero time point control samples were collected and the remaining inoculated surfaces were placed in a humidified 28^0C incubator for either low oxidation treatment or ozone free treatment. A set of four inoculated samples for each surface

After the specific times of exposure had been reached, the surfaces were immersed into 10 mls DMEM containing 10ug/ml ciprofloxacin. Stainless steel surfaces were scraped with a sterile cell scraper to remove virus from the cassette surface. Carpet and cloth samples were placed in a sterile bag and homogenized for 1 minute in a Stomacher Lab Blender. Samples were removed from the bag and placed in a 15 ml conical centrifuge tube and spun at 1000 x g for 10 minutes to remove residual carpet and cloth fragments. As controls, each surface was inoculated with an equivalent amount of virus and placed in a 28^0 incubator without treatment to serve as the 24 hour untreated controls. Each of the samples subjected to the decontamination system was tested in quadruplicate at each time point. Controls were also tested in quadruplicate. Data are expressed as an average of all data points.

Calculation of viral titer and viral reduction

After neutralization of the disinfectant in specified volumes of DMEM, stainless steel surfaces were thoroughly scraped with a sterile cell scraper to elute the virus into the DMEM. Carpet and cloth samples were suspended in sterile DMEM and homogenized using a Stomacher blender to release the virus. The viral titer of each eluate was determined

inoculating cell cultures with serial ten-fold dilutions of the eluates, and calculating the tissue culture infective dose 50 (TCID50) based on observations of characteristic cytopathic effects associated with MNV. The final titer was calculated by averaging the individual titers calculated from each replicate and the decrease in viral titer was then calculated.

Results

The following tables summarize the results of these experiments.

Table 1. Reduction in Murine Norovirus Titer Following CIMR® Treatment

	Stainless steel			Carpet			Cloth		
Treatment time	Untreated ($TCID_{50}$/ml)	Treated ($TCID_{50}$/ml)	Percent decrease from t=0	Untreated ($TCID_{50}$/ml)	Treated ($TCID_{50}$/ml)	Percent decrease from t=0	Untreated ($TCID_{50}$/ml)	Treated ($TCID_{50}$/ml)	Percent decrease from t=0
0 hrs	1.2×10^6			1.6×10^6			4.0×10^5		
2 hrs		3.5×10^5	70.8		3.4×10^5	78.8		2.1×10^4	94.8
4 hrs		3.6×10^4	97.0		7.5×10^4	95.3		1.7×10^4	95.8
6 hrs		1×10^2	99.9		$<1 \times 10^3$	>99.9		$<1 \times 10^3$	>99.8
24 hrs	1×10^3	1×10^2	99.9	$<1 \times 10^3$	$<1 \times 10^3$	>99.9	8.6×10^2	$<1 \times 10^3$	>99.8

Figure 1. Survival of MNV following CIMR® Treatment

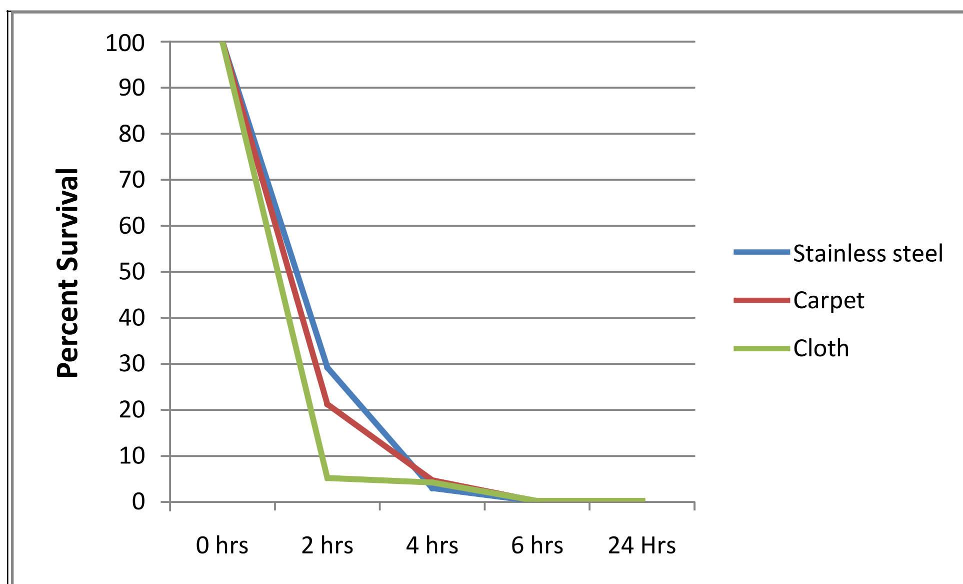
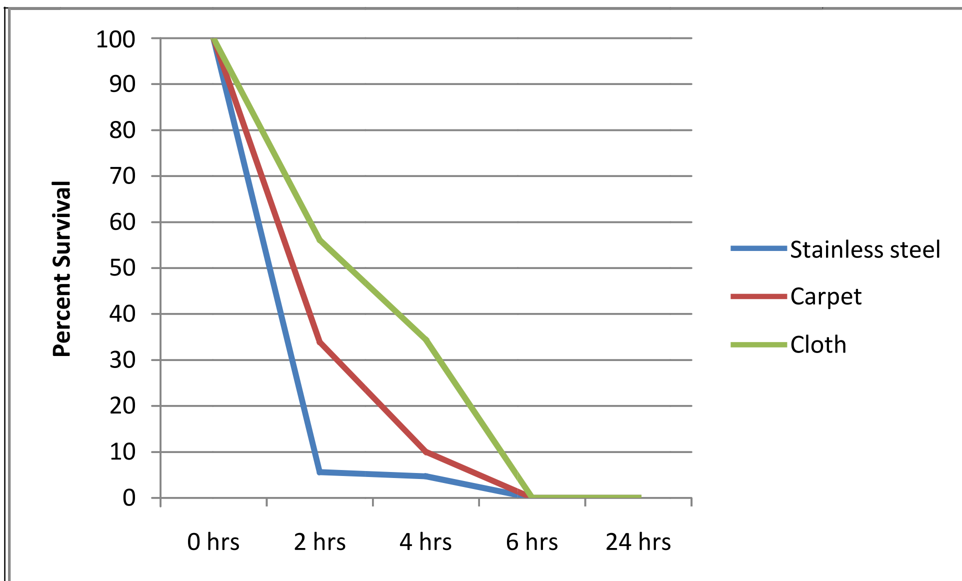


Table 2. Survival of Murine Norovirus following Low-oxidation(ozone) Treatment

	Stainless steel			Carpet			Cloth		
Treatment time	Untreated (TCID 0/ml)	Treat d (TCID50/ml)	Percent decrease from t=0	Untreated (TCID50/ml)	Treated (TCID50 ml)	Percent decrease from t=0	Untreated (TCID50/ml)	Treated (TCID50/ l)	Percent decrease from t=0
0 hrs	1.6×10^5			2.8×10^5			2.5×10^4		
2 hrs		9.03×10^3	94.4		9.5×10^4	66.1		1.4×10^4	44.0
4 hrs		7.6×10^3	95.3		2.8×10^4	90.0		8.6×10^3	65.6
6 hrs		$<1 \times 10^2$	>99.9		$<1 \times 10^3$	>99. 9		$<1 \times 10^2$	>99.6
24 hrs	9.3×10^3	$<1 \times 10^2$	>99.9	$<1 \times 10^3$	$<1 \times 10^3$	>99.9	$<1 \times 10^2$	$<1 \times 10^2$	>99.6

Figure 2. Survival of MNV following Low-Oxidation(ozone)Treatment



This report prepared by:

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Date

MNV-4 Titers Following Two Hour CIMR® Treatment

TCID50/ml

1.8e+6
1.6e+6
1.4e+6
1.2e+6
1.0e+6
8.0e+5
6.0e+5
4.0e+5
2.0e+5
0.0

Stainless Steel
Carpet
Cloth

Untreated Control
CIMR® Treated

MNV-4 Titers Following Two Hour Lo Oxidation (ozone) Active Pure Cell Treatment

TCID50/ml

3e+5
3e+5
2e+5
2e+5
1e+5
5e+4
0

Stainless Steel
Carpet
Cloth

Untreated Control
Lo Oxidation Treated

MNV-4 Titers Following CIMR® Treatment

TCID50/ml

1.8e+6
1.6e+6
1.4e+6
1.2e+6
1.0e+6
8.0e+5
6.0e+5
4.0e+5
2.0e+5
0.0

2
4
6
24

Hours of treatment

Untreated stainless steel
Treated stainless steel
Untreated carpet
Treated carpet
Untreated cloth
Treated cloth

MNV-4 Titers Following Low Oxidation (ozone) Active Pure Cell Treatment

TCID50/ml

3e+5
3e+5
2e+5
2e+5
1e+5
5e+4
0

2
4
6
24

Hours of treatment

Untreated stainless steel
Treated stainless steel
Untreated carpet
Treated carpet
Untreated cloth
Treated cloth