Norovirus Study Summary

General Details

Study title: Assessment of PathO3Gen Solutions Ozone + UVC (UVZone) Shoe Sanitizing Station for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing against Murine Norovirus (Strain S99) as a representative Healthcare-Associated Pathogen

Organism tested: Murine Norovirus (Strain S99)

Performing laboratory: CREM Co. Labs, Ontario, Canada

Date Performed: February 22, 2021

Summary

The initial challenge on each carrier in **Test 1** was 4.15 log 10. The PathO3Gen Solutions Ozone + UVC Shoe Sanitizing Station achieved the maximum attainable result of 4.15 log 10 at 6,8 and 10 seconds, leaving behind zero plaque. Similarly, the initial challenge on each carrier in **Test 2** was 4.36 log 10, and the PathO3Gen Solutions Ozone + UVC Shoe Sanitizing Station also achieved the maximum attainable result at 6, 8 and 10 seconds leaving zero plaque behind. Lastly, the initial challenge on each carrier in **Test 3** was 4.31 log 10, and the Ozone + UVC Shoe Sanitizing Station also achieved the maximum attainable result at 6, 8 and 10 seconds leaving zero plaque behind.

Overall, the results were as follows:

6 seconds: 0 PFU remaining (PFU = Plaque forming unit = pathogen)
8 seconds: 0 PFU remaining
10 seconds: 0 PFU remaining

Concluding statement

"The PathO3Gen Solutions' Ozone + UVC (UVZone) Shoe Sanitizing Station left 0 Norovirus residue on the bottom of footwear, in 8 seconds."

STUDY TITLE

Assessment of PathO3Gen Solutions™ Footwear Sanitizing Station for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing against Murine Norovirus (Strain S99) as a representative Healthcare-Associated Pathogen

TEST ORGANISM

Murine Norovirus (Strain S99)

TEST SAMPLE IDENTITY

PathO3Gen Solutions™ Footwear Sanitizing Station

TEST Method

Modified Quantitative Disk Carrier Test Method (ASTM 2197) to Determine the Virucidal Activity of an Ozone-Generating Whole-Shoe Disinfection Device

AUTHOR

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Study Director

STUDY COMPLETION DATE

Feb 22, 2021

PERFORMING LABORATORY

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SPONSOR

PathO3Gen Solutions™

STUDY NUMBER

PTGS200219-02

STUDY PERSONNEL

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TEST SYSTEM

1. Test Microorganism

Murine Norovirus (MNV): MNV is a non-enveloped RNA virus in the family Caliciviridae. It is the most prevalent viral infection in mice. There are 4 described strains designated MNV-1, MNV-2, MNV-3, and MNV-4, as well as multiple field strains. The virus causes enteric infections and can also exit the gut to replicate in macrophages and dendritic cells in multiple organs, including mesenteric lymph nodes and liver.

Since human noroviruses are difficult to culture in the lab, MNV is frequently used as its surrogate.

2. Host Cell Line

RAW 264.7 cells were used as host cell to support replication of MNV. This cell line is a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice. This cell line is a commonly used model of mouse macrophages for the study of efficacy test of disinfectants.

The cells were seeded into 12-well cell culture plates containing modified Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and maintained at 36±1°C in a humidified atmosphere of 5% CO_2. Efficacy test was performed when the cell monolayer reached >90% confluency.

3. Preparation of Test Inocula

To prepare the virus for inoculation, the virus stock was mixed directly with the soil load (5% FBS). Dilution of the mixture was prepared using Earle's balanced salt solution (EBSS; pH 7.2-7.4).

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TEST METHOD

1. Preparation of Test Substance

The efficacy tests were performed on PathO3Gen Solutions™ Footwear Sanitizing Station following the instruction in the device's user manual at three exposure times (6, 8 and 10 seconds).

2. Test Procedure

A quantitative test system to closely simulate the field-application of the environmental surface decontamination process (modified quantitative carrier test – Tier 2 or QCT-2 (ASTM 2197)) was applied. The protocol was adapted to test the UV LED-based technology. Disks (2 cm diameter) from croc sole shoe were used as archetypical environmental surfaces. Sterile disks were placed on a small platform which was the same size as a shoe at three different positions (middle, back and front). The platform was taped at the bottom of the shoe. The platforms with the disks were exposed to the UV without touching the glass cover of the device. The disks were retrieved in an eluent/neutralizer immediately at the end of the exposure time. The disks were then eluted and the eluates

assayed for viable virus.

Each disk on the platform was contaminated with 20 µL of the virus inoculum with a soil load (5% FBS) and left to dry (contaminated platform) under an operating biosafety cabinet (BSC) for 30±10 minutes. Three disks were contaminated and used as controls.

Experimental Design

a) Input

The stock virus utilized in the testing was titrated by 10-fold serial dilutions and plaque assayed for infectivity to determine the starting titer of the virus. The results of this control were for informational purposes only.

b) Efficacy Test

1. Disks (2 cm diameter) from crocs sole shoes were used in testing of this method, 3 disks were assessed as control without exposure to UV.
2. Disks were left inside an operating BSC to dry.
3. Disks were inserted on a platform with the same size of the shoe at three different locations (front, middle and back).
4. The platform was taped to the bottom of a shoe.
5. The experimenter put on the shoes with the platforms at their bottom.
6. The experimenter stepped on the device which was already on for 10 minutes.
7. After the specific exposure time, the experimenters stepped out of the device.
8. The disks were removed from the platform and each disk was inserted into a Nalgene vial containing 2 mL of an eluent.
9. The RAW 264.7 cells in multi-well culture plates were inoculated with 100 µL of the dilutions prepared from test and control samples. Uninfected indicator cell cultures (cell controls) were inoculated with 100 µL EBSS alone. The cultures were incubated at 36±1°C in a humidified atmosphere of 5% CO_2 for 44-48 hrs before fixing and staining them for counting the plaque-forming units (PFU).

10. Three control disks were included in each test to estimate the initial contamination on the platform. The test was initiated with processing one control before the processing test carriers, one in the middle of the test and ended up with the third control. This was done to take into the account the changes in the input level of the test organisms during the experiment.

DATA ANALYSIS

Calculation of Log_{10} Reduction

Log_{10} Reduction = Log_{10} of average PFU from control carriers – log_{10} of average PFU the test carriers.

STUDY ACCEPTANCE CRITERIA

No product acceptance criterion was specified for this range-finding study.

TEST RESULTS

The initial levels of challenge on each carrier were 4.15, 4.36 and 4.41 log_{10} PFU in three different tests performed on PathO3Gen Solutions™ Footwear Sanitizing Station. Table 1 show the result of log_{10} reductions for each contact time. In this test, the drying time of inoculated disks was reduced to 1 hr. In all contact times the log10 reduction was more than 4.31. No plaque was detected for 6, 8 and 10 seconds contact times.

Table 1: Virucidal Efficacy Test of PathO3Gen Solutions™ Footwear Sanitizing Station against Murine Norovirus (Strain S99) with three different contact times

Contact times	Log Reduction in PFU			
	Test #1	Test #2	Test #3	Average of Three tests
6 seconds	>4.15	>4.36	>4.41	>4.31
8 seconds	>4.15	>4.36	>4.41	>4.31
10 seconds	>4.15	>4.36	>4.41	>4.31

APPENDIX

Result of efficacy test on the device with three different contact times (6, 8 and 10 seconds) against Murine Norovirus (Strain S99) dried on carriers representing shoe soles.

Test #1												
Contact Time	6 seconds			8 seconds			10 seconds			Control		
Dilution	Front	Middle	Back	Front	Middle	Back	Front	Middle	Back	C1	C2	C3
10^{-0}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-1}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	40,40,39	34,28,32	TNTC
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	3,2,3	4,5,4	29,31,24
10^{-3}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	2,1,2	2,3,2
10^{-4}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0

C= Control
TNTC= Too numerous to count

Test #2												
Contact Time	6 seconds			8 seconds			10 seconds			Control		
Dilution	Front	Middle	Back	Front	Middle	Back	Front	Middle	Back	C1	C2	C3
10^{-0}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-1}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	12,11,12	10,10,9	12,11,11
10^{-3}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	2,1,2	2,1,2	2,1,2
10^{-4}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
Test #3												
Contact Time	6 seconds			8 seconds			10 seconds			Control		
Dilution	Front	Middle	Back	Front	Middle	Back	Front	Middle	Back	C1	C2	C3
10^{-0}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-1}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	13,10,10	11,8,10	10,11,12
10^{-3}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	3,2,4	1,1,1	1,1,1
10^{-4}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0