STUDY TITLE

Assessment of HealthySole UV Shoe Sanitizer Device for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing against Human Respiratory Coronavirus 229E (ATCC VR-740) as a representative Healthcare-Associated Pathogen

TEST ORGANISM

Coronavirus 229E (ATCC VR-740)

TEST SAMPLE IDENTITY

HealthySole UV Shoe Sanitizer Device

TEST Method

Modified Quantitative Disk Carrier Test Method (ASTM 2197) to Determine the Virucidal Activity of HealthySole UV Shoe Sanitizer Device

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STUDY COMPLETION DATE

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SPONSOR

HealthySole

STUDY NUMBER

HLS200219-01

STUDY PERSONNEL

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TEST SYSTEM

1. Test Microorganism

Coronavirus 229E (ATCC VR-740): Coronavirus 229E (ATCC VR-740) is an enveloped virus in the genus Coronavirus. Members of this genus can cause acute respiratory infections such as SARS-1 and SARS-2 (19-nCOV). Unlike Coronavirus 229E, SARS-1, SARS-2 and Middle-East Respiratory Syndrome (MERS) virus require Biosafety Level 3 labs. Therefore, Coronavirus 229E is frequently used as a surrogate for them to assess the activity of different technologies for infection prevention and control (IPAC).

2. Host Cell Line

L-132 cells were used as hosts to support the replication and quantitation of 229E.

The cells were seeded into 12-well multi-well cell culture plates containing modified Eagle's 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 HealthySole UV Shoe Sanitizer device following the instruction in the devices user manual at one exposure times 8 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 shoes were used as archetypical environmental surfaces. Sterile disks were placed on a small platform which was the same size as that of 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) Cytotoxicity Control

Prior to the test, cytotoxicity control and control for interference with virus infectivity were performed to determine if the shoe material caused any apparent degeneration (cytotoxicity) of the host cell line. Control monolayers received an equivalent volume of EBSS (without any neutralizer) only.

c) Efficacy Test

1. Disks (2 cm diameter) from croc 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) in duplicate, one on the right and the other on the left.
4. The platform was taped to the bottom of a shoe.
5. The experimenter put on the shoes with the platforms installed on the sole.
6. The experimenter stepped on the device which was already on for 10 minutes.
7. After the specific exposure time, the experimenter stepped away from the device.
8. The disks were removed from the platform and each disk was placed into a Nalgene vial containing 2 mL of an eluent.
9. The L-132 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 33±1°C in a humidified atmosphere of 5% CO_2 for 40-44 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 challenge on each carrier in three tests were 3.94, 3.77 and 3.79 log_{10} PFU. Table 1 show the result of log_{10} reduction with 8-second contact time for each test. In this test, the drying time of inoculated disks was reduced to 1 hr. The average log_{10} reduction was 2.53.

Table 1: Virucidal Efficacy Test of HealthySole UV Shoe Sanitizer device against Coronavirus 229E (ATCC VR-740) with 8-seconds of contact

Contact times	**Log Reduction in PFU**			
Test	**Test #1**	**Test #2**	**Test #3**	**Average of the three tests**
8 seconds	2.79	2.52	2.38	**2.53**

APPENDIX

Result of efficacy test on the device with an 8-second contact against Coronavirus 229E dried on carriers representing shoe soles, Test #1.

Contact Time	8 seconds						Control		
Dilution	Front Left	Front Right	Middle Left	Middle Right	Back Left	Back Right	C1	C2	C3
10^{-0}	2,5,4	7,8,4	1,0,0	4,10,5	1,0,1	13,16,13	TNTC	TNTC	TNTC
10^{-1}	0,0,0	1,1,0	0,0,0	1,1,1	0,0,0	1,1,2	TNTC	25,24, 25	11,16, 23
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	7,4,5	8,5,6	5,8,7
10^{-3}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	1,0,0	1,0,0	1,0,0
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

C= Control
TNTC= Too numerous to count

Result of efficacy test on the device with an 8-second contact against Coronavirus 229E dried on carriers representing shoe soles, Test #2.

Contact Time	8 seconds						Control		
Dilution	Front Left	Front Right	Middle Left	Middle Right	Back Left	Back Right	C1	C2	C3
10^{-0}	2,2,1	0,1,2	5,2,1	5,8,5	45,46 ,44	0,0,0	TNTC	TNTC	TNTC
10^{-1}	0,0,0	0,0,0	0,0,0	0,0,0	4,4,4,	0,0,0	31,30,31	25,37, 34	37,32, 31
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	3,2,3	3,3,3	3,3,3
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
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

Result of efficacy test on the device with an 8-second contact against Coronavirus 229E dried on carriers representing shoe soles, Test #3.

Contact Time	8 seconds						Control		
Dilution	Front Left	Front Right	Middle Left	Middle Right	Back Left	Back Right	C1	C2	C3
10^{-0}	4,4,2	1,1,4	7,7,7	3,3,0	3,6,4	6,8,5	TNTC	TNTC	TNTC
10^{-1}	0,0,0	1,1,0	0,0,0	1,1,1	0,0,0	1,1,2	34,33,32	36,34, 26	36,31, 32
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	2,3,3	3,3,3	3,3,3
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
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