MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Patho$_3$gen Solutions' UVZone Shoe Sanitizing Station Device

Test Method

Custom Device Study Based on: Modified ASTM E1153

Study Identification Number

NG19887

Study Sponsor

Scott Beal
Patho$_3$gen Solutions
scott@patho3gen.com
(502) 930-6526

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378
Testing performed by: Kyra Christensen

ASTM E1153: General Information

ASTM International, formerly the American Society for Testing and Materials (ASTM), is an internationally recognized organization that develops and publishes product and testing standards. ASTM E1153 is a quantitative test method designed to evaluate the antimicrobial efficacy of sanitizers on pre-cleaned inanimate, nonporous, non-food contact surfaces. The method is typically used with a maximum contact time of 5 minutes, during which the sanitizer reduces the concentration of viable test microorganisms. ASTM E1153 utilizes non-antimicrobial agents as controls to establish baselines for microbial reductions. The ASTM E1153 method is a benchmark method for non-food contact surface sanitizers and is recognized by several regulatory agencies as an approved method for claim substantiation. See study modifications for changes made to the study method to accommodate a device.

Laboratory Qualifications Specific to ASTM E1153

Microchem Laboratory began conducting the ASTM E1153 test method in 2007. Since then, the laboratory has performed hundreds of ASTM E1153 tests on a broad array of test substances, against a myriad of bacterial and fungal species. The laboratory is also experienced with regard to modifying the test method as needed in order to accommodate customer needs. Every ASTM E1153 test at Microchem Laboratory is performed in a manner appropriate for the test substances submitted by the Study Sponsor, while maintaining the integrity of the method.

Study Timeline

Test Device Received	Cultures Initiated	Treatment	Enumeration Plates Incubated	Enumeration Plates Evaluated	Report Delivered
08JUL2022	20JUL2022	21JUL2022	21JUL2022	25JUL2022	26JUL2022

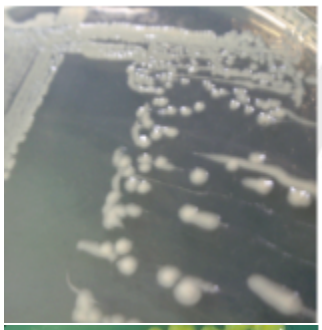
1304 W. INDUSTRIAL BLVD ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Test Device Information

Name of Test Device: UVZone Shoe Sanitizing Station (received 08JUL2022)
Manufacturer: Patho$_3$gen Solutions
Mode of Disinfection: UVC and Ozone

Test Microorganism Information

The test microorganism(s)selected for this test:



Salmonella enterica

This bacteria is Gram-negative, rod-shaped, facultative anaerobe. Like the closely related *Escherichia* genus, *Salmonella* are common to all parts of the world and share habitats in the digestive systems of cold and warm-blooded animals. *S. enterica* is one of the most common bacteria associated with zoonotic and foodbourne illness. Because of it's regular occurrence and pathogenicity, *S. enterica* is a common bacteria for measuring disinfectant efficacy.

***Cronobacter sakazakii* ATCC 29004**

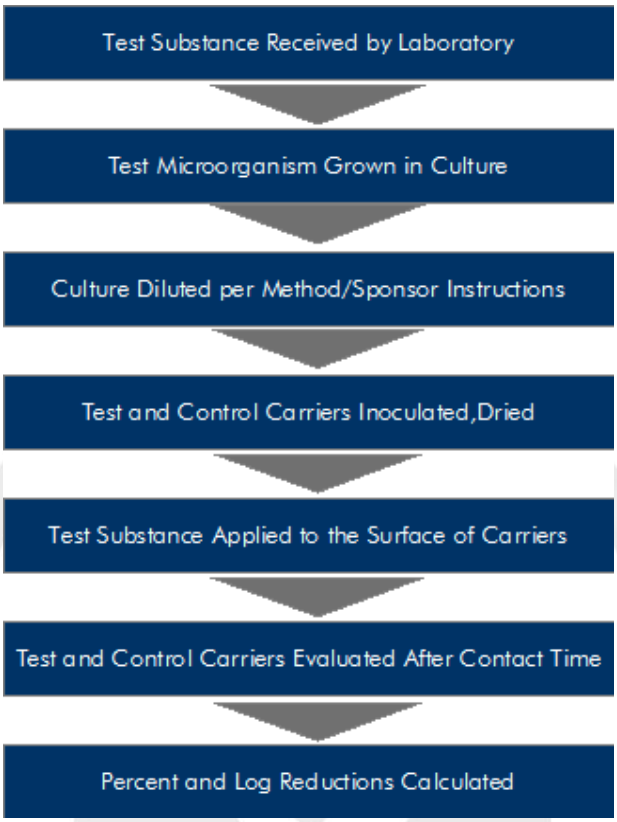
This bacteria is a Gram-negative, rod-shaped, pathogenic bacterium that can live in very dry places. It is described as a ubiquitous and opportunistic pathogen that currently contaminates a wide spectrum of foods and poses a lethal threat to neonates, the elderly, and persons with immune deficiencies.

Listeria monocytogenes

This bacteria is a Gram-positive, rod shaped, facultative anaerobe that is motile due to the presence of flagella. These bacteria are common cause of the foodbourne illness listeriosis, which can be fatal. Listeriosis can cause meningitis and sepsis and is particularly dangerous to pregnant women and unborn infants. *Listeria monocytogenes* is pervasive and can be found in soil, water, and certain livestock animals. They can resist both heat and freezing and can survive for several years.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Diagram of the Procedure



Summary of the Procedure

- The test microorganisms were prepared in liquid culture.
- Sterilized carriers were inoculated with 0.20 mL of the test cultures. Inoculated carriers were allowed to dry completely prior to testing.
- Control carriers were harvested prior to testing.
- Test carriers were treated with the device for 6, 8, and 10 seconds.
- At the conclusion of the contact time, test carriers were harvested.
- Dilutions of the harvested carriers were enumerated to determine the surviving microorganisms at the respective contact times.
- To determine log and percent reductions, the microbial concentrations of the carriers treated by the test device were compared to the that of the untreated parallel controls.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Criteria for Scientific Defensibility of a Custom Device Study

For Microchem Laboratory to consider a Device Study to be scientifically defensible, the following criteria must be met:

1. The average number of viable bacteria recovered from the time zero samples must be approximately 1 x 10^5 CFU/carrier or greater.
2. Positive/Growth controls must demonstrate growth of the appropriate test microorganism.
3. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

Due to the modified nature of the study, passing criteria may be determined by the Study Sponsor.

Testing Parameters

Carriers (Size):	1" x 3" Glass Slides		**Replicates:**	Triple
Culture Growth Media:	Tryptic Soy Broth, Nutrient Broth for CS29004		**Culture Growth Time:**	18-24 hours
Culture Dilution Media:	Phosphate Buffered Saline		**Inoculum Volume:**	0.20 mL
Inoculum Concentration:	Approx. 1.0 x 10^7 CFU/Carrier		**Contact Temperature:**	Ambient
Contact Time:	6, 8 and 10 seconds		**Enumeration Media:**	Tryptic Soy Agar (TSA), Nutrient Agar (NTA) for CS29004
Neutralizer (Vol.):	Phosphate Buffered Saline (PBS) w/ 0.1% Tween 80		**Enumeration Plate Incubation Time:**	24-48 hours
Enumeration Plate Incubation Temperature:	36□ ± 1□, 30□ ± 1□ for CS29004			

PARAMETERS

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Study Notes

The glass carriers were placed inverted for the microorganisms to be in contact with the device's surface, to simulate the sole of a shoe.

The device was disinfected with ethanol and allowed to dry in between each test run.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Control Results

Neutralization Method:	N/A	Media Sterility:	Confirmed
Growth Confirmation:	Confirmed		

Calculations

$$\text{Percent Reduction} = \left(\frac{B - A}{B}\right) \times 100$$

Where:

B = Number of viable test microorganisms on the control carriers immediately after inoculation

A = Number of viable test microorganisms on the test carriers after the contact time

$$\text{Log}_{10}\text{Reduction} = \text{Log}\left(\frac{B}{A}\right)$$

Where:

B = Number of viable test microorganisms on the control carriers immediately after inoculation

A = Number of viable test microorganisms on the test carriers after the contact time

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Results of the Study

Table 1: Results for *Cronobacter sakazakii*

Test Microorganism	Device	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Average Percent Reduction Compared to Parallel Controls	Average Log10 Reduction Compared to Parallel Controls
Cronobacter sakazakii ATCC 29004	N/A	Time Zero	Control 1	1.12E+05	1.10E+05	N/A	
			Control 2	7.50E+04			
			Control 3	1.44E+05			
	UVZone Shoe Sanitizing Station	6 seconds	Replicate 1	<1.00E+01	<1.00E+01	>99.991%	>4.04
			Replicate 2	<1.00E+01			
			Replicate 3	<1.00E+01			
		8 seconds	Replicate 1	1.00E+01	<3.67E+01	>99.97%	>3.48
			Replicate 2	9.00E+01			
			Replicate 3	<1.00E+01			
		10 seconds	Replicate 1	<1.00E+01	<1.00E+01	>99.991%	>4.04
			Replicate 2	1.00E+01			
			Replicate 3	<1.00E+01			
The limit of detection for this assay was 1.00E+01 CFU/carrier. For all values below this amount, the value is recorded as <1.00E+01.							

Table 2: Results for *Salmonella enterica*

Test Microorganism	Device	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Average Percent Reduction Compared to Parallel Controls	Average Log10 Reduction Compared to Parallel Controls
Salmonella enterica ATCC 10708	N/A	Time Zero	Control 1	3.30E+05	6.39E+05	N/A	
			Control 2	2.06E+05			
			Control 3	1.38E+06			
	UVZone Shoe Sanitizing Station	6 seconds	Replicate 1	<1.00E+01	<1.00E+01	>99.998%	>4.81
			Replicate 2	<1.00E+01			
			Replicate 3	<1.00E+01			
		8 seconds	Replicate 1	<1.00E+01	<1.67E+01	>99.997%	>4.58
			Replicate 2	<1.00E+01			
			Replicate 3	3.00E+01			
		10 seconds	Replicate 1	3.50E+02	<1.23E+02	>99.98%	>3.71
			Replicate 2	<1.00E+01			
			Replicate 3	<1.00E+01			
The limit of detection for this assay was 1.00E+01 CFU/carrier. For all values below this amount, the value is recorded as <1.00E+01.							

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Results of the Study continued

Table 3: Results for *Listeria monocytogenes*

Test Microorganism	Device	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Average Percent Reduction Compared to Parallel Controls	Average Log10 Reduction Compared to Parallel Controls
Listeria monocytogenes ATCC 19115	N/A	Time Zero	Control 1	1.07E+05	1.62E+05	N/A	
			Control 2	2.06E+05			
			Control 3	1.73E+05			
	UVZone Shoe Sanitizing Station	6 seconds	Replicate 1	2.30E+02	<8.33E+01	>99.95%	>3.29
			Replicate 2	<1.00E+01			
			Replicate 3	<1.00E+01			
		8 seconds	Replicate 1	<1.00E+01	<1.00E+01	>99.994%	>4.21
			Replicate 2	<1.00E+01			
			Replicate 3	<1.00E+01			
		10 seconds	Replicate 1	<1.00E+01	<1.67E+01	>99.9897%	>3.99
			Replicate 2	<1.00E+01			
			Replicate 3	3.00E+01			
The limit of detection for this assay was 1.00E+01 CFU/carrier. For all values below this amount, the value is recorded as <1.00E+01.							

The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378