ANTIMICROBIAL TEST LABORATORIES

Study Report

www.AntimicrobialTestLabs.com - Info@AntimicrobialTestLabs.com - (512) 310-8378

Study Title

Quantitative Antibacterial Activity and Efficacy of Stephen Kong Consulting Test Substances

Test Method

ASTM International Method E2197
Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporocidal Activities of Chemicals

Study Identification Number

NG6297

Study Sponsor

Stephen Kong
Stephen Kong Consulting
20 Alamo Lane
Alamo, CA 94507
(925) 324-9288
StephenBKong@gmail.com

Test Facility

Antimicrobial Test Laboratories
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378

History of the Laboratory

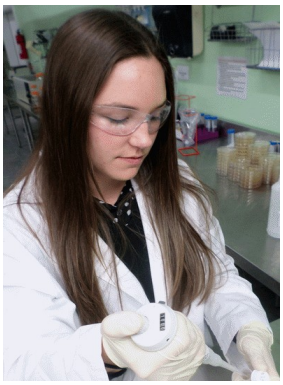
Antimicrobial Test Laboratories was launched in 2006 to provide testing services to the antimicrobial industry. The company has grown considerably since then but its focus remains the same: Test antimicrobial agents, test them well, and test them fast! Antimicrobial Test Laboratories operates a 15,000+ square foot facility near Austin, Texas, where hundreds of studies are conducted annually by a staff of friendly, knowledgeable, and experienced microbiologists and virologists.

Laboratory Qualification Statement

Antimicrobial Test Laboratories was founded by microbiologist Dr. Benjamin Tanner. The laboratory ensures consistent, reproducible results by utilizing a well-trained and educated scientific staff who work from a comprehensive system of Standard Operating Procedures, official standard methods from ASTM, AOAC, and other organizations, and custom study protocols. The laboratory provides testing services to dozens of Fortune 500 companies and has been inspected for GLP compliance by the US government.

Scientist Qualifications

Your Study was designed, conducted and reported by: Kelli Jo Kuntzman B.S.



Kelli Jo graduated from Texas State University with a Bachelors of Science degree in Microbiology.

Kelli Jo is a hardworking, detail oriented, and dedicated microbiologist. As a Microbiology Associate at Antimicrobial Test Laboratories, she participates in a broad array of studies and test methods. Her strong work ethic and interest in comprehensive and accurate testing make her an asset to the laboratory and clients alike.

If you have any questions about your study, please don't hesitate to contact Kelli Jo at:

KelliJo@AntimicrobialTestLabs.com
or
(512) 310-8378

ASTM E2197: 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. Known also as the quantitative disk-carrier method, ASTM E2197 is designed to evaluate antimicrobial efficacy of germicides on hard, nonporous environmental and medical surfaces. The method is versatile and be conducted using contact times ranging from ten seconds to several hours. The ASTM E2197 test method utilizes non-antimicrobial agents as controls to establish baselines for microbial reductions. The ASTM E2197 method is a benchmark method for bactericidal, virucidal, fungicidal, mycobactericidal, and sporicidal activites of chemicals and is recognized by several regulatory agencies as an approved method for substantiation of certain claims, such as disinfection of *Clostiridium difficile*.

Laboratory Qualifications Specific to ASTM E2197

Antimicrobial Test Laboratories has a great deal of experience with the ASTM E2197 method. The laboratory has performed numerous tests across a broad array of treated substances, including under GLP conditions for submission to the United States Environmental Protection Agency (EPA). In addition, the laboratory is experienced with regard to modifications of the method as needed to accommodate customer needs, such as by using different or additional bacterial or fungal species. Every ASTM E2197 test at Antimicrobial Test Laboratories is performed in a manner appropriate to the test substances submitted by the Study Sponsor, while maintaining the integrity of the method.

Study Timeline

Culture Initiated	Carriers Inoculated	Carriers Treated	Carriers Evaluated	Report Delivered
N/A Spore Prep	15SEP2015	15SEP2015	18SEP2015	18SEP2015

Test Substance Information

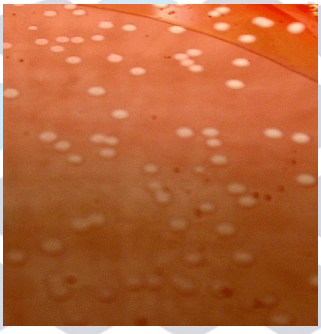
The test substances were received on 20AUG2015 and the following picture was taken.
(note: photo depicts the test substances that were analyzed in this study)

Test Substance Received: 2-Part ClO_2 Product with Surfactant Yield1023PPM ClO_2 and 2-Part ClO_2 Product with Surfactant Yields 1300 PP ClO_2

Test Substances arrived ready to use for conduct of the study. Test substances were not diluted prior to use in the study.

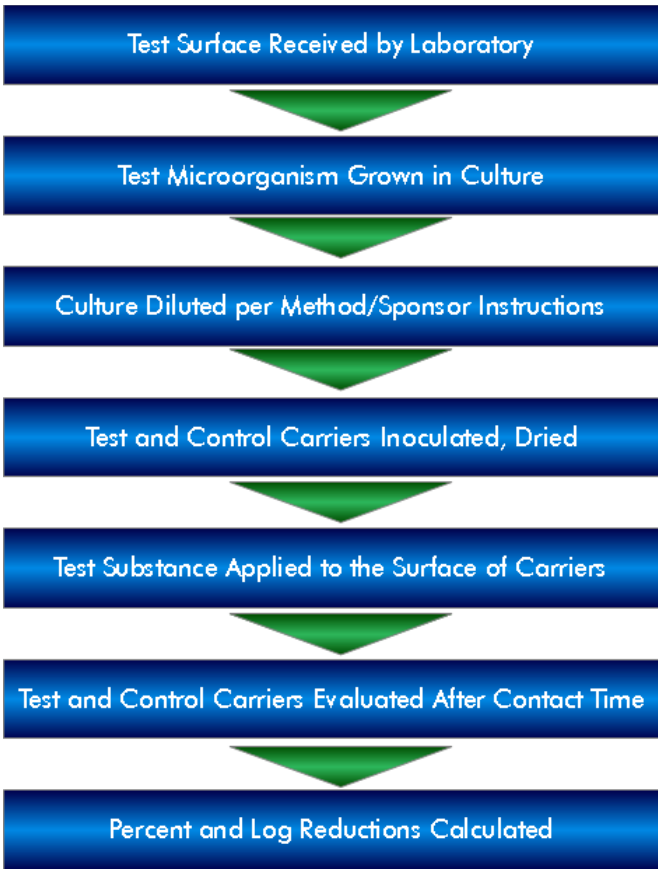
Test Microorganism Information

The test microorganism(s) selected for this test:



Clostridium difficile 43598 (Endospores)
This bacteria is a Gram-positive, rod shaped, endospore generating obligate anaerobe. *Clostridium* species are part of the normal human gut flora that produce spores which are highly resistant to chemical and environmental conditions. *C. diff* is commonly associated with hospital acquired infections and is know to cause antibiotic assisted colitis. Because of it's high resistance to antimicrobials, *C. difficile* is a benchmark bacteria for sporicidal and sterilant activity of chemicals.

Diagram of the Procedure



Summary of the Procedure

- The test microorganism is prepared, usually by growth in liquid culture medium.
- The test culture may be supplemented with an artificial soil load, such as is required for one-step cleaner/sanitizer claims.
- Sterilized disk carriers are inoculated directly in the center with the test culture, and the inoculated carriers are dried in a vacuum desiccator. Only completely dried carriers are used in the test.
- Test carriers are treated with the test substance and allowed to sit for the predetermined contact time.
- Control carriers are treated with phosphate buffered saline and are allowed to sit for the predetermined contact time.
- At the conclusion of the contact time, test and control carriers are chemically neutralized and vortex mixed. Membrane filtration is used to increase recovery, as appropriate.
- Reductions of microorganisms by the test substance are compared to the surviving microorganisms on control carriers.

Criteria for Scientific Defensibility of an ASTM E2197 Study

For Antimicrobial Test Laboratories to consider an ASTM E2197 study to be scientifically defensible, the following criteria must be met:

1. The average number of viable microorganisms recovered from the control carriers must be approximately 1.0 x 10^6 spores/carrier or greater.
2. Ordinary consistency between replicates must be observed for the control carriers.
3. Positive/Growth controls must demonstrate growth of appropriate test microorganism.
4. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

The US Environmental Protection Agency specifies a passing criteria of 6 Log_{10} or 99.9999% reduction of viable spores.

Testing Parameters used in this Study

Test Carrier Size:	10 mm diameter disk	Number of Carriers:	3 per Test Substance
Test Substance Volume:	0.050 ml		

Culture Growth Media:	N/A spore Prep	Culture Growth Time:	N/A Spore Prep
Culture Supplement:	Tri-Part Soil	Carrier Inoculum Volume:	0.010 ml
Inoculum Concentration:	1 x 10^6 CFU/Carrier	Carrier Dry Time:	1 Hour
Carrier Dry Temp:	25°C ± 2°C	Contact Temp.:	Ambient (25°C ± 2°C)
Contact Time:	10 Minutes	Neutralizer:	See Notes
Enumeration Plate Incubation Temperature:	36°C ± 1°C	Enumeration Plate Incubation Time:	72 Hours
Incubation Conditions:	Anaerobic		

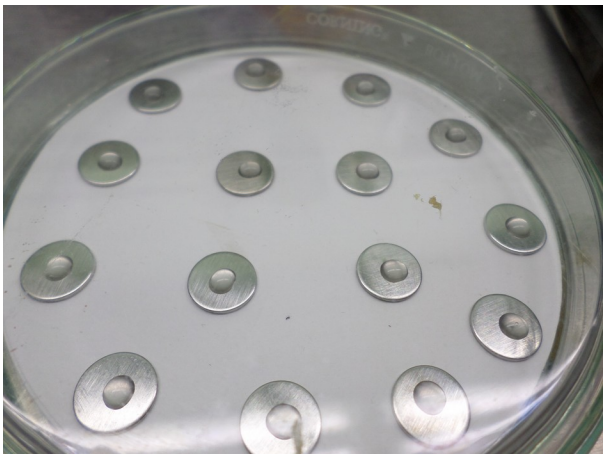
Study Modifications

No further modifications were made to the method for this study.

Study Notes

The neutralizer used in this assay was 10 ml volumes of D/E Broth supplemented with 0.1% Sodium Thiosulfate and 0.1% Lecithin.

Study Photographs



The photo above depicts inoculated carriers before drying.

Control Results

Neutralization Method: Verified
Media Sterility: Sterile
Growth Confirmation: Confirmed, morphology on BHIY-HT agar

Calculations

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

Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

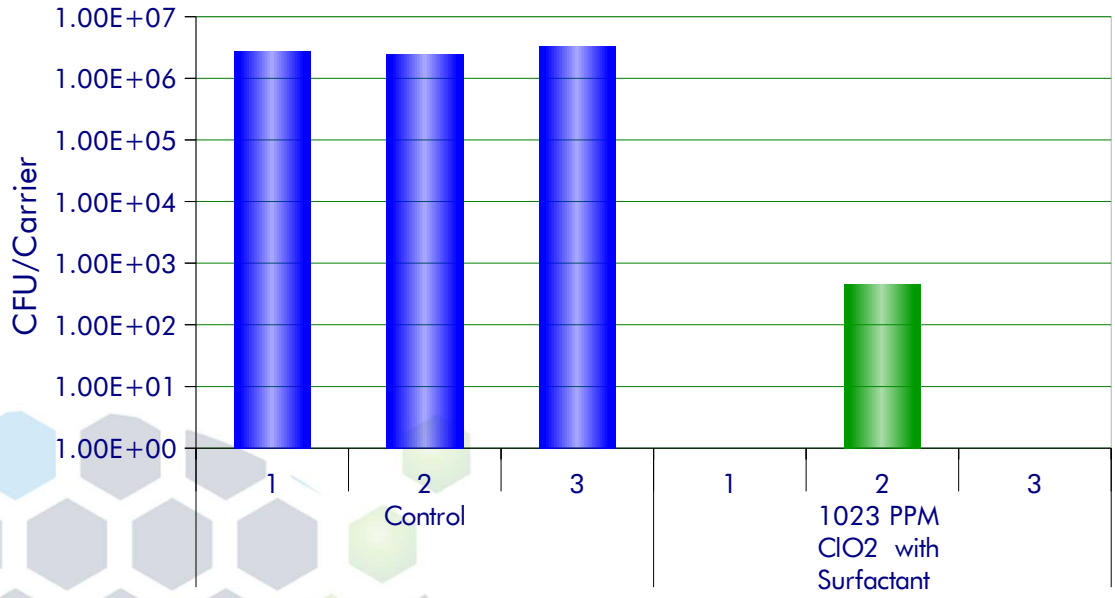
$$\text{Log}_{10}\text{Reduction} = \text{Log}\left(\frac{B}{A}\right)$$

Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

Results of the Study

Results for 1023 PPM ClO_2 with Surfactant:

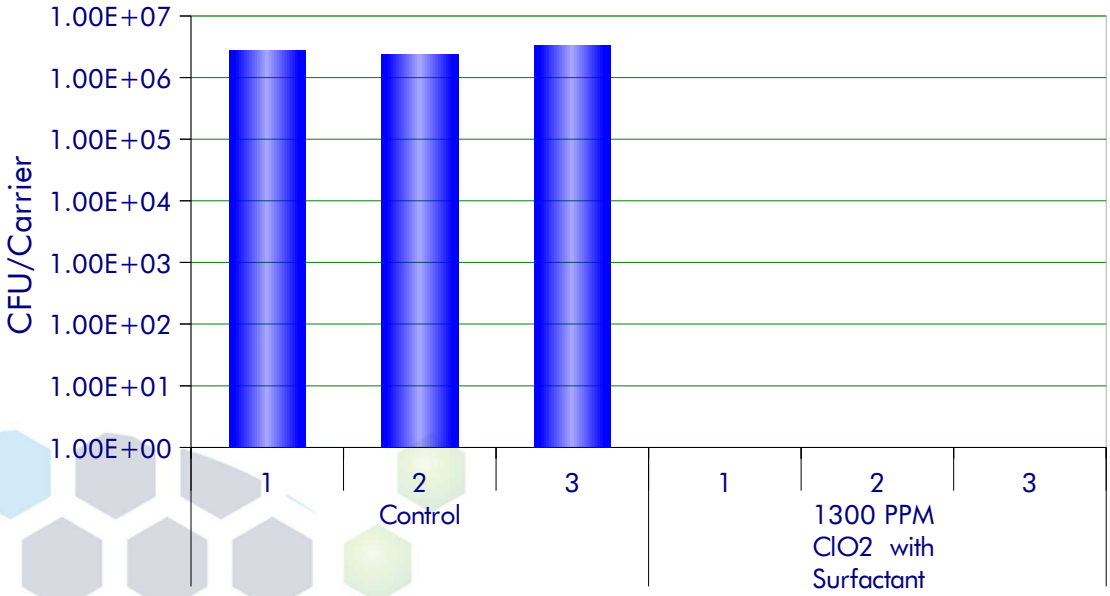
Test Microorganism	Contact Time	Test Substance	Carrier Number	Replicate CFU/Carrier	Geometric Average CFU/Carrier	Geometric Percent Reduction Compared to Control after Contact Time	Geometric Log_{10} Reduction Compared to Control after Contact Time
C. difficile 43598 (endospores)	10 minutes	Control	1	2.70E+06	2.76E+06	N/A	
			2	2.40E+06			
			3	3.25E+06			
		1023 PPM ClO_2 with Surfactant	1	<1.00E+00	<4.52E+02	>99.98%	>3.79
			2	4.50E+02			
			3	<1.00E+00			



The limit of detection for this assay was 1 CFU/ml. Counts below this limit of detection are presented as 1.00E+00 in the table and zero in the graph above.

Results for 1300 PPM ClO_2 with Surfactant:

Test Microorganism	Contact Time	Test Substance	Carrier Number	Replicate CFU/Carrier	Geometric Average CFU/Carrier	Geometric Percent Reduction Compared to Control after Contact Time	Geometric Log_{10} Reduction Compared to Control after Contact Time
C. difficile 43598 (endospores)	10 minutes	Control	1	2.70E+06	2.76E+06	N/A	
			2	2.40E+06			
			3	3.25E+06			
		1300 PPM ClO_2 with Surfactant	1	<1.00E+00	<1.00E+00	>99.99996%	>6.44
			2	<1.00E+00			
			3	<1.00E+00			



The limit of detection for this assay was 1 CFU/ml. Counts below this limit of detection are presented as 1.00E+00 in the table and zero in the graph above.