MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Zoono Limited's Test Substance

Test Method

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

Study Identification Number

NG7368

Study Sponsor

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Test Facility

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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 actives 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

Microchem Laboratory 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 Microchem Laboratory 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)	21JUL2016	21JUL2016	25JUL2016	28JUL2016

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Test Substance Information

The test substances were received on 28JUN2016

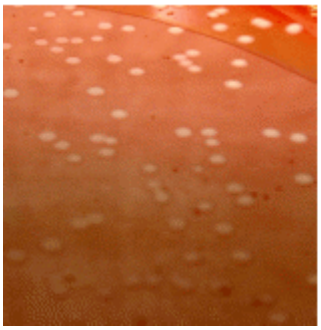
(note: photo depicts the test substances that were analyzed in this study)

Test Substances Received: Zoono Z-71 Microbe Shield Hospital Grade Disinfectant

Test substances arrived as ready to use for the conduct of the study. Test substances were not diluted for the study.

Test Microorganism Information

The test microorganism(s) selected for this test:



***Clostridium difficile* 43598**

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.

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.

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Criteria for Scientific Defensibility of an ASTM E2197 Study

For Microchem Laboratory 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 cells/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 U.S. 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
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:	See notes
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:	48 ± 6 Hours
Incubation Conditions:	Anaerobic		

PARAMETERS

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Study Modifications

No modifications were made to the method for this study.

Study Notes

Carriers were dried in a laminar flow hood for 30 minutes, then transferred to a desiccator and dried for an additional 2 hours.

Neutralizer used in the study was Dey Engley broth additionally supplemented to contain 0.5% sodium thiosulfate and 0.1% lecithin.

Enumeration plates were incubated for a period of ~48 hours, however, plates were evaluated 72 hours after initial incubation.

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Control Results

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

Calculations

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

Where:
B = Number of viable test microorganisms in the control substance immediately after inoculation
A = Number of viable test microorganisms in the test substance after the contact time

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

Where:
B = Number of viable test microorganisms in the control substance immediately after inoculation
A = Number of viable test microorganisms in the test substance after the contact time

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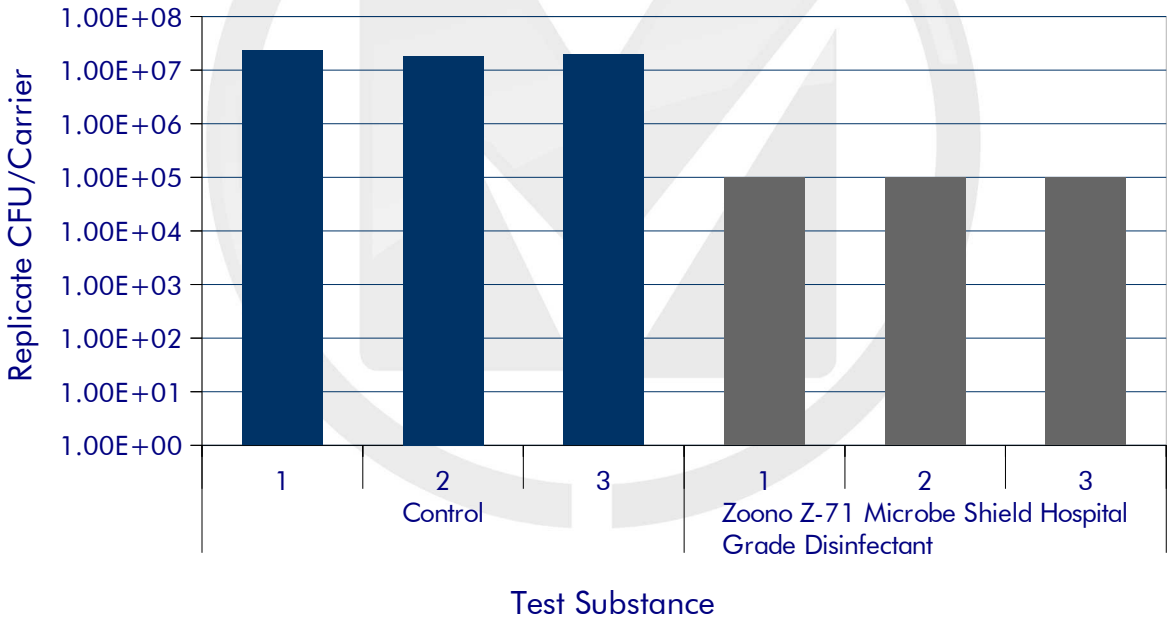
Results of the Study

Test Microorganism	Contact Time	Test Substance	Carrier Number	Replicate CFU/Carrier	Average Log_{10} Density CFU/Carrier	Percent Reduction Compared to Control	Log_{10} Reduction Compared to Control
C. difficile 43598 (endospores)	10 minutes	Control	1	2.35E+07	7.31	N/A	
			2	1.85E+07			
			3	1.95E+07			
		Zoono Z-71 Microbe Shield Hospital Grade Disinfectant	1	≥1.00E+05	≥5.00	≤99.51%	≤2.31
			2	≥1.00E+05			
			3	≥1.00E+05			



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

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