ANTIMICROBIAL TEST LABORATORIES

Study Report

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Study Title

Antibacterial Activity and Efficacy of SteriWeb Medical, LLC Test Substance Using a Suspension Time-Kill Procedure

Test Method

ASTM International Method E2315
Assessment of Antimicrobial Activity using a Time-Kill Procedure

Study Identification Number

NG5395

Study Sponsor

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Test Facility

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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: Nicholas Garcia, B.S.

Nicholas graduated from Texas A&M University with a Bachelors of Science in Biomedical Science.

Nicholas is an experienced microbiologist with a keen eye for documentation and experimental quality. He has taken part in or overseen hundreds of studies and mastered several test methods. He takes on new projects with enthusiasm and can be counted on to see them through to completion. Nick is known in the laboratory as a hard-working, ethical professional with an upbeat attitude.

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

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ASTM E2315: 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 E2315 is a quantitative test method designed to assess changes in the population of microorganisms in an antimicrobial liquid suspension. The method is versatile and can be conducted using contact times ranging from ten seconds to 24 hours. The ASTM E2315 test method uses non-antimicrobial agents as controls to establish baselines for microbial reductions. Because ASTM E2315 allows a great degree of latitude with regard to how the procedure is carried out, some scientists consider it to be more similar to a testing guideline than a test method.

Laboratory Qualifications Specific to ASTM E2315

Antimicrobial Test Laboratories began conducting the ASTM E2315 test method in 2007. Since then, the laboratory has performed thousands of ASTM E2315 tests on a broad array of test substances, against a myriad of bacterial, fungal, and viral species. The laboratory is also experienced with regard to modifying the method as appropriate to accommodate unique test substances. Every ASTM E2315 test at Antimicrobial Test Laboratories is performed in a manner appropriate to the test substance submitted by the Study Sponsor, while maintaining the integrity of the method.

Study Timeline

Test Substance Information

The test substance was received on 22 AUG 2014 and the following picture was taken.

Test Substances Received: Omnicide Ointment Formula# LM-100-142A 6/24/14

Test Substances arrived ready to use for the conduct of the Study. Test substances were not diluted for the Study.

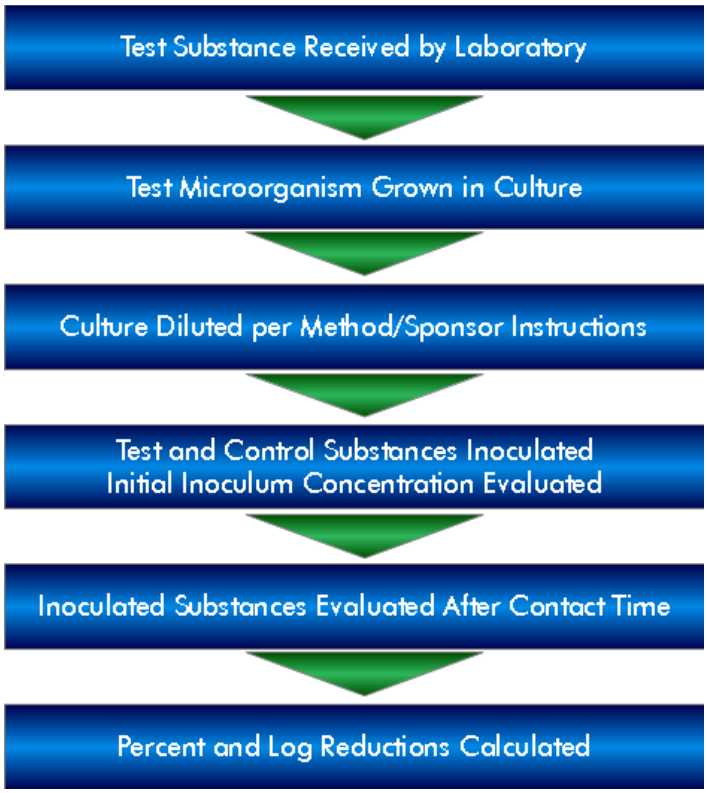
Test Microorganism Information

The test microorganism(s) selected for this test:

Staphylococcus aureus (MRSA) 33592

This bacteria is a Gram-positive, cocci shaped, aerobe which is resistant to the penicillin-derivative antibiotic methicillin. MRSA can cause troublesome infections, and their rapid reproduction and resistance to antibiotics makes them more difficult to treat. MRSA bacteria are resistant to drying and can therefore survive on surfaces and fabrics for an extended period of time and therefore makes this bacteria an excellent representative for antimicrobial efficacy testing on surfaces.

Diagram of the Procedure



Summary of the Procedure

- Test microorganisms are prepared in liquid culture medium for bacteria or on agar for fungi.
- The suspension of test microorganism is standardized, as needed, by dilution in a buffered saline solution.
- Test and control substances are dispensed in identical volumes to sterile vessels.
- Independently, Test and Control substances are inoculated with each test microorganism, then mixed and incubated.
- Control substances are immediately harvested and represent the concentration present at the start of the test, or time zero.
- At the conclusion of the contact time, a volume of the liquid test solution is harvested and chemically neutralized.
- Dilutions of the neutralized test solution are assayed using appropriate growth media to determine the surviving microorganisms at the respective contact times.
- Reductions of microorganisms are calculated by comparing initial microbial concentrations to final microbial concentrations.

Criteria for Scientific Defensibility of an ASTM E2315 Study

For Antimicrobial Test Laboratories to consider a Suspension Time Kill study to be scientifically defensible, the following criteria must be met:

1. The average number of viable fungi recovered from the time zero samples must be approximately 1 x 10^6 cells/ml or greater.
2. Ordinary consistency between replicates must be observed for the time zero samples.
3. Positive/Growth controls must demonstrate growth of appropriate test microorganism.
4. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

ASTM International does not specify performance criteria, therefore it may be established by the Study Sponsor.

Testing Parameters used in this Study

Test Substance Volume:	10 ml	Replicates:	Two
Control Substance Volume:	10 ml	Control Substance:	PBS

Culture Dilution Media:	N/A	Inoculum Volume:	0.200 ml
Inoculum Concentration:	10^6 CFU/ml	Contact Temp.:	Ambient (25°C ± 2°C)
Contact Time:	30 seconds, 2 minutes	Volume Harvested:	1.0 ml
Neutralizer (Vol.):	Letheen Broth (19 ml)	Plating Media:	Tryptic Soy Agar
Enumeration Plate Incubation Temperature:	36°C ± 1°C	Enumeration Plate Incubation Time:	~48 hours

Study Modifications

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

Study Notes

In order to aide in inoculation, aliquots of test substance were warmed in a water bath prior to inoculation.

Study Photographs

No photographs were taken during this study.

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Control Results

Neutralization Method: Validated
Media Sterility: Sterile
Growth Confirmation: Colony morphology on TSA

Calculations

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

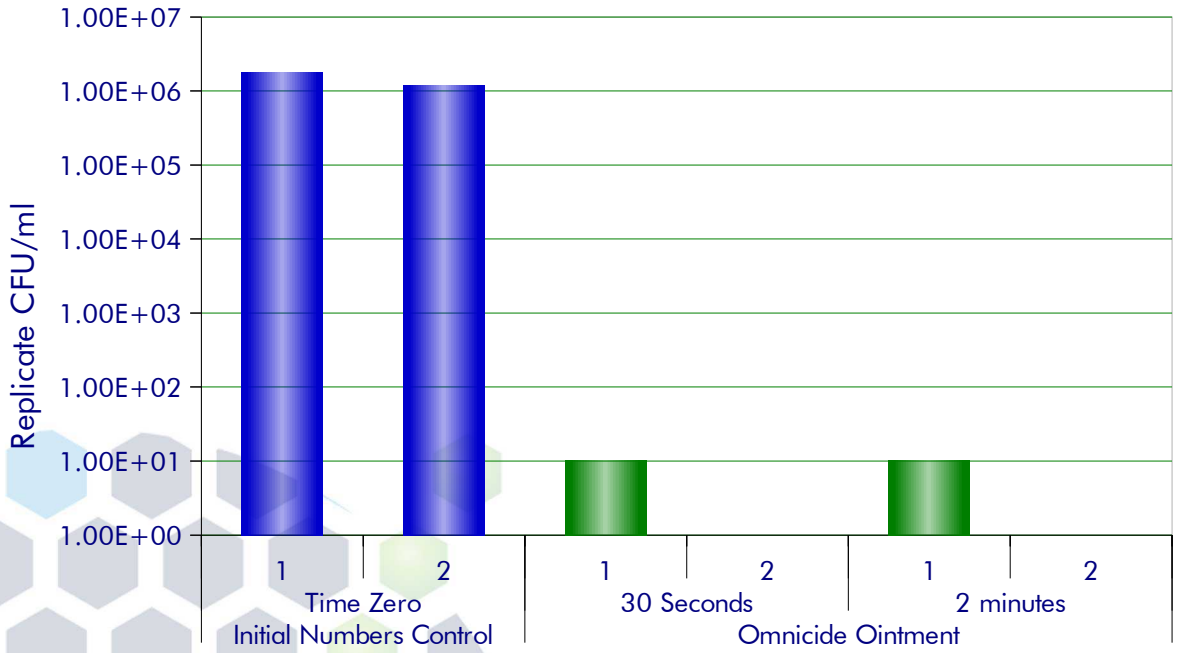
Where:
B = Number of viable test microorganisms in the control substance immediately after inoculation
A = Number of viable test microorganisms in the test substance after the contact time

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

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

Results of the Study

Test Microorganism	Test Substance	Contact Time	Replicate	Replicate CFU/ml	Average CFU/ml	Percent Reduction vs. Control at Time Zero	Log_{10} Reduction vs. Control at Time Zero
S. aureus 33592 (MRSA)	Initial Numbers Control	Time Zero	1	1.75E+06	1.48E+06	N/A	
			2	1.20E+06			
	Omnicide Ointment	30 Seconds	1	1.00E+01	<1.00E+01	>99.9993%	>5.17
			2	<1.00E+01			
		2 minutes	1	1.00E+01	<1.00E+01	>99.9993%	>5.17
			2	<1.00E+01			



The limit of detection for this assay is 1.00E+01 CFU/ml. Values below the limit of detection are noted as <1.00E+01 in the table and zero in the chart.

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