MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Kismet Holdings Group's Test Device Using a Custom Suspension Time-Kill Procedure

Test Method

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

Study Identification Number

NG13978

Study Sponsor

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Report Author

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Date Reported

06NOV2019

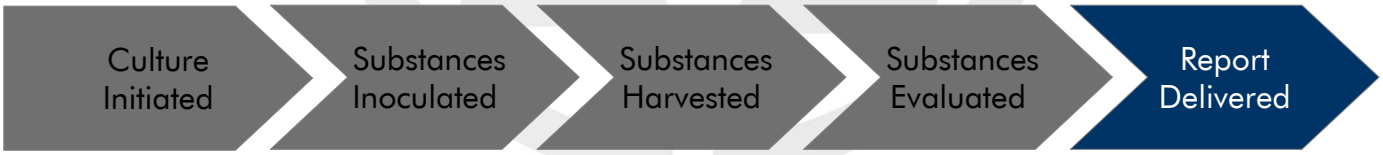
ASTM E2315: General Information

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

Microchem Laboratory 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 Microchem Laboratory is performed in a manner appropriate to the test substance submitted by the Study Sponsor, while maintaining the integrity of the method.

Study Timeline



28OCT2019	29OCT2019	29OCT2019	01NOV2019	06NOV2019

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Test Substance Information

The test device was received on 29OCT2019.

Test Device Received: Kismet UV Splint Bath

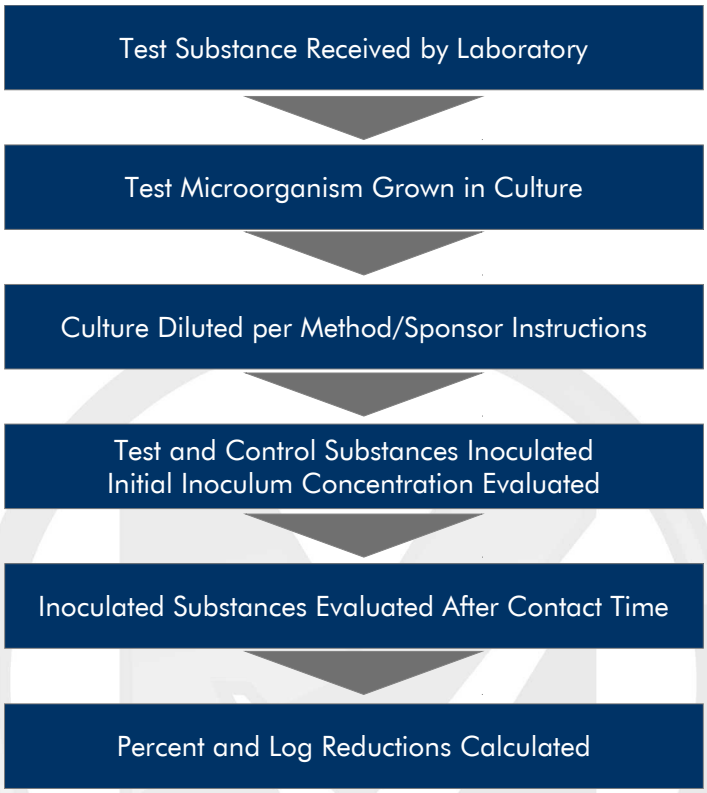
Test device arrived and was set up on-site by Study Sponsor prior to testing.

Test Microorganism Information

The test microorganisms selected for this test:

Escherichia coli ATCC 11229
Staphylococcus epidermidis ATCC 12228
MS2 Bacteriophage ATCC 15597-B1
Mycobacterium terrae ATCC 15755

Diagram of the Procedure



Summary of the Procedure

- Test microorganisms are prepared in liquid culture medium for bacteria; MS2 virions were pulled from frozen laboratory stock.
- The suspension of test microorganism is standardized, as needed, by dilution in a buffered saline solution.
- Test device is inoculated with each test microorganism, then mixed.
- Control substances are immediately harvested and represent the concentration present at the start of the test, or time zero.
- At the conclusion of the specified contact times, a volume of the liquid test solution is harvested.
- Dilutions of the 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.

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Criteria for Scientific Defensibility of an ASTM E2315 Study

For Microchem Laboratory to consider a Suspension Time Kill 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 10^6 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

Test Substance Volume:	15 L	Replicates:	Single
Control Substance Volume:	N/A	Control Substance:	N/A

Culture Growth Media:	TSB, M7H9 Slurry	Culture Growth Time:	24 hours, 21 days
Culture Dilution Media:	PBS	Inoculum Volume:	9.7 ml, 19.5 ml
Inoculum Concentration:	≥ 1.0 x 10^6 CFU/ml	Contact Temp.:	140 ± 1 °F, 185 ±1 °F
Contact Time:	Various (See Results)	Volume Harvested:	1.0 ml
Neutralizer (Vol.):	N/A	Plating Media:	TSA, M7H9 Agar
Enumeration Plate Incubation Temperature:	36°C ± 1 °C	Enumeration Plate Incubation Time:	48 hours, 12 days

Note: Testing parameters listed are relevant to vegetative and bacteriophage cultures (E. coli, S. epidermidis, and MS2) and M. terrae, respectively.

PARAMETERS

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Study Modifications

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

Study Notes

"Maintenance Cycle":

Microorganisms: *E. coli* ATCC 11229, *S. epidermidis* ATCC 12228
Contact Temperature: 140 ± 1 °F
Contact Times: Time Zero, 1 minute, 10 minutes, 30 minutes, and 60 minutes

"Sanitization Cycle":

Microorganisms: MS2 Bacteriophage ATCC 15597-B1, *M. terrae* ATCC 15755
Contact Temperature: 185 ± 1 °F
Contact Times: Time Zero, 2 minutes, 5 minutes, and 10 minutes

Test results for *M. terrae* ATCC 15755 are pending and will be included in an amended report once available.

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Control Results

Neutralization Method: N/A
Media Sterility: Sterile
Growth Confirmation: Confirmed; morphology on growth media

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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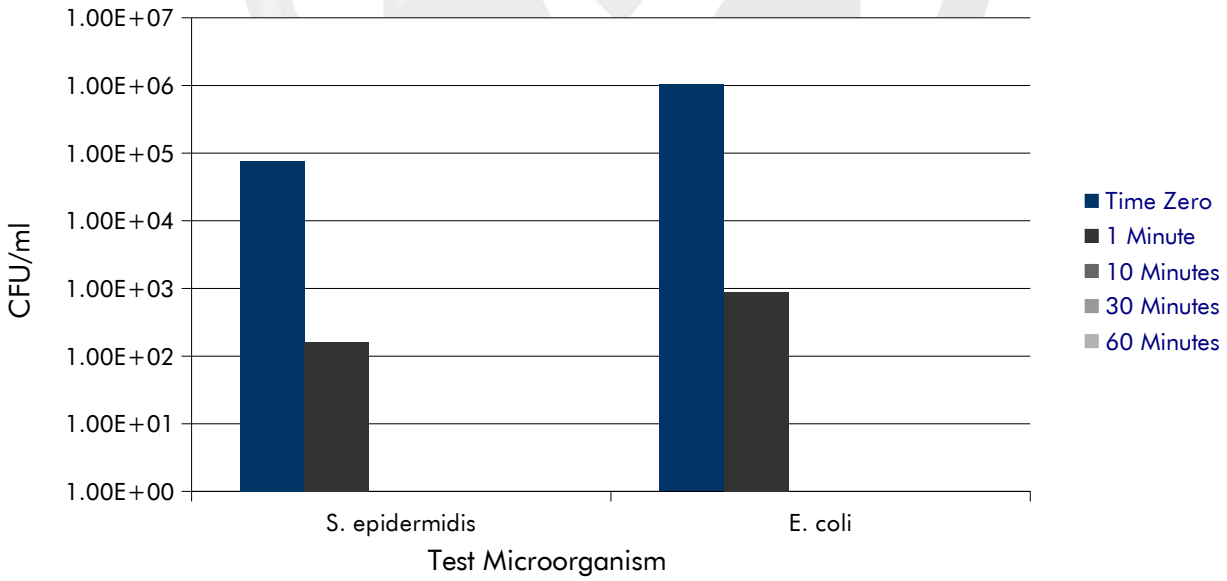
Results of the Study: "Maintenance Cycle"

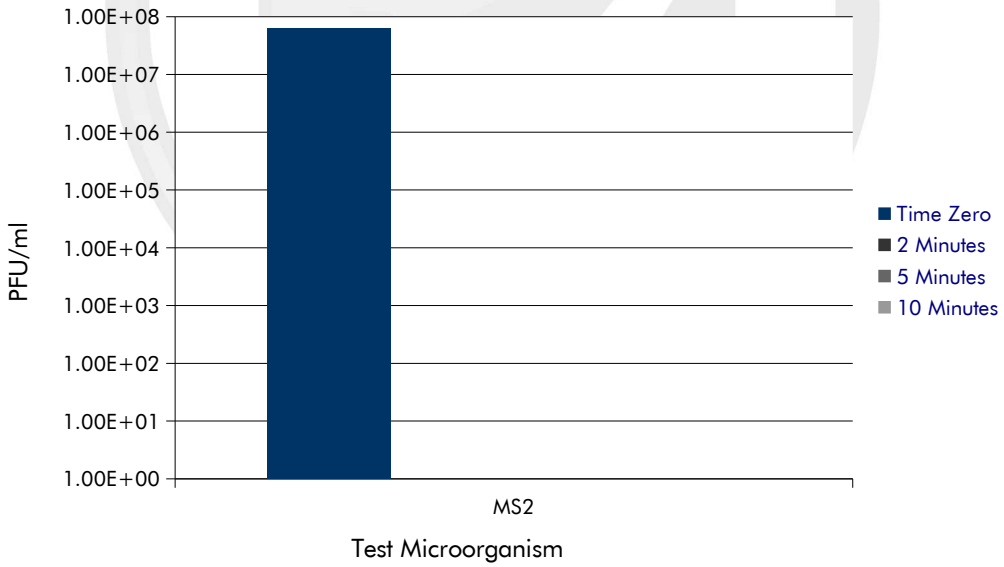
Test Device	Contact Time	Data Description	Test Microorganism	
			S. epidermidis ATCC 12228	*E. coli* ATCC 11229
Kismet UV Splint Bath	Time Zero	CFU/ml	7.67E+04	1.03E+06
	1 Minute	CFU/ml	1.63E+02	8.95E+02
		Log_{10} Reduction	2.67	3.06
	10 Minutes	CFU/ml	<1.00E+00	<1.00E+00
		Log_{10} Reduction	>4.88	>6.01
	30 Minutes	CFU/ml	<1.00E+00	<1.00E+00
		Log_{10} Reduction	>4.88	>6.01
	60 Minutes	CFU/ml	<1.00E+00	<1.00E+00
		Log_{10} Reduction	>4.88	>6.01



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

Results of the Study: "Sanitization Cycle" (MS2)

Test Device	Contact Time	Data Description	Test Microorganism MS2 ATCC 15597-B1
Kismet UV Splint Bath	Time Zero	PFU/ml	6.23E+07
	2 Minutes	PFU/ml	<1.00E+00
		Log_{10} Reduction	>7.79
	5 Minutes	PFU/ml	<1.00E+00
		Log_{10} Reduction	>7.79
	10 Minutes	PFU/ml	<1.00E+00
		Log_{10} Reduction	>7.79



The limit of detection for this assay is 1 PFU/ml. Values observed below this limit are presented as <1.00E+00 in the table and zero in the graph above.

The results of this study apply to the tested device only. Extrapolation of findings to related materials is the responsibility of the Sponsor.