MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of KHG FiteBac Technology 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

NG6838

Study Sponsor

Kirk Kimmerling
KHG FiteBac Technology
3698 Largent Way Ste. 202
Marietta, GA 30064
(770)-423-4900
kirkkimmerling@fitebac.com

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-837

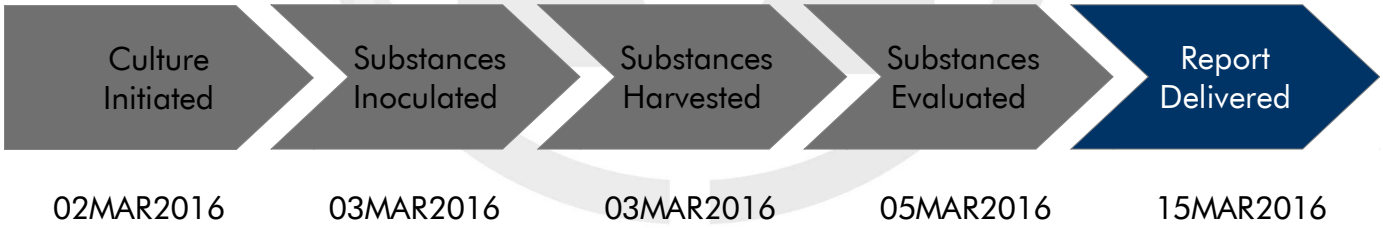
ASTM E2315: General Information

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



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Test Substance Information

The test substances were received on 15 FEB 2016.

Test Substances Received: fiteBac Germicidal Hand Softening Gel (exp: 03/2014), fiteBac Germicidal Hand Softening Gel (exp: 03/2018)

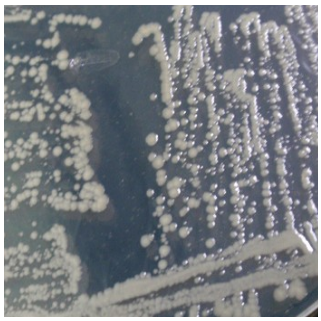
Test Substances arrived ready to use for the conduct of the Study. Test substances were not diluted for the Study.

Test Microorganism Information

The test microorganism(s) selected for this test:

Staphylococcus aureus 6538

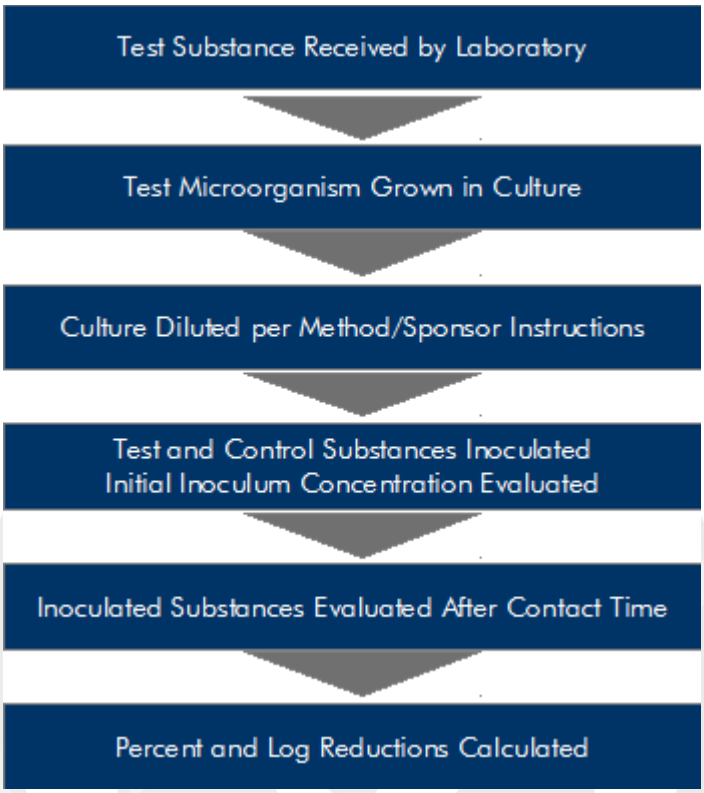
This bacterium is a Gram-positive, spherical-shaped, facultative anaerobe. *Staphylococcus* species are known to demonstrate resistance to antibiotics such as methicillin. *S. aureus* pathogenicity can range from commensal skin colonization to more severe diseases such as pneumonia and toxic shock syndrome (TSS). *S. aureus* is commonly used in several test methods as a model for gram positive bacteria. It can be difficult to disinfect but does demonstrate susceptibility to low level disinfectants.



Escherichia coli 8739

This bacteria is a Gram-negative, rod shaped, facultative anaerobe commonly found in the gastrointestinal tract of mammals. Although most serotypes of this microorganism are harmless there are pathogenic groups of *E. coli* such as enterohemorrhagic (EHEC), verocytotoxin producing (VTEC) and Shiga-like toxin producing (STEC) that can cause a multitude of illnesses. *E. coli* is relatively susceptible to disinfection when dried on a surface, yet it can be a challenging microorganism to mitigate in solution.

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 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 10^5 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:	1.0 gram	Replicates:	Singlet
Control Substance Volume:	1.0 ml	Control Substance:	PBS

Culture Growth Media:	Tryptic Soy Broth	Culture Growth Time:	24 hours
Culture Dilution Media:	PBS	Inoculum Volume:	0.010 ml
Contact Time:	30 Sec., 60 Sec.	Contact Temperature:	Ambient
Neutralizer (Vol.):	9.0 ml D/E Broth	Volume Harvested:	1.0 gram
Enumeration Plate Incubation Temperature:	36°C ± 2 °C	Plating Media:	TSA
Enumeration Plate Incubation Time:	48 hours		

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Study Modifications

The received test and control substances were observed to be highly viscous. To facilitate study performance the test and control substances were tested on a per weight basis. 1.0 gram of the test or control substance was weighed directly into a sterile 50 ml conical tube and centrifuged at approximately 2500 rpm for 3 minutes to collect.

Neutralization was achieved by application of 9.0 ml of Dey Engley broth at the conclusion of the selected contact times.

This report was amended to include data from a previous study (NG6677) in which the vehicle control was assessed under similar testing conditions.

Study Notes

The inoculated test substances were manually mixed during each contact time to ensure sufficient contact between the inoculum and the test and control substances.

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Control Results

Neutralization Method: Verified
Media Sterility: Sterile
Growth Confirmation: Confirmed, Morphology on TSA

Calculations

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

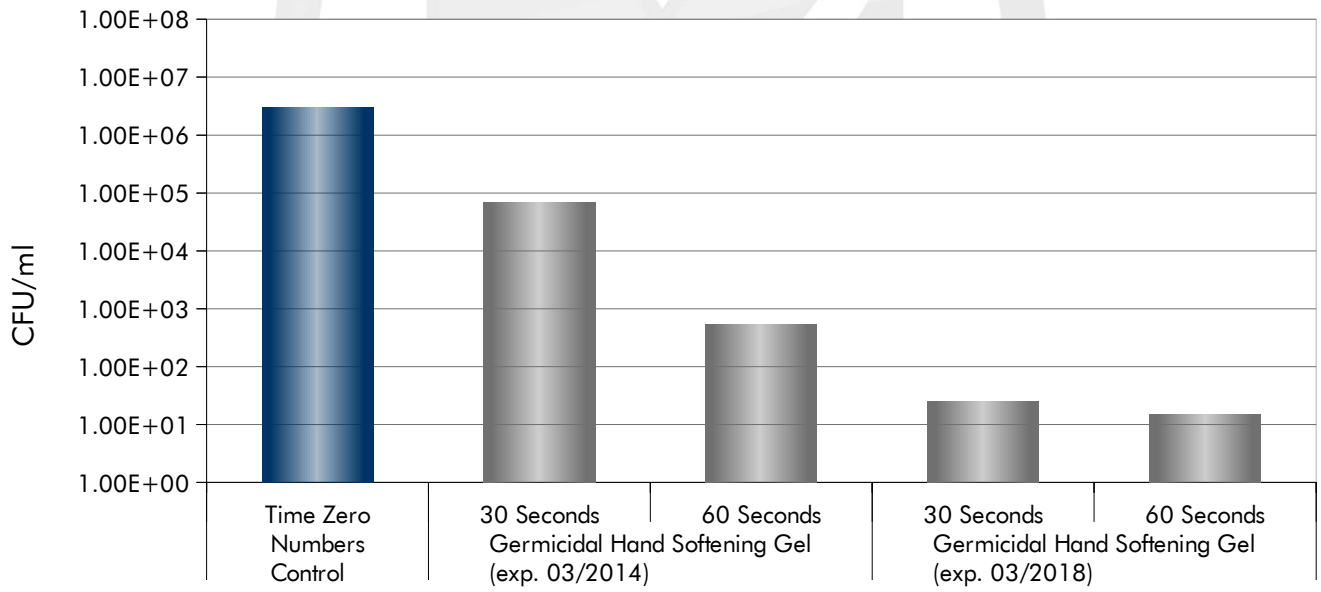
Where:
B = Number of viable test microorganisms in the control substance immediately after inoculation
A = Number of viable test microorganisms in the test substance after the contact time

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

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- *S. aureus* ATCC 6538

Test Microorganism	Test Substance	Contact Time	CFU/ml	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
S. aureus ATCC 6538	Numbers Control	Time Zero	2.90E+06	N/A	
	Germicidal Hand Softening Gel (exp. 03/2014)	30 Seconds	6.75E+04	97.67%	1.63
		60 Seconds	5.30E+02	99.98%	3.74
	Germicidal Hand Softening Gel (exp. 03/2018)	30 Seconds	2.50E+01	99.9991%	5.06
		60 Seconds	1.50E+01	99.9995%	5.29



RESULTS

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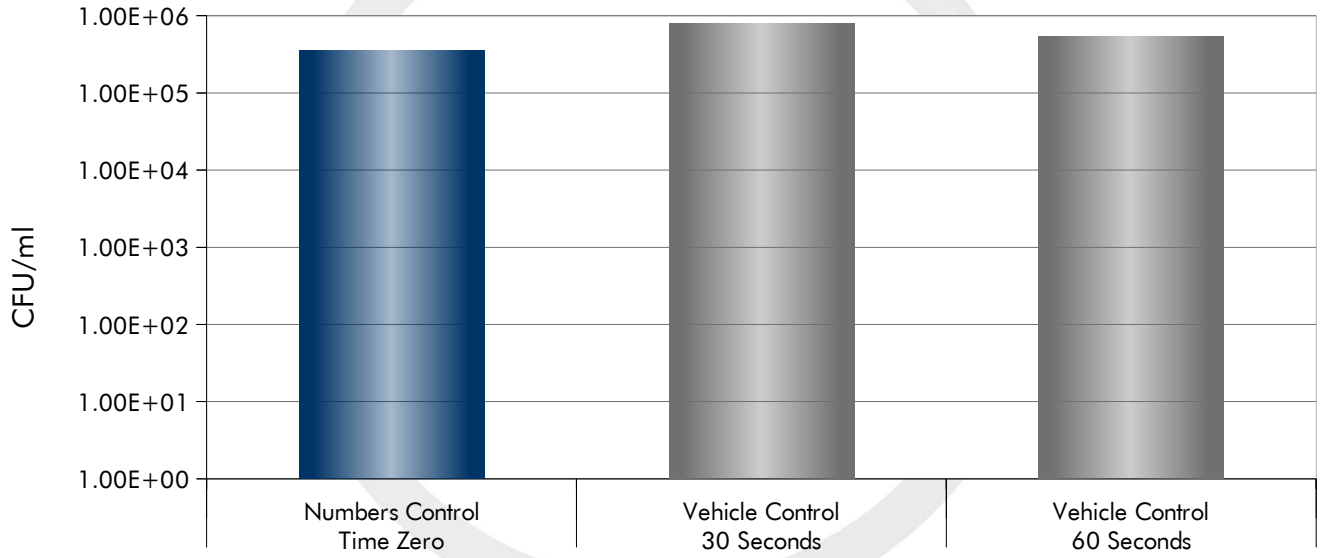
Results of the Study- *S. aureus* ATCC 6538

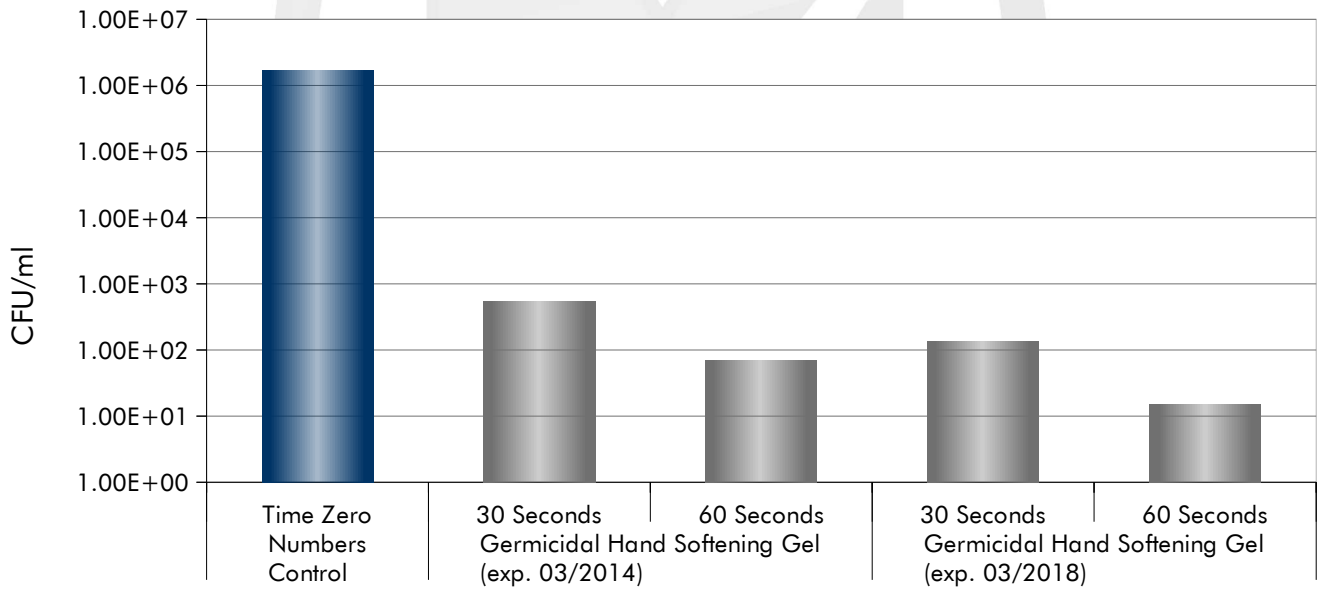
Test Microorganism	Contact Time	Test Substance	CFU/ml	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
S. aureus ATCC 6538	Time Zero	Numbers Control	3.55E+05	N/A	
	30 Seconds	Vehicle Control	7.85E+05	None	None
	60 Seconds	Vehicle Control	5.30E+05	None	None



RESULTS

Results of the Study- *E. coli* ATCC 8739

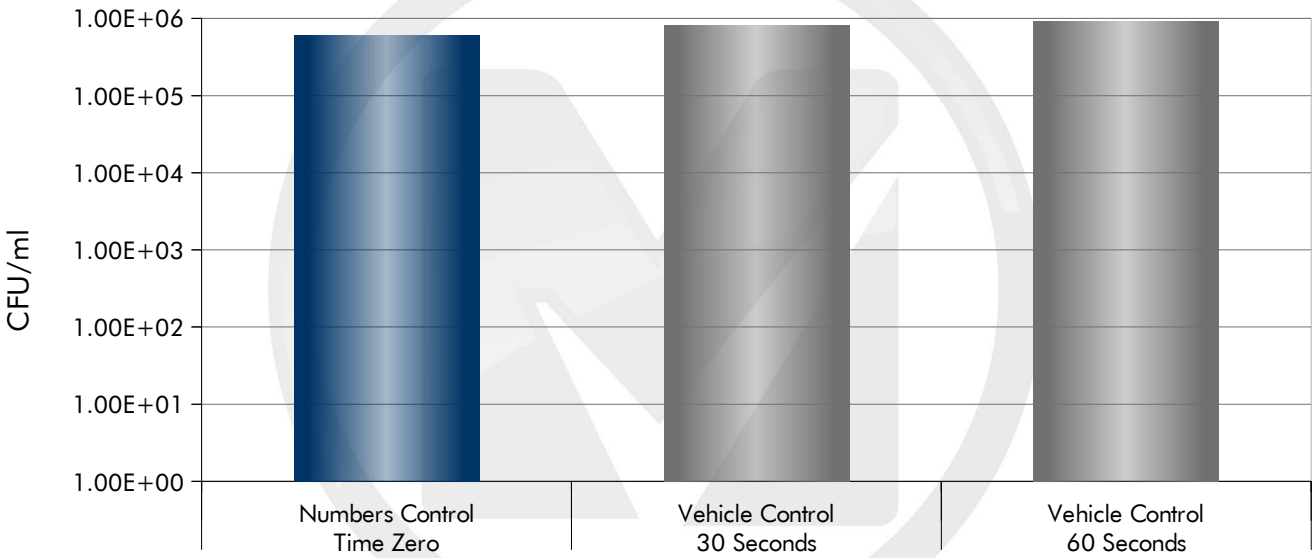
Test Microorganism	Test Substance	Contact Time	CFU/ml	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
E.coli ATCC 8739	Numbers Control	Time Zero	1.65E+06	N/A	
	Germicidal Hand Softening Gel (exp. 03/2014)	30 Seconds	5.35E+02	99.97%	3.49
		60 Seconds	7.00E+01	99.9958%	4.37
	Germicidal Hand Softening Gel (exp. 03/2018)	30 Seconds	1.35E+02	99.992%	4.09
		60 Seconds	1.50E+01	99.9991%	5.04



RESULTS

Results of the Study- *E. coli* ATCC 8739

Test Microorganism	Contact Time	Test Substance	CFU/ml	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
E. coli ATCC 8739	Time Zero	Numbers Control	6.00E+05	N/A	
	30 Seconds	Vehicle Control	8.10E+05	None	None
	60 Seconds	Vehicle Control	9.05E+05	None	None



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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