MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Stella Life's Test Substance Using a Suspension Time-Kill Procedure

Test Method

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

Study Identification Number

NG8899

Study Sponsor

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Test Facility

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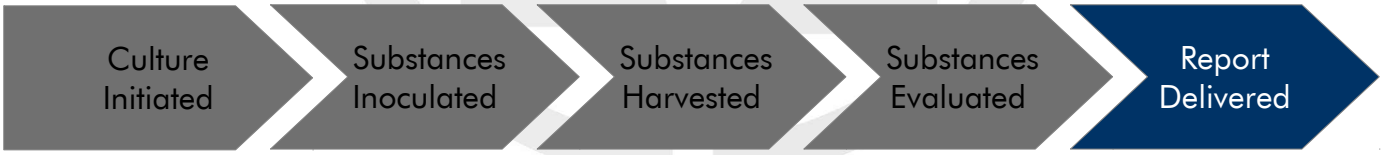
ASTM E2315: General Information

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

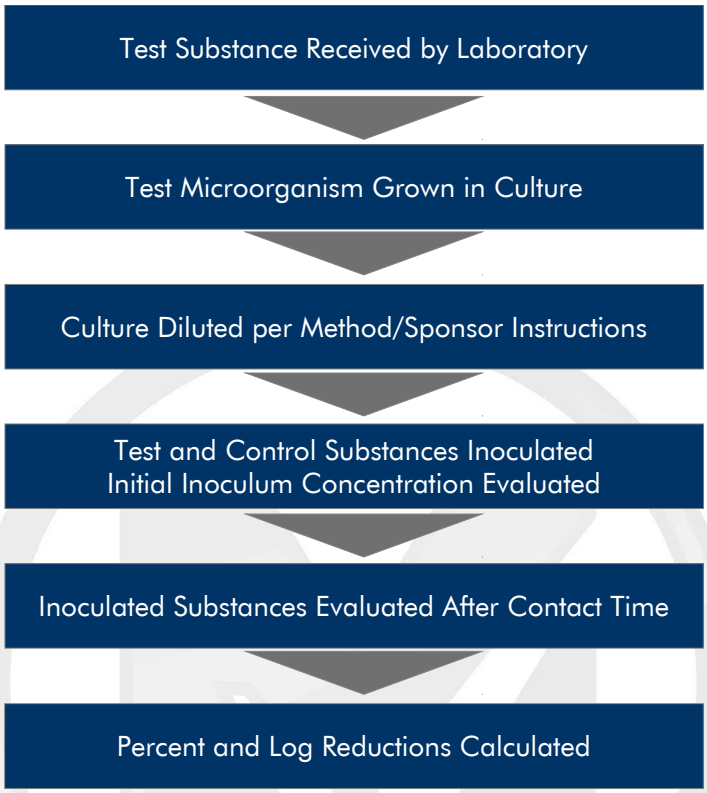
Microchem Laboratory began conducting the ASTM E2315 test method in 2007. Since then, the laboratory has performed thousands of ASTM E2315 tests on a broad array of test substances, against a myriad of bacterial, fungal, and viral species. The laboratory is also experienced with regard to modifying the 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



		B. fragilis 43858		
24MAY2017	26MAY2017	26MAY2017	30MAY2017	05JUN2017
		P. gingivalis 33277		
10MAY2017	26MAY2017	26MAY2017	05JUN2017	05JUN2017

Diagram of the Procedure



Summary of the Procedure

- Test microorganisms are prepared on appropriate agar plates or liquid culture medium.
- The suspension of test microorganisms are 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.

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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 used in this Study

Control Substance Volume:	10 ml	Control Substance:	PBS
Culture Growth Media:	TSA with 5% sheep's blood	Culture Growth Time:	See Study Notes
Culture Dilution Media:	PBS	Inoculum Volume:	0.100 ml
Inoculum Concentration:	> 1 x 10^6	Contact Temp.:	Ambient
Contact Time:	30 minutes	Volume Harvested:	1.0 ml
Neutralizer (Vol.):	PBS (9 ml)	Plating Media:	TSA with 5% sheep's blood
Enumeration Plate Incubation Temperature:	36°C ± 1°C under Anaerobic conditions	Enumeration Plate Incubation Time:	See Study Notes

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Study Modifications

The test substance was neutralized by adding 1 ml of the inoculated test substance to PBS and serially diluted.

Study Notes

- Enumeration Plate Incubation Time:
    - *B. fragilis (*ATCC 43858): 24-48 hours
    - *P. gingivalis* (ATCC 33277): 10 days

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Control Results

Neutralization Method: Verified Media Sterility: Sterile
Growth Confirmation: Confirmed, morphology on appropriate 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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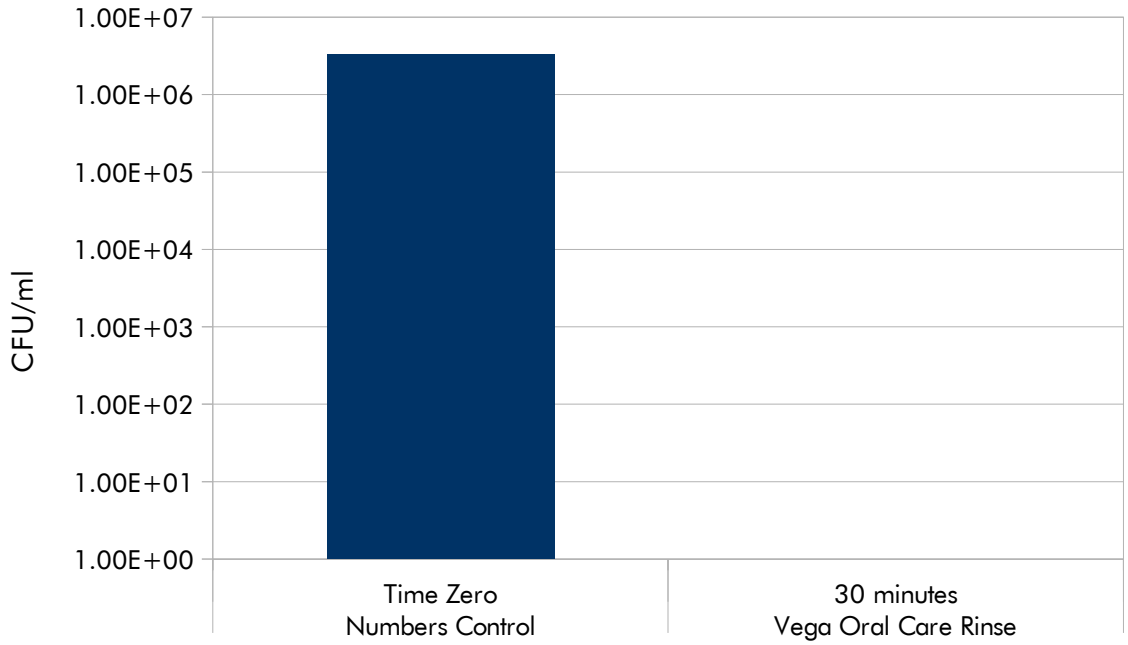
Results of the Study: *P. gingivalis* (33277)

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
P. gingivalis ATCC 33277	Numbers Control	Time Zero	3.30E+06	N/A	
	Vega Oral Care Rinse	30 minutes	<5.00E+00	>99.9998%	>5.82



Note: The limit of detection for this assay was 5.00E+00 and is noted as <5.00E+00 in the table and as 0 in the graph.

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