MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antimicrobial Activity and Efficacy of BOI Environmental's 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

NG17637

Study Sponsor

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Test Facility

Microchem Laboratory
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Round Rock, TX 78681
(512) 310-8378
Testing Performed By: Kyra Christensen and Sage Tuchsen

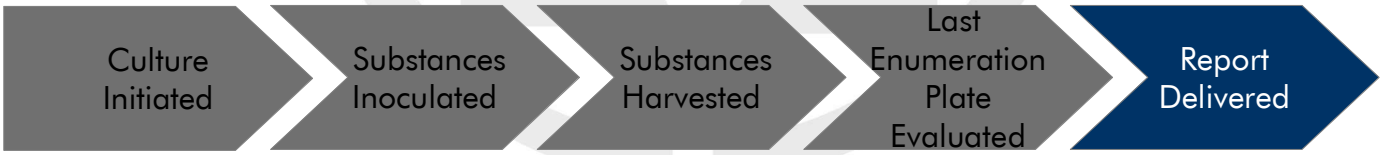
ASTM E2315: General Information

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



Phage Stock	29JUN2021	29JUN2021	02JUL2021	08JUL2021

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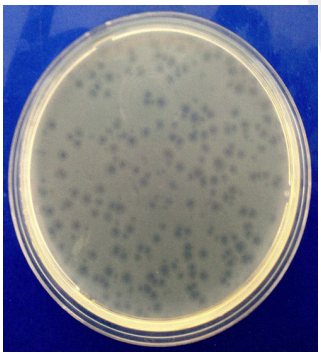
Test Substance Information

The test substance was received on 29MAR2021
Test Substances Received: Always Ready Ozone Spray Device, 3 L Water, Ozone Test Strips

Test Substances arrived ready to use for the conduct of the Study.

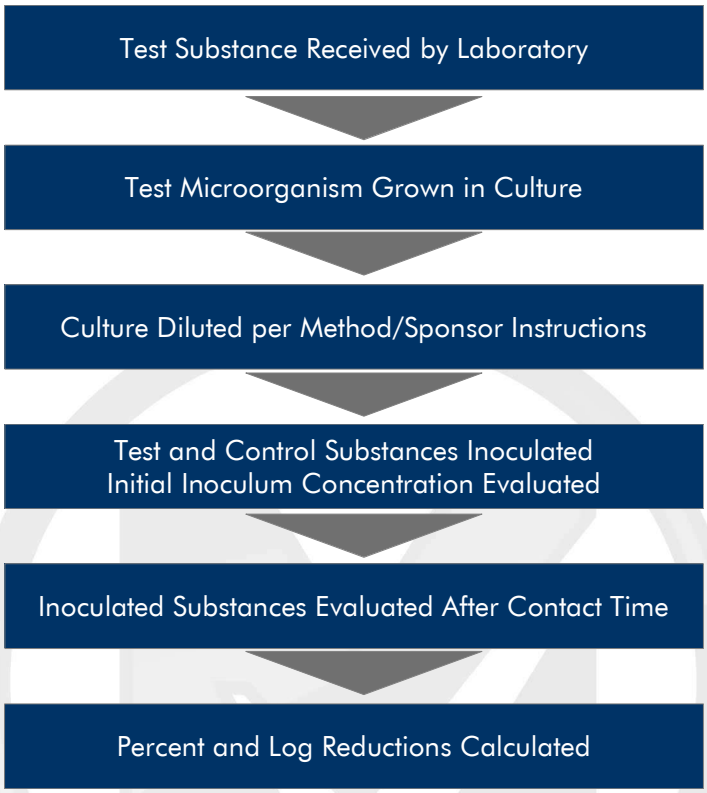
Test Microorganism Information

The test microorganism(s) selected for this test:



MS2 Bacteriophage (MS2), ATCC 15597-B1
This virus is a non-enveloped positive-stranded RNA virus of the bacteriophage family Leviviridae. Bacterial cells are the hosts for bacteriophages, and *E. coli* 15597 serves this purpose for MS2 bacteriophage. Its small size, icosohedral structure, and environmental resistance has made MS2 ideal for use as a surrogate virus (particularly in place of picornaviruses such as poliovirus and human norovirus) in water quality and disinfectant studies.
Permissive Host Cell System for MS2: *Escherichia coli*, 15597

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.

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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

Culture Growth Media:	Tryptic Soy Broth	**Host Culture Growth Time:**	6-24 hours
Culture Diluent	Phosphate Buffered Solution	**Inoculum Volume**	0.100 ml
Target Concentration	≥1.0 x 10^6 CFU/ml	**Contact Temperature**	Ambient
Contact Time	30 seconds, 1 minute, 2 minutes	**Volume Harvested**	0.100 ml
Neutralizer (Vol.)	D/E Broth (9.0 ml)	**2° Neutralizer (Vol.)**	N/A
Incubation Temperature	36 ± 1°C	**Incubation Time**	12 -24 hours
Enumeration Media	50% Tryptic Soy Agar		

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Study Notes

The neutralizer used was Dey Engley Broth.

MS2 host culture, *E. coli* ATCC 15597, was incubated for ~6 hours. A 0.100 mL aliquot was used per plate.

All conicals and serological pipettes used were pre-exposed to ozone.

Before each run, ozone spray was tested to be within the 1.5 ppm range.

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Control Results

Neutralization Method: Valid
Growth Confirmation: Pure and Viable
Media Sterility: No Growth

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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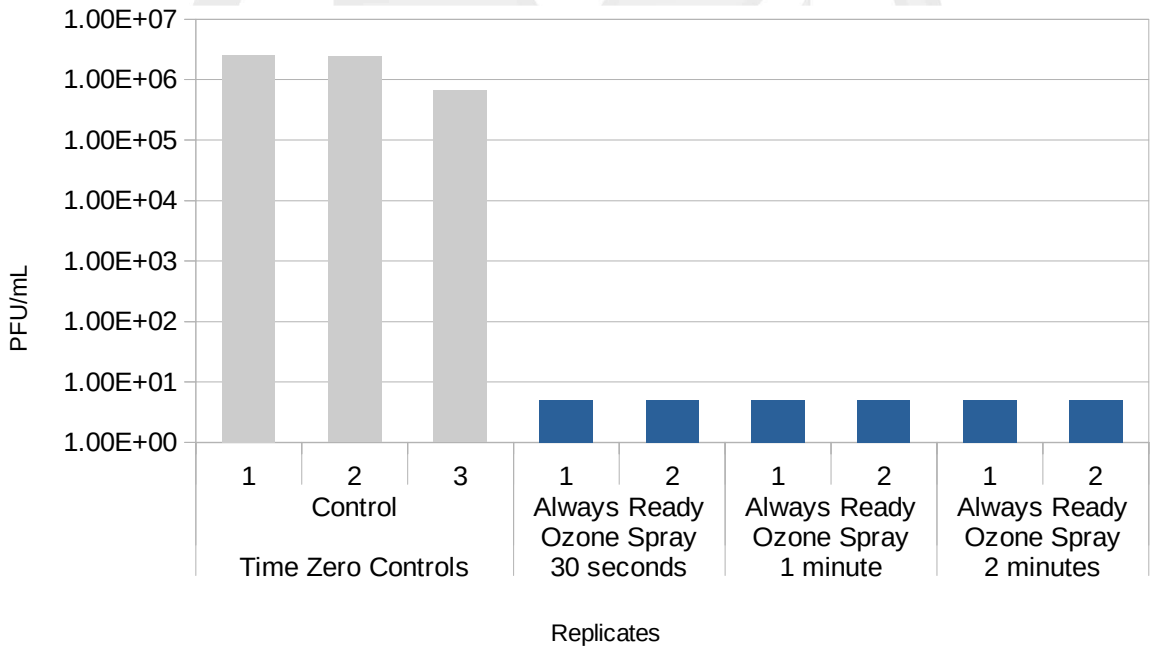
Results of the Study

Test Microorganism	Contact Time	Substance	Replicate	PFU/ml	Average PFU/ml	Percent Reduction Compared to Control at Time Zero	Log Reduction Compared to Control at Time Zero
MS2 Bacteriophage ATCC 15597	Time Zero Controls	Control	1	2.55E+06	2.48E+06	N/A	
			2	2.40E+06			
			3	6.65E+05	6.65E+05		
	30 seconds	Always Ready Ozone Spray	1	<5.00E+00	<5.00E+00	>99.9998%	>5.69
			2	<5.00E+00			
	1 minute	Always Ready Ozone Spray	1	<5.00E+00	<5.00E+00	>99.9998%	>5.69
			2	<5.00E+00			
	2 minutes	Always Ready Ozone Spray	1	<5.00E+00	<5.00E+00	>99.9995%	>5.41
			2	<5.00E+00			

The limit of detection of this assay is 10 CFU per ml of test substance. Values below the limit of detection and calculations that use values below the limit of detection are designated with "<" or ">" as appropriate.



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