MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of CleanCores's Test Device
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

NG14065

Study Sponsor

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Report Author

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Date Reported

19NOV2019

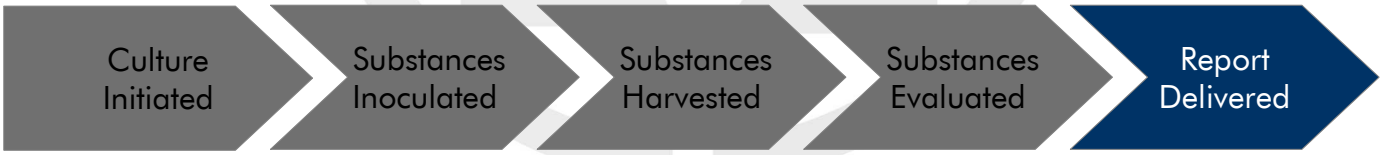
ASTM E2315: General Information

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



L. pneumophila ATCC 33153				
13NOV2019	15NOV2019	15NOV2019	19NOV2019	19NOV2019
E. coli ATCC 8739				
17NOV2019	18NOV2019	18NOV2019	19NOV2019	19NOV2019

Test Substance Information

The test device was received on 28OCT2019.

Test Device Serial Number: 17853

Test device arrived ready to use.

Test Microorganism Information

The test microorganisms selected for this test:

E. coli ATCC 8739
L. pneumophila ATCC 33153

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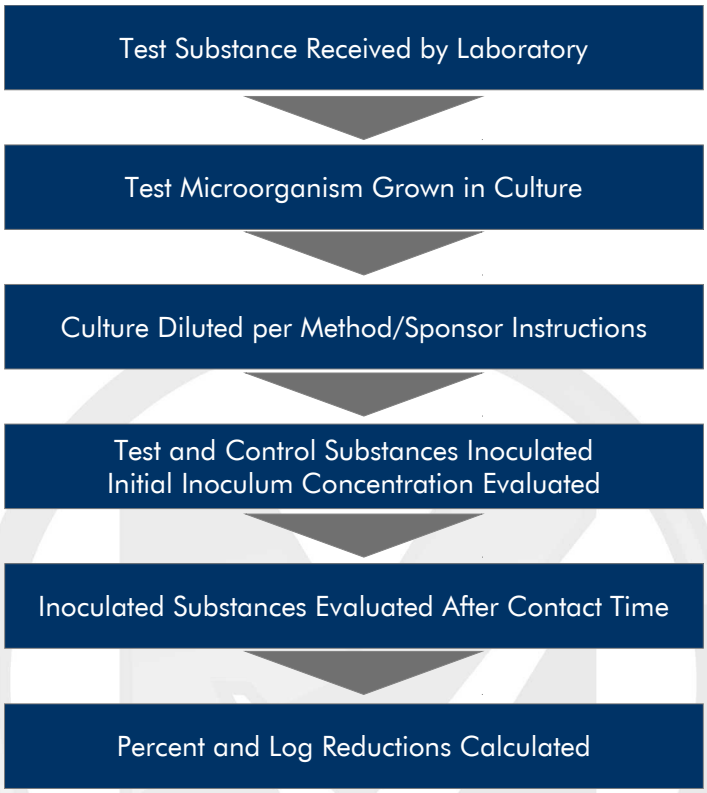
Diagram of the Procedure



Summary of the Procedure

- Test microorganisms are prepared in liquid culture medium for *E. coli. L. pneumophila* is grown on buffered charcoal yeast extract agar (BCYEA).
- The suspension of test microorganism is standardized, as needed, by dilution in a buffered saline solution.
- Test substance (the ozonated water) 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:	100 ml	Replicates:	Single
Control Substance Volume:	N/A	Control Substance:	N/A

L. pneumophila ATCC 33153:

Culture Growth Media:	BCYEA	Culture Growth Time:	53 hours
Culture Dilution Media:	PBS	Inoculum Volume:	0.100 ml
Inoculum Concentration:	≥ 1.0 x 10^{10} CFU/ml	Contact Temp.:	22.5 °C
Contact Time:	5 min, 10 min	Volume Harvested:	1.000 ml
Neutralizer (Vol.):	09 ml	Plating Media:	BCYEA
Enumeration Plate Incubation Temperature:	36°C ± 1 °C	Enumeration Plate Incubation Time:	86 hours

E. coli ATCC 8739:

Culture Growth Media:	TSB	Culture Growth Time:	24 hours
Culture Dilution Media:	PBS	Inoculum Volume:	0.100 ml
Inoculum Concentration:	≥ 1.0 x 10^{10} CFU/ml	Contact Temp.:	22.5 °C
Contact Time:	5 min, 10 min	Volume Harvested:	1.000 ml
Neutralizer (Vol.):	09 ml	Plating Media:	TSA
Enumeration Plate Incubation Temperature:	36°C ± 1 °C	Enumeration Plate Incubation Time:	16 hours

Study Modifications

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

Study Notes

No sterility controls were plated the day of testing for *Legionella pneumophila* ATCC 33153, 15NOV2019. This is not thought to impact the study outcome because no contamination was observed on the test or control plates for this day of testing.

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Control Results

Neutralization Method: Confirmed Media Sterility: Sterile*
Growth Confirmation: Confirmed; morphology on growth media
**Note: See note on page 6.*

Calculations

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

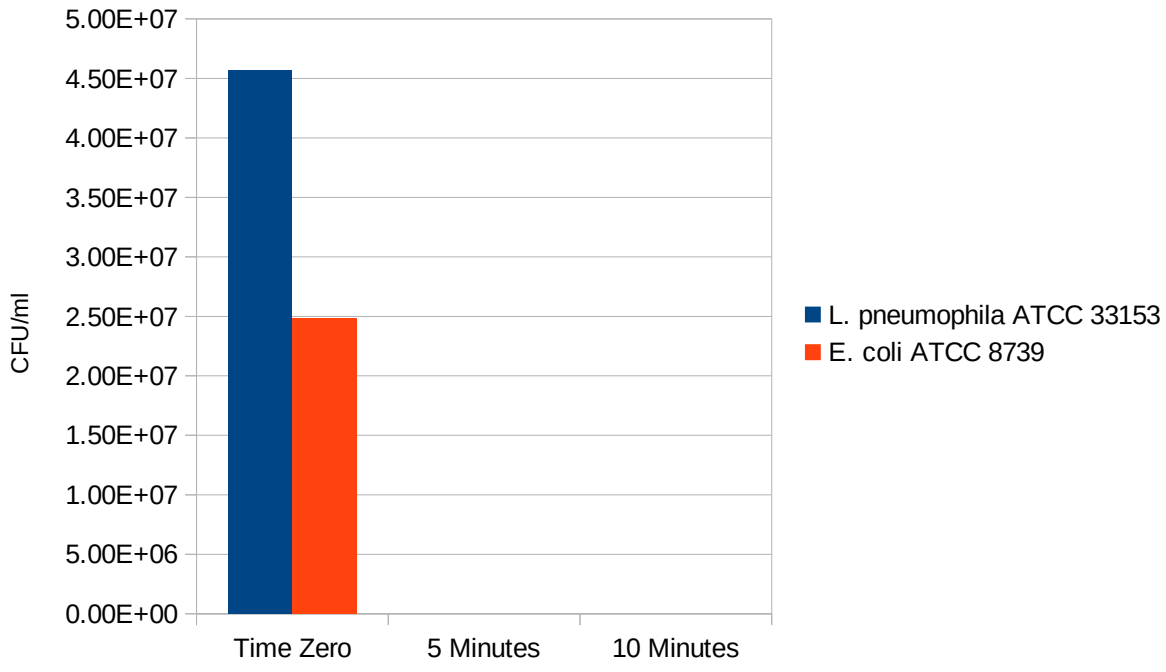
Where:
B = Number of viable test microorganisms in the control substance immediately after inoculation
A = Number of viable test microorganisms in the test substance after the contact time

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

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 Device	Contact Time	Data Description	Test Microorganism	
			L. pneumophila ATCC 33153	*E. coli* ATCC 8739
CleanCore Ozone Water Device	Time Zero	CFU/ml	4.57E+07	2.48E+07
	5 Minutes	CFU/ml	<1.00E+01	<1.00E+01
		Log_{10} Reduction	>6.66	>6.39
	10 Minutes	CFU/ml	<1.00E+01	<1.00E+01
		Log_{10} Reduction	>6.66	>6.39



The limit of detection for this assay is 10 CFU/ml. Values observed below this limit are presented as <1.00E+01 in the table and zero in the graph above.

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Results of the Study (cont.)

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