Mobile Outfitters
Pruvia Copper Spray

Antimicrobial Test

ASTM E1153 and E2315 Time Kill Study

Study Title

Antibacterial Activity and Sanitizing Efficacy of Pruvia Test Substance

Test Method

Modified ASTM International Method E1153 Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces and ASTM International Method E2315 Assessment of Antimicrobial Activity using a Time-Kill Procedure

Study Identification Number

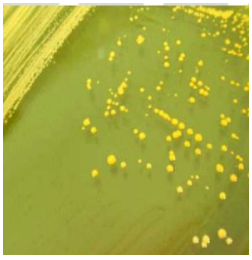
NG5817 & NG5818

Study Timeline - E2315

Study Timeline - E1153

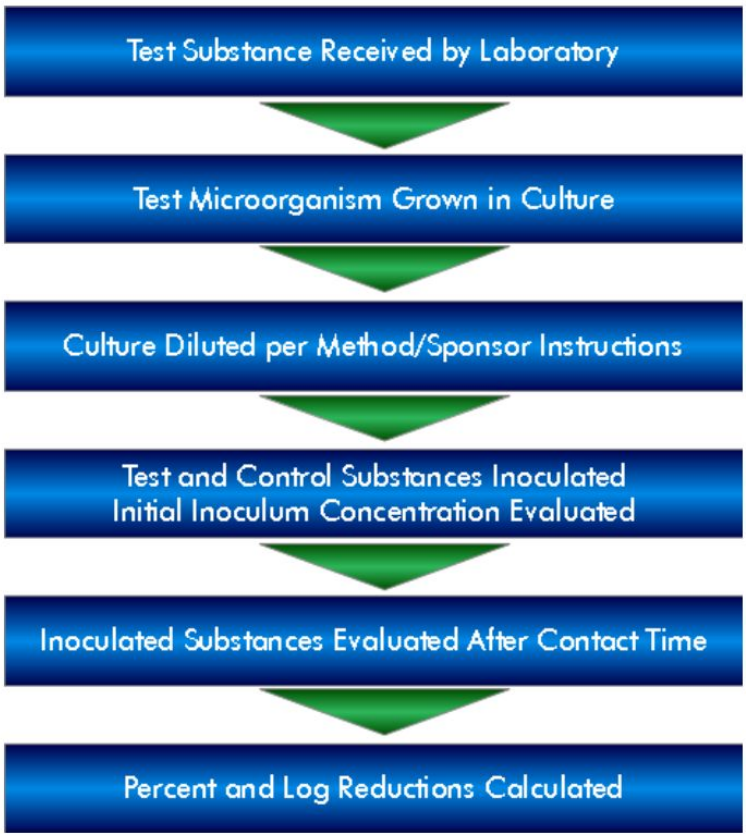
Test Microorganism Information

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.

Diagram of the Procedure - E2315



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

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 E2315 Study

ASTM International does not specify performance criteria, therefore it may be established by the Study Sponsor.

Testing Parameters used in this Study ASTM E2315 Study

Test Substance Volume: 10 ml
Control Substance Volume: 10 ml
Replicates: Single
Control Substance: PBS
Culture Growth Time: 18-24 Hours
Inoculum Volume: 0.04ml
Contact Temp.: Ambient (25°C +- 2°C)
Volume Harvested: 1.0ml
Plating Media: Tryptic Soy Agar
Enumeration Plate Incubation Time: 24-48 hours

Control Results ASTM E2315 Study

Neutralization Method: Validated
Media Sterility: Sterile
Growth Confirmation: Confirmed, morphology on TSA

Calculations ASTM E2315 Study

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

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

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

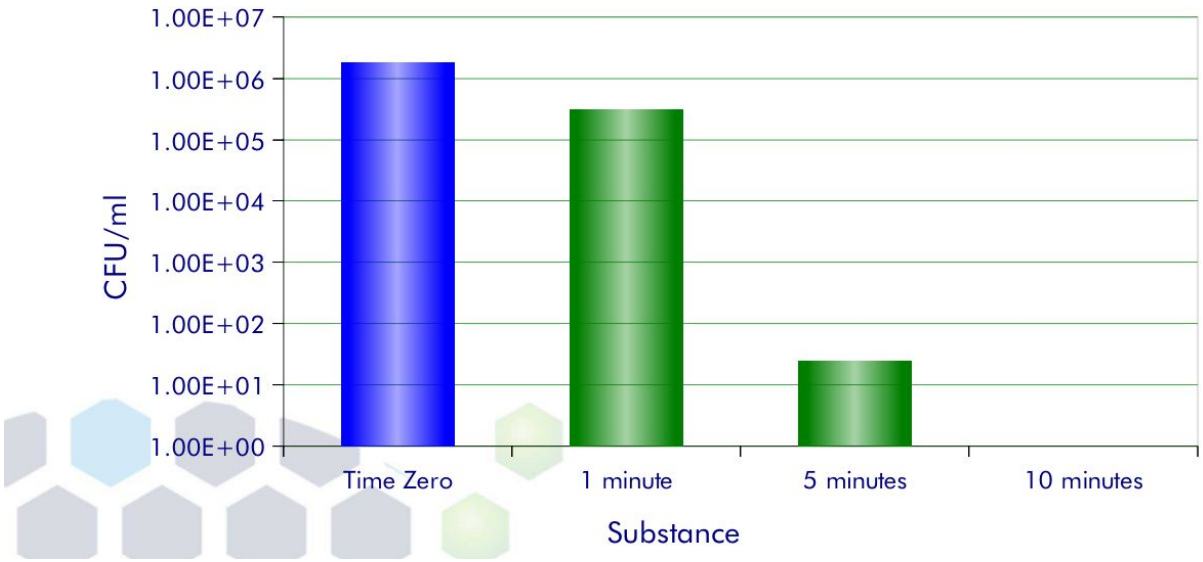
Where:

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 ASTM E2315 Study

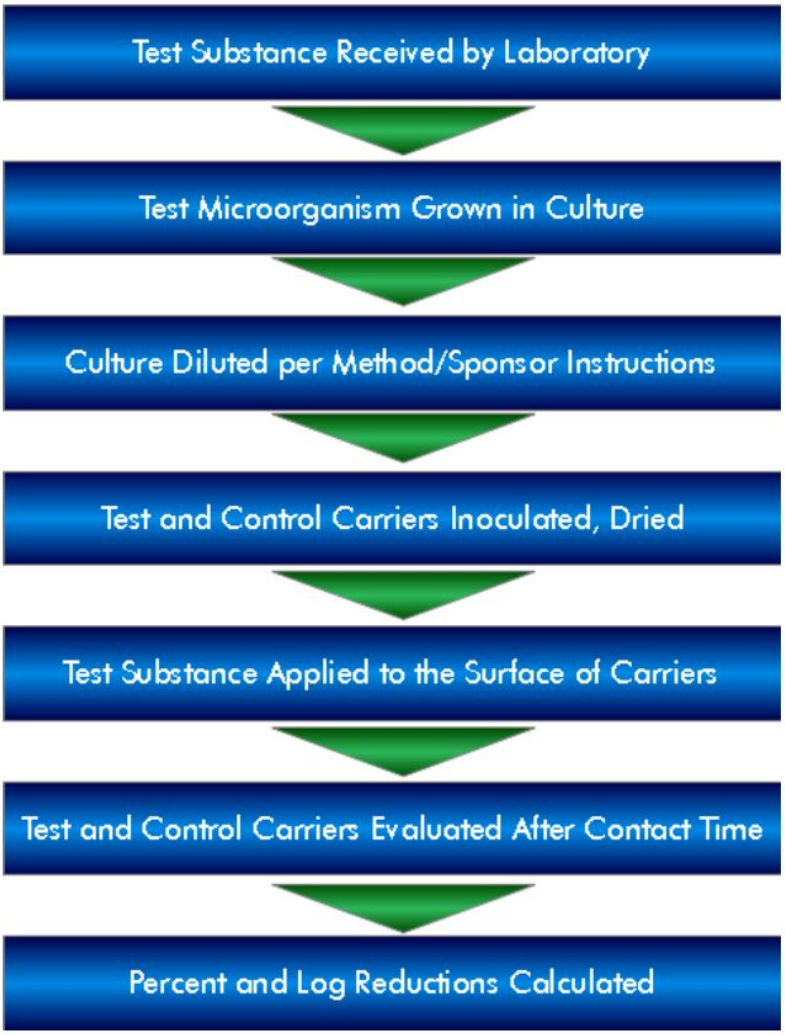
Test Microorganism	Contact Time	Test Substance	CFU/ml	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
S. aureus 6538	Time Zero	Control	1.80E+06	N/A	
	1 minute	Copper Spray	3.15E+05	82.53%	0.76
	5 minutes	Copper Spray	2.50E+01	99.9986%	4.86
	10 minutes	Copper Spray	<5.00E+00	>99.9997%	>5.56



The limit of detection for the assay was 5 CFU/ml. Results observed below the limit of detection are represented as <5.00E+00 in the graph and zero in the chart.

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

Diagram of the Procedure ASTM E1153 Study



Summary of the Procedure ASTM E1153 Study

- *The test microorganism is prepared, usually by growth in liquid culture medium.*
- *The test culture may be supplemented with an artificial soil load, such as horse or fetal bovine serum, for one-step cleaner/sanitizer claims.*
- *Sterilized carriers are inoculated with a volume of the test culture. Inoculated slides are dried in an incubator. Only completely dried carriers are used in the test.*
- *Test carriers are treated with the test substance and incubated for the predetermined contact time.*
- *Control carriers are treated with a buffered saline solution and are allowed to sit for the predetermined contact time.*

- *At the conclusion of the contact time, test and control carriers are chemically neutralized.*
- *Dilutions of the neutralized test substance are evaluated using appropriate growth media to determine the surviving microorganisms at the respective contact time.*
- *The effect of the test substance is compared to the effect of the control substance in order to determine microbial reductions.*

Criteria for Scientific Defensibility of an ASTM E1153 Study

To consider an ASTM E1153 study to be scientifically defensible, the following criteria must be met:

1. *The average number of viable microorganisms recovered from the control carriers must be approximately 7.5 x 10_5 cells/carrier or greater.*
2. *Ordinary consistency between replicates must be observed for the control carriers.*
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 E1153 Study

ASTM International defines passing criteria to be a 3 Log_{10} or 99.9% reduction in the treated test carriers when compared to the control carriers.

Testing Parameters used ASTM E1153 Study

Test Carrier Size:1 inch x 3 inc
Replicates: Single
Test Substance Volume:2 Sprays followed by a wipe with microfiber towel
Culture Growth Media: Tryptic Soy Broth
Culture Supplement: 0.01% Triton X-100
Inoculum Concentration: 1 x 10_5 CFU/Carrier
Carrier Dry Temp: 25°C ± 2°C
Contact Temp.: Ambient (25°C ± 2°C)
Contact Time: 2 hours

Enumeration Plate: 36°C ± 1°C
Incubation Temperature:
Incubation Conditions: Aerobic
Culture Growth Time: 24 hours
Carrier Inoculum Volume: 0.020 ml
Carrier Inoculum Area: 1 inch x 1 inch
Carrier Dry Time: 30 minutes
Contact Humidity: 45.00%
Neutralizer: Dey/Engley Broth (20 ml)
Enumeration Plate Incubation Time: 24-48 hours

Control Results ASTM E1153 Study

NeutralizationMethod: Validated

MediaSterility: Sterile

Growth Confirmation: Confirmed, morphology on TSA

Calculations ASTM E1153 Study

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

Where:

B = Number of viable test microorganisms on the control carriers after the contact time A = Number of viable test microorganisms on the test carriers after the contact time

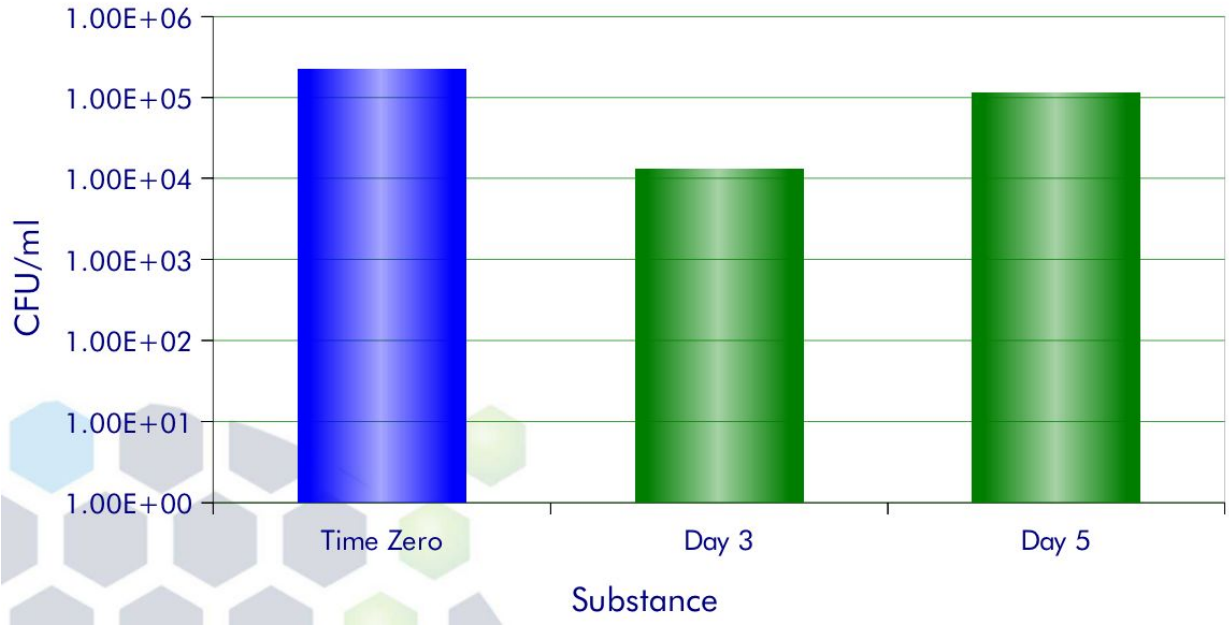
$$\text{Log}_{10}\text{Reduction} = \text{Log}\left(\frac{B}{A}\right)$$

Where:

B = Number of viable test microorganisms on the control carriers after the contact time A = Number of viable test microorganisms on the test carriers after the contact time

Results of the Study ASTM E1153 Study

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 6538	Time Zero	Control	2.25E+05	N/A	
	Day 3	Copper Spray	1.30E+04	94.22%	1.24
	Day 5	Copper Spray	1.15E+05	48.89%	0.29



Testing Performed By:

Antimicrobial Test Laboratories 1304 W. Industrial Blvd Round Rock, TX 78681 (512) 310-8378