MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity of AtmosAir's Bipolar Ion Producing Device: Matterhorn

Test Method

ASTM International Method E1153 Modified for Devices
Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces

Study Identification Number

NG7257

Study Sponsor

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Test Facility

Microchem Laboratory
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Testing performed by: B. Richard

ASTM E1153: 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 E1153 is a quantitative test method designed to evaluate the antimicrobial efficacy of sanitizers on pre-cleaned inanimate, nonporous, non-food contact surfaces. The method is typically used with a maximum contact time of 5 minutes, during which the sanitizer reduces the concentration of viable test microorganisms. ASTM E1153 utilizes non-antimicrobial agents as controls to establish baselines for microbial reductions. The ASTM E1153 method is a benchmark method for non-food contact surface sanitizers and is recognized by several regulatory agencies as an approved method for claim substantiation. See study modifications for changes made to the study method to accommodate a device.

Laboratory Qualifications Specific to ASTM E1153

Microchem Laboratory began conducting the ASTM E1153 test method in 2007. Since then, the laboratory has performed hundreds of ASTM E1153 tests on a broad array of test substances, against a myriad of bacterial and fungal species. The laboratory is also experienced with regard to modifying the test method as needed in order to accommodate customer needs. Every ASTM E1153 test at Microchem Laboratory is performed in a manner appropriate for the test substances submitted by the Study Sponsor, while maintaining the integrity of the method.

Study Timeline

C. difficile 43598 (Endospores)

Spore Stock (N/A)	07 JUN 2016	07 JUN 2016	14 JUN 2016	23 JUN 2016

Test Device Information

The test device was received on 12 AUG 2015. The following is a picture of the device as it was received.

Test device was operated by the scientist according to instructions provided by the study sponsor.

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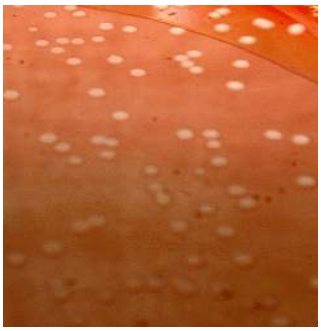
Test Microorganism Information

The test microorganism(s) selected for this test:



Clostridium difficile 43598

This bacteria is a Gram-positive, rod shaped, endospore generating obligate anaerobe. *Clostridium* species are part of the normal human gut flora that produce spores which are highly resistant to chemical and environmental conditions. *C. diff* is commonly associated with hospital acquired infections and is know to cause antibiotic assisted colitis. Because of it's high resistance to antimicrobials, *C. difficile* is a benchmark bacteria for sporicidal and sterilant activity of chemicals.

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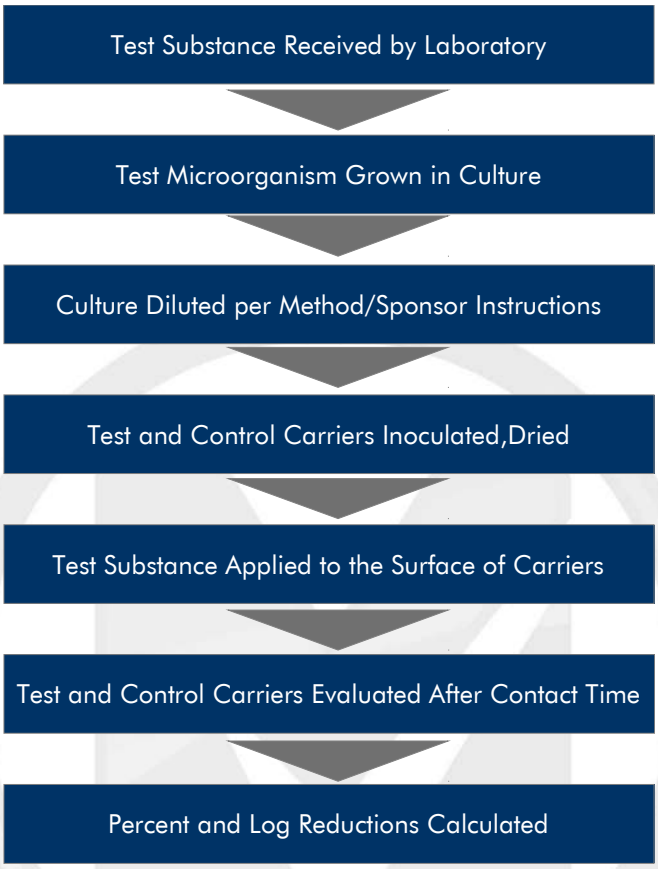
Diagram of the Procedure



Summary of the Procedure

- 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 surfaces are dried. Only completely dried carriers are used in the test.
- Test carriers are treated with the test device and incubated 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.

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Criteria for Scientific Defensibility of an ASTM E1153 Study

For Microchem Laboratory 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

Due to the modified nature of testing, the study sponsor may determine success criteria.

Testing Parameters used in this Study

Test Device	Matterhorn	Replicates	Triple Rep
Carrier	1" x 3" glass slides		

C. difficile 43598 (Endospores)

Culture growth media	Spore Stock (N/A)	Culture growth time	Spore Stock (N/A)
Culture dilution media	Phosphate buffered saline	Culture supplement	Tri-part (=5% FBS)
Inoculum concentration	≥1.0 x 10^6 CFU/carrier	Inoculum volume	0.010 ml
Contact time(s)	6, 18, and 24 hours	Contact temperature	Ambient
Neutralizer (Volume)	Dey Engley Broth (20ml)	Enumeration media	BHI-YHT
Enumeration plate incubation temp	36°C ± 1°C (Anaerobic)	Enumeration plate Incubation time	48-72 hours

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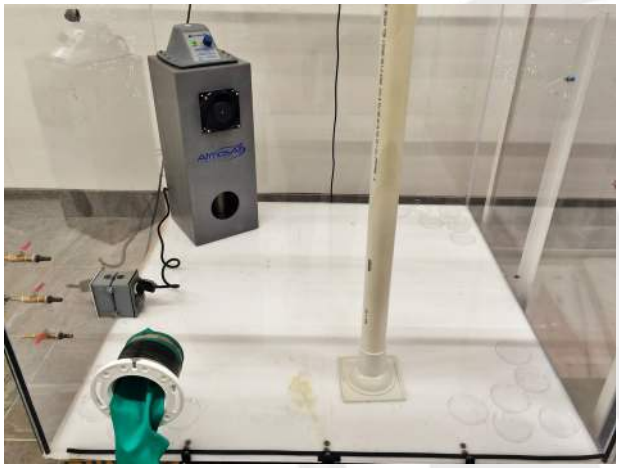
Study Modifications

Due to the device's technology, the surface time kill was ran in a chamber measuring 4' x 4' x 4'.

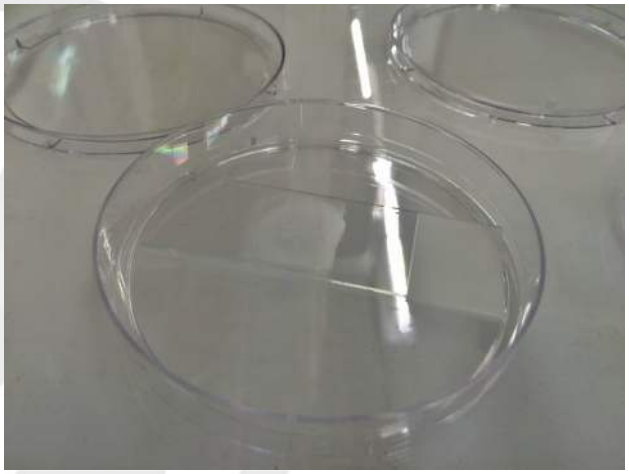
Study Notes

There are no additional study notes.

Study Photographs



The photo above shows the Matterhorn treating inoculated slides inside the test chamber



The photo above shows glass carriers inoculated with microorganisms

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Control Results

Neutralization Method: Not applicable
Growth Confirmation: Confirmed
Media Sterility: Confirmed

Calculations

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

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

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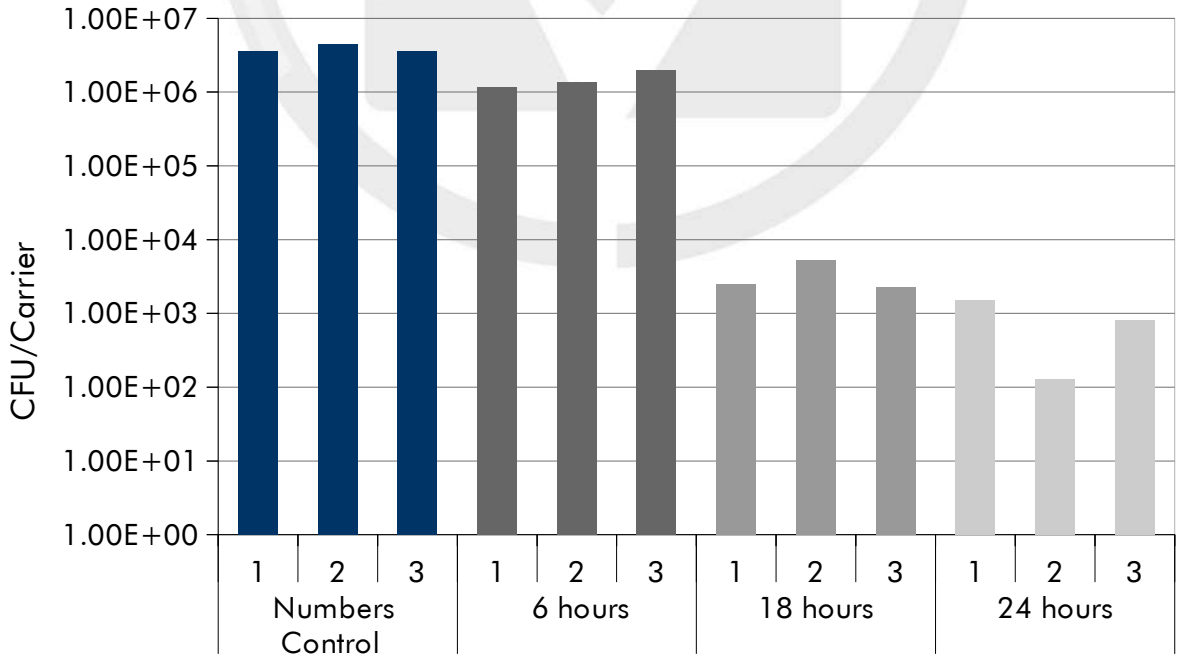
Results of the Study

The following graph and table are the calculated results for *C. difficile* 43598 (Endospores) when treated with Matterhorn in a closed chamber measuring 4' x 4'.

Test Device	Test Microorganism	Carrier Control/ Treatment	Replicate or Control Time Point	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Contact Time	Log_{10} Reduction Compared to Control at Contact Time
Matterhorn	*C. difficile* 43598 (Endospores)	Numbers Control	6 hours	3.60E+06	N/A		
			18 hours	4.50E+06			
			24 hours	3.60E+06			
		6 hours	1	1.19E+06	1.53E+06	57.59%	0.37
			2	1.38E+06			
			3	2.01E+06			
		18 hours	1	2.50E+03	3.33E+03	99.93%	3.13
			2	5.20E+03			
			3	2.30E+03			
		24 hours	1	1.51E+03	8.17E+02	99.98%	3.64
			2	1.30E+02			
			3	8.10E+02			

The limit of detection for this assay is 1.00E+01 results below the limit of detection are reported as <1.00E+01.



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