MICROCHEM LABORATORY

STUDY REPORT

Study Title

Measurement of the Antimicrobial Efficacy of Atlantic Microbial Technologies' Fogging Device

Test Method

Custom Device Study Based on: ASTM E1153 and AOAC 961.02, Modified for use with Fogging Device

Study Identification Number

NG20231

Study Sponsor

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Test Facility

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Purpose of the Study

The purpose of this study was to determine the antimicrobial efficacy of Atlantic Microbial Technologies, LLC's device. Evaluation of microbial kill over a short term was performed along with evaluation of inhibition of future microbial growth on treated surfaces.

Brief History of the Performing Laboratory

Microchem Laboratory is located in the greater Austin, Texas area. It is owned and operated by microbiologist Dr. Benjamin Tanner. The core of the company was founded by Dr. Tanner as Antimicrobial Test Laboratories in 2006. Antimicrobial Test Laboratories was later combined with a niche cosmetic testing lab and Microchem Laboratory, founded in 1988 by Dr. Norman Miner. The combined labs have operated under one roof as Microchem Laboratory since 2016. Microchem Laboratory is ISO 17025 accredited and offers testing in compliance with current Good Laboratory Practice (GLP) regulations as stipulated by EPA and FDA. Clients are always welcome to tour the lab, observe studies, and audit the lab's quality systems.

Study Timeline

Test Substance Received	Cultures Initiated	Carriers Inoculated	Carriers Treated	Enumeration Plates Evaluated	Report Delivered
13SEP2022	20SEP2022	21SEP2022	21SEP2022	22-26SEP2022	30SEP2022

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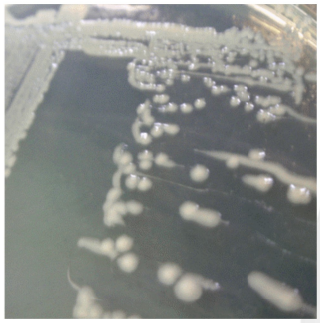
Test Device Information

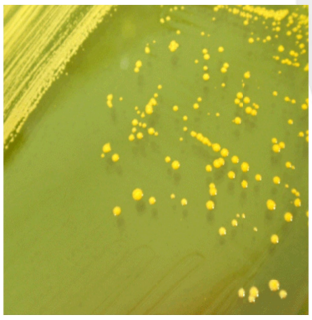
Name of Test Device:	Dry Decon 50
Manufacturer:	Atlantic Microbial Technologies, LLC
Name of Test Substance:	7.5% Food Grade Hydrogen Peroxide
Manufacturer:	Bulk Peroxide
Mode of Active:	Hydrogen Peroxide via Fogging

Test Microorganism Information



Salmonella enterica

This bacteria is Gram-negative, rod-shaped, facultative anaerobe. Like the closely related *Escherichia* genus, *Salmonella* are common to all parts of the world and share habitats in the digestive systems of cold and warm-blooded animals. *S. enterica* is one of the most common bacteria associated with zoonotic and foodbourne illness. Because of it's regular occurrence and pathogenicity, *S. enterica* is a common bacteria for measuring disinfectant efficacy.



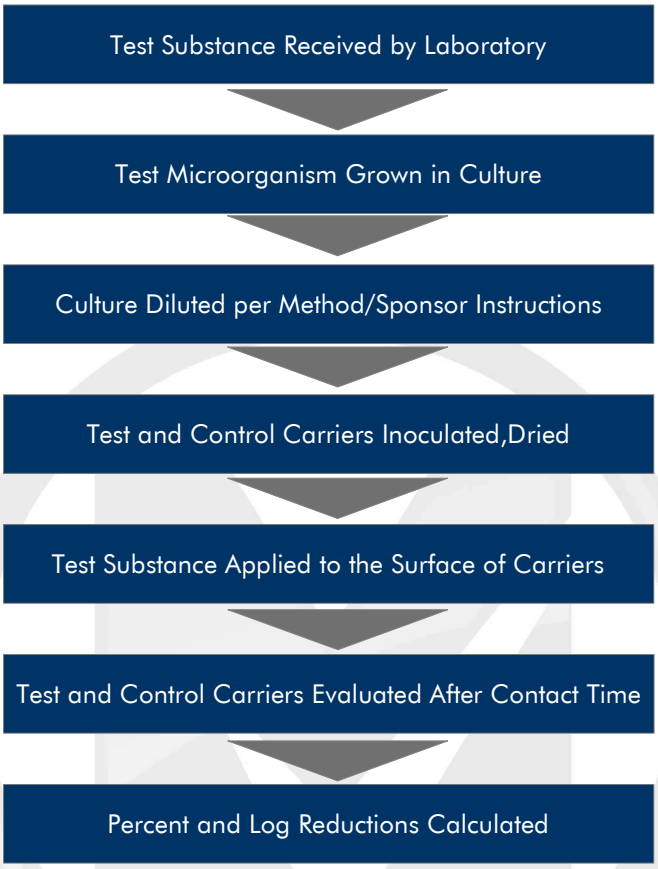
Staphylococcus aureus 6538

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.

Aspergillus brasiliensis 16404

This fungi is a conidiophore, or a sexual spore generating aerobic fungus. *A. brasiliensis*, formerly listed as a strain of *A. niger*, is related to other *Aspergillus* species in that they produce spores which are highly resistant to chemical and environmental conditions. *A. brasiliensis* is commonly used as a benchmark fungus for antimicrobial fungicides and preservatives used in pharmaceutical and personal care products.

Diagram of the Procedure



Summary of the Procedure

- Test microorganisms were prepared in appropriate liquid broth or harvested as spores from mature agar plates, as appropriate and the resulting suspension was diluted to achieve $\geq 1x10^7$ CFU/mL.
- Test and control carriers were inoculated with ten microliters of the microbial preparation and allowed to dry in optimal conditions for test microorganism.
- Test carriers were treated for the Sponsor-determined contact time.
- Test carriers were harvested into liquid media and plated in optimal incubation conditions and time for the test microorganism.
- After incubation, microbial concentrations were determined and reductions were calculated relative to pre-treatment controls.
- TSA, NTA and PDA agar plates, for *Salmonella enterica, Staphylococcus aureus and Aspergillus brasiliensis,* respectively, were inoculated directly from cultures (heavily inoculated) by spreading and treated with fog, then removed and placed under ideal growth conditions.

Study Design and Intent

This study was designed to assess the short/intermediate term microbial kill potential of the device and treatment, as well as the inhibitory action of the treatment on future microbial growth. Ordinary surface-based time-kill testing was used to evaluate short and intermediate term efficacy. Heavily inoculated petri dishes, placed in the chamber for treatment then incubated under ideal conditions thereafter, were used to evaluate inhibition of microbial growth after fogging treatment.

Passing Criteria

Due to the modified nature of the study, passing criteria may be determined by the Study Sponsor prior to test initiation. If no passing criteria is established, a conclusion about the data is not provided by Microchem Laboratory, but the Study Sponsor may determine significance based on statistical interpretation or other means.

Testing Parameters

Culture Growth Media:	TSB (Bacteria) PDA (Fungi)	**Culture Growth Time:**	18-24 hours (Bacteria) >5 days (Fungi)
Culture Dilution Media	Sterile DI Water	**Inoculum Volume**	0.010 mL
Carrier Type	1" x 3" Glass Slides	**Carrier Dry Temp. and Humidity**	Ambient
Contact Time	10 minutes	**Contact Temperature and Humidity**	N/A*
Harvest Media (Volume)	Dey-Engley Neutralizing Broth with 0.1% Catalase	**Enumeration Media**	Tryptic Soy Agar, Nutrient Agar, Potato Dextrose Agar
Incubation Temperature	36 ±1 °C (bacteria) 30 ±1 °C (mold)	**Incubation Time**	18-24 hours (SE10708) 24-48 hours (SA6538) 48-72 hours(AB16404)

*Usage of Hygrometer was disallowed by the test conditions possibly damaging the equipment.

Key Study Details

- The test device was connected directly to a port on the $1m^3$ chamber via ~2ft of flexible PVC tubing. The Study-Sponsor provided "S-shaped" hard tubing was not included. A fan was connected to the opposite end using a vent tube to evacuate the chamber after treatment. Carriers were placed on the floor of the chamber, elevated by test tube racks during treatment. All microorganisms and contact times were evaluated simultaneously.

- All bacterial test cultures were purified via sterile DI water wash prior to inoculation of carriers.

- To facilitate comparable environmental conditions, the control carriers were kept in sealed, moistened bags to more closely resemble the conditions of the test chamber. This was performed for all controls except the time zero controls, which were harvested prior to treatment.

- The test substance was diluted and observed to be at approximately 7.5% Hydrogen Peroxide using Bartovation Hydrogen Peroxide Test Strips.

- Prior to treatment, sterile DI Water was run through the test device for approximately 5 minute and then the test substance was run through the test device for approximately 5-10 minutes to clear the device of any leftover test substance or rinse water.

- The test substance was discarded by the test device after turning on the power. A new batch of test substance was made and confirmed to be approximately 7.5% Hydrogen Peroxide. The test device was then activated again and testing resumed.

- Carrier treatment was performed by fogging for 1 minute until the chamber was saturated, then initiating the corresponding contact time. The device was allowed to run for the duration of the contact time with passive venting of excess test substance. At the conclusion of the contact time, the timer was paused, the device was deactivated, the vent fan was turned on, and the chamber was evacuated prior to carrier harvesting.

- Additional growth prevention reference (GPR) plates containing the appropriate agar type for each test microorganism were inoculated and placed within the chamber for the duration of testing to evaluate microorganism inactivation. After treatment, the GPR plates were incubated alongside the test materials. See table in Results section (pg. 9) for observations.

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Control Results

Neutralization Method: Neutralization Confirmed
Media Sterility: Sterile
Growth Confirmation: Pure and Viable

Calculations

CFU/mL = (Average plate count) x 1:10 serial dilution factor

CFU/carrier = (Average plate count) x 1:10 serial dilution factor x media dilution factor

CFU/carrier = CFU/mL x total harvest media volume

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

$$\text{Log}_{10}\ \text{Reduction} = \text{Log}(B/A)$$

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

Results: Efficacy – *S. enterica*

Test Microorganism	Test Substance	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Controls	Log_{10} Reduction Compared to Controls
S. enterica ATCC 10708	Control	Time Zero	1	6.30E+05	5.36E+05	N/A	
			2	2.99E+05			
			3	6.80E+05			
		10 minutes	1	8.78E+04	7.13E+04	86.71%	0.88
			2	5.00E+04			
			3	7.60E+04			
	Hydrogen Peroxide (Diluted to 7.5%)	10 minutes	1	<1.00E+01	<1.00E+01	>99.99%	>3.85
			2	<1.00E+01			
			3	<1.00E+01			

Note 1: The lower limit of detection for this study was 1.00E+01 CFU/carrier. Values observed less than the limit are reported as "<1.00E+01" in the results table.
Note 2: The control reductions are compared to the Time Zero controls, while the test carrier reductions are compared to the corresponding parallel controls.

Results: Efficacy – *S. aureus*

Test Microorganism	Test Substance	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Controls	Log_{10} Reduction Compared to Controls
S. aureus ATCC 6538	Control	Time Zero	1	3.77E+06	5.56E+06	N/A	
			2	7.06E+06			
			3	5.84E+06			
		10 minutes	1	4.26E+06	4.03E+06	27.53%	0.14
			2	5.21E+06			
			3	2.61E+06			
	Hydrogen Peroxide (Diluted to 7.5%)	10 minutes	1	<1.00E+01	<1.00E+01	>99.9998%	>5.60
			2	<1.00E+01			
			3	<1.00E+01			

Note 1: The lower limit of detection for this study was 1.00E+01 CFU/carrier. Values observed less than the limit are reported as "<1.00E+01" in the results table.
Note 2: The control reductions are compared to the Time Zero controls, while the test carrier reductions are compared to the corresponding parallel controls.

Results: Efficacy – *A. brasiliensis*

Test Microorganism	Test Substance	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Controls	Log_{10} Reduction Compared to Controls
A. brasiliensis ATCC 16404	Control	Time Zero	1	1.01E+05	1.01E+05	N/A	
			2	8.30E+04			
			3	1.18E+05			
		10 minutes	1	1.07E+05	1.08E+05	No Reduction	
			2	1.07E+05			
			3	1.11E+05			
	Hydrogen Peroxide (Diluted to 7.5%)	10 minutes	1	<1.00E+01	<1.00E+01	>99.991%	>4.03
			2	<1.00E+01			
			3	<1.00E+01			

Note 1: The lower limit of detection for this study was 1.00E+01 CFU/carrier. Values observed less than the limit are reported as "<1.00E+01" in the results table.
Note 2: The control reductions are compared to the Time Zero controls, while the test carrier reductions are compared to the corresponding parallel controls.

Results: Prevention of Growth on Treated Surfaces Under Ideal Growth Conditions

Test Microorganism	Media Type	Observations
S. enterica ATCC 10708	Tryptic Soy Agar	No Growth
S. aureus ATCC 6538	Nutrient Agar	No Growth
A. brasiliensis ATCC 16404	Potato Dextrose Agar	No Growth

Note: The table above describes the results of the Growth Prevention Reference plates after concurrent incubation with test materials.

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