

ANTIMICROBIAL TEST LABORATORIES

Custom Antimicrobial Persistence Evaluation Study Report NG4180

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Client Information

Company Name:	<u>Microbial Disinfecting Solutions</u>	Sponsor:	<u>Lloyd Starks</u>
		E-mail:	<u>ls@chemcotech.com</u>

Test Information

Test(s) Performed:	<u>Efficacy and Persistence Evaluation of an Antimicrobial Applied via a Steam Vapor Device (NG4180)</u>		
SOP Followed:	<u>Testing Facility Operation 011.1</u>	Performed by:	<u>D. Sowersby</u>

Sample Information

Date Received:	<u>02 MAY 2013</u>	Test Substance ID:	<u>FTC Proprietary Applied Vapor Technologies (TANCS)</u>
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Parameters

Microorganisms:	<u><i>S. aureus</i> ATCC 6538</u>	Exposure Temp.	<u>Ambient (25 ± 5°C)</u>
# of Replicates:	<u>2</u>	Carrier Types:	<u>1" x 3" Glass Slide, 4.8 cm cotton discs</u>
Culture Age:	<u>18-24 Hours</u>	Inoculum Volume:	<u>0.200 ml, 0.020 mL, 0.050 mL</u>
Growth Medium:	<u>Tryptic Soy Broth</u>	Target Conc:	<u>2.0x10⁶ CFU/carrier for initial sanitation</u>
Contact Times:	<u>10 seconds for initial sanitation</u>		<u>8x10⁵ CFU/carrier for persistence (Phase I)</u>
	<u>2 hours for persistence studies</u>		<u>1x10⁵ CFU/carrier for persistence (Phase II)</u>
Neutralizer Used:	<u>Dey/Engley (D/E) Broth</u>	Enumeration Agar:	<u>Tryptic Soy Agar</u>
Treatment Method:	<u>Steam deposition</u>	Plate Incub. Temp.:	<u>36.0 ± 1°C</u>
Treatment Volume:	<u>~ 10 mL</u>	Incubation Time:	<u>24 ± 4hours</u>

Controls

Neutralized:	<u>Yes</u>	Growth Control:	<u>Passed</u>
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Test Results

Test(s) Valid?:	<u>See Results</u>	Confirmation:	<u>Morphology on TSA</u>
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Notes: This study was broken into 2 phases. The first phase of the study validated the initial sanitation of the steam and FTC proprietary solution Test System using a modified surface time-kill testing procedure. The surface of slides containing dried residue left after the steam treatment were then re-inoculated with the Test Microorganism using a modified JIS Z 2801 procedure. The inoculum dwelled on slides for several contact times to determine the amount of time necessary to reduce CFU by at least 99.9%. The second phase of the study was conducted to determine if the dried residue from the initial steam application demonstrated residual antimicrobial properties against the Test Microorganism after several re-inoculations. Summaries of the procedures for both phases of the study are detailed on page 2.

Tests Completed:	<u>19 JUN 2013</u>	Report Sent:	<u>27 JUN 2013</u>
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Summary of Test Procedure

Phase I: Initial Sanitization Efficacy and Determination of Antimicrobial Persistence Evaluation Contact Time

1. Sterile glass carriers were inoculated with 0.020 ml of an overnight culture of *S. aureus* ATCC 6538 and dried in ambient conditions for 30 minutes before treatment with Test System.
2. Test System was created by thoroughly mixing 1 part FTC proprietary solution with 21 parts tap water and pouring it into the Commercial Steam Cleaning unit.
3. After equilibrating for ~ 15 minutes, steam from the highest power setting was deposited to inoculated carriers at approximately a 45° angle and 8 inches from glass surfaces for 10 seconds.
4. A set of carriers were harvested in 10 mL D/E neutralization buffer 30 seconds after the end of steam treatment. These slides along with untreated controls were enumerated using standard plating techniques.
5. Remaining treated samples were tilted to drain most liquid. Treated and untreated carriers dried in ambient conditions for ~ 1 hour.
6. Visibly dry carriers were then inoculated with sterile 200 µL RO water containing ~ 8.0 X 10⁵ CFU of Test Microorganism. These carriers were incubated under high humidity (> 85% RH) for 2, 6, and 24 hours.
7. Upon reaching each contact time, treated and control samples were harvested and enumerated as mentioned above.

Phase II: Antimicrobial Persistence Evaluation

1. Sterile glass and cotton carriers were treated with Test System as described in Phase I of the study with one caveat. The angle of deposition was 90° so test pieces did not become dislodged from Petri dishes.
2. Excess liquid was removed from carriers, which were then allowed to dry for ~ 1 hour before the first inoculation (50 µL of RO water containing ~ 1.0 X 10⁵ CFU). All subsequent inoculations were the same.
3. Samples remained in ambient conditions for remaining persistence times of 1, 2, 3, 7, 14, and 21 days.
4. The contact time chosen for each persistence inoculation was 2 hours as determined from Phase I of this study.
5. All treated and non-treated samples were harvested and enumerated using standard plating techniques.

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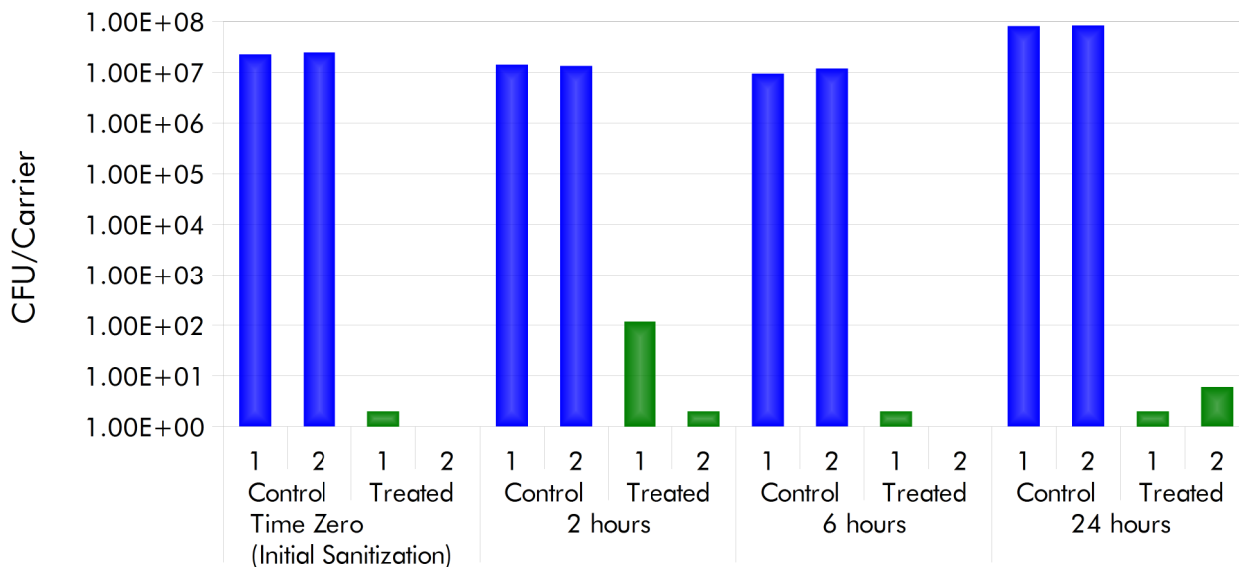
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Results (Phase I)

FTC Proprietary Solution (1:21 Dilution)							
Test Microorganism	Contact Time	Sample	Replicate Number	CFU/Carrier	Average CFU/Carrier	Percent Reduction	
<i>S. aureus</i> ATCC 6538	Time Zero (Initial Sanitization)	Control	1	2.25E+07	2.36E+07	N/A	
			2	2.46E+07			
		Treated	1	2.00E+00	1.50E+00		99.999994%
			2	1.00E+00			
	2 hours	Control	1	1.41E+07	1.37E+07	N/A	
			2	1.32E+07			
		Treated	1	1.18E+02	6.00E+01		99.999560%
			2	2.00E+00			
	6 hours	Control	1	9.30E+06	1.06E+07	N/A	
			2	1.18E+07			
		Treated	1	2.00E+00	1.50E+00		99.999986%
			2	1.00E+00			
	24 hours	Control	1	8.10E+07	8.20E+07	N/A	
			2	8.30E+07			
		Treated	1	2.00E+00	4.00E+00		99.999995%
			2	6.00E+00			



Note: Results down to the limit of detection (2 CFU) are represented as zero on this chart

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Results (Phase II)

Antimicrobial Persistence Evaluation on 2 Surfaces FTC Proprietary Solution (1:21 Dilution)								
Test Microorganism	Contact Time	Sample	Surface Type	Replicate Number	CFU/Carrier	Average CFU/Carrier	Percent Reduction	
<i>S. aureus</i> ATCC 6538	Time Zero	Control	Glass	1	1.00E+05	1.05E+05	N/A	
				2	1.10E+05			
			Cotton	1	1.70E+04	2.08E+04		
				2	2.45E+04			
	24 hours (2 Challenges)	Control	Glass	1	1.90E+05	1.98E+05		
				2	2.05E+05			
			Cotton	1	2.25E+04	3.43E+04		
				2	4.60E+04			
		Treated	Glass	1	<2.00E+00	<2.00E+00		>99.9990%
				2	<2.00E+00			
			Cotton	1	<2.00E+00	<2.00E+00		>99.994%
				2	<2.00E+00			
	48 hours (3 Challenges)	Control	Glass	1	1.80E+05	1.93E+05	N/A	
				2	2.05E+05			
			Cotton	1	8.45E+04	9.73E+04		
				2	1.10E+05			
		Treated	Glass	1	<2.00E+00	<2.00E+00		>99.9990%
				2	<2.00E+00			
			Cotton	1	<2.00E+00	<2.00E+00		>99.998%
				2	<2.00E+00			
	72 hours (4 Challenges)	Control	Glass	1	2.00E+05	2.55E+05		N/A
				2	3.10E+05			
			Cotton	1	2.45E+04	1.75E+04		
				2	1.05E+04			
		Treated	Glass	1	<2.00E+00	<2.00E+00	>99.9992%	
				2	2.00E+00			
			Cotton	1	<2.00E+00	<2.00E+00	>99.989%	
				2	<2.00E+00			
	1 week (5 Challenges)	Control	Glass	1	1.70E+05	N/A		
			Cotton	1	5.70E+04			
Treated		Glass	1	<2.00E+00	>99.9994%			
		Cotton	1	<2.00E+00	>99.998%			
2 weeks (6 Challenges)	Control	Glass	1	3.00E+05	N/A			
		Cotton	1	7.00E+04				
	Treated	Glass	1	<2.00E+00			>99.9997%	
		Cotton	1	<2.00E+00			>99.9986%	
3 weeks (7 Challenges)	Control	Glass	1	3.30E+05			N/A	
		Cotton	1	5.05E+04				
	Treated	Glass	1	<2.00E+00				>99.9997%
		Cotton	1	<2.00E+00				>99.998%

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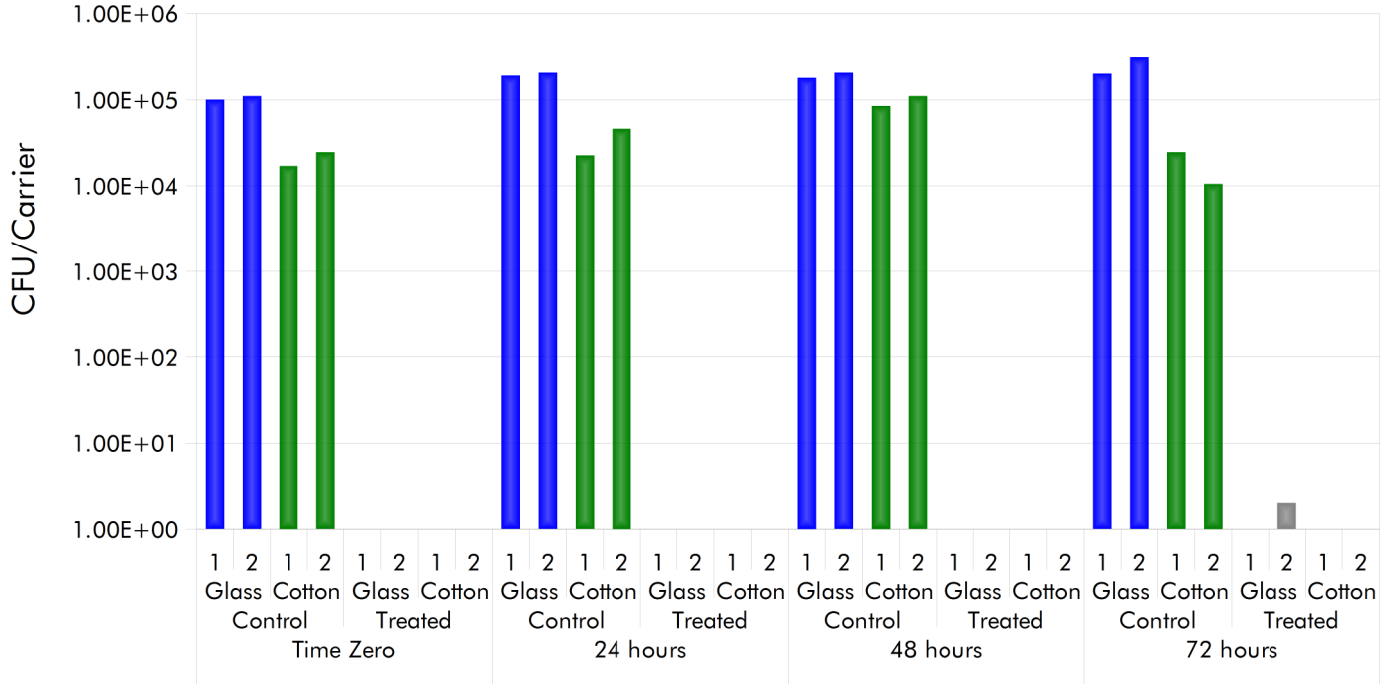
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Results Cont'd (Phase II)

Duplicate Testing for the First 72 hours of Testing



Single Replicate Testing from 1-3 Weeks



Note: Results down to the limit of detection (2 CFU) are represented as zero on charts above

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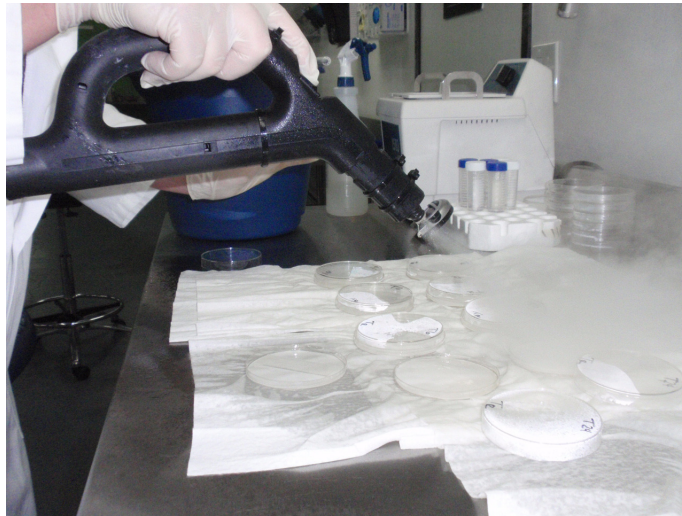
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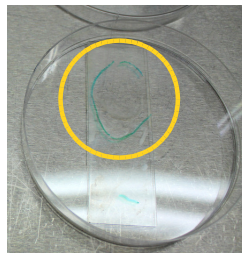
Photographs of Study

Scheme of Basic Procedure

Treatment

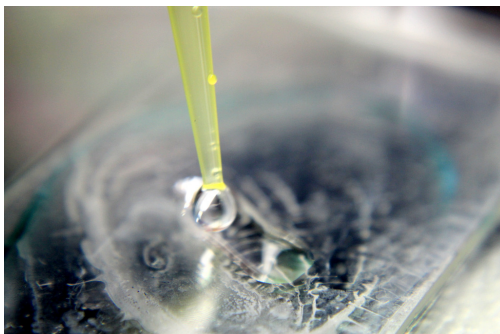


Drying



Application

Incubation



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Photographs of Study

Miscellaneous



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Additional Test Information

Neutralization Validation

Microorganism	Treated Surface	CFU/mL before Neutralization	CFU/mL after Neutralization	Result
<i>S. aureus</i> ATCC 6538	Dried Glass	56	52	Pass
	Dried Cotton		52	Pass

Method of Calculation of Percent Reduction:

Percent Reduction = $(A-B)/A \times 100$, where:

A = Average number of viable CFU on control samples at each contact time.

B = Average number of viable CFU on treated samples after each contact time.