MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Paul Dabney's Light Device and Test Substance

Test Method

Custom Device Study: Modified ASTM E1153

Study Identification Number

NG11778

Study Sponsor

Paul Dabney
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Test Facility

Microchem Laboratory
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Round Rock, TX 78681
(512) 310-8378
Testing performed by: S. Kappel

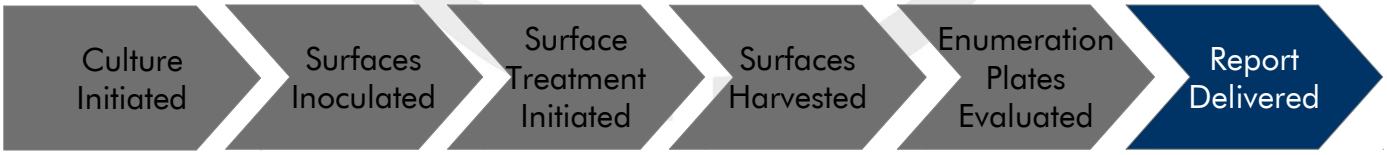
ASTM E1153: General Information

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 notes 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



S. epidermidis ATCC 12228					
27 NOV 2018	28 NOV 2018	28 NOV 2018	28 NOV 2018	30 NOV 2018	
28 NOV 2018	29 NOV 2018	29 NOV 2018	29 NOV 2018	01 DEC 2018	
29 NOV 2018	30 NOV 2018	30 NOV 2018	30 NOV 2018	02 DEC 2018	10 DEC 2018
02 DEC 2018	03 DEC 2018	03 DEC 2018	03 DEC 2018	05 DEC 2018	
04 DEC 2018	05 DEC 2018	05 DEC 2018	05 DEC 2018	07 DEC 2018	

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Test Substance Information

The test device and test substance were received on 26 OCT 2018 and the following picture was taken.
(note: photo depicts the test device and test substance that were analyzed in this study)

Test Device Received: 400-420 nm light device
Test Substance Received: 3% Hydrogen Peroxide

Test Device arrived ready to use for the conduct of the Study.

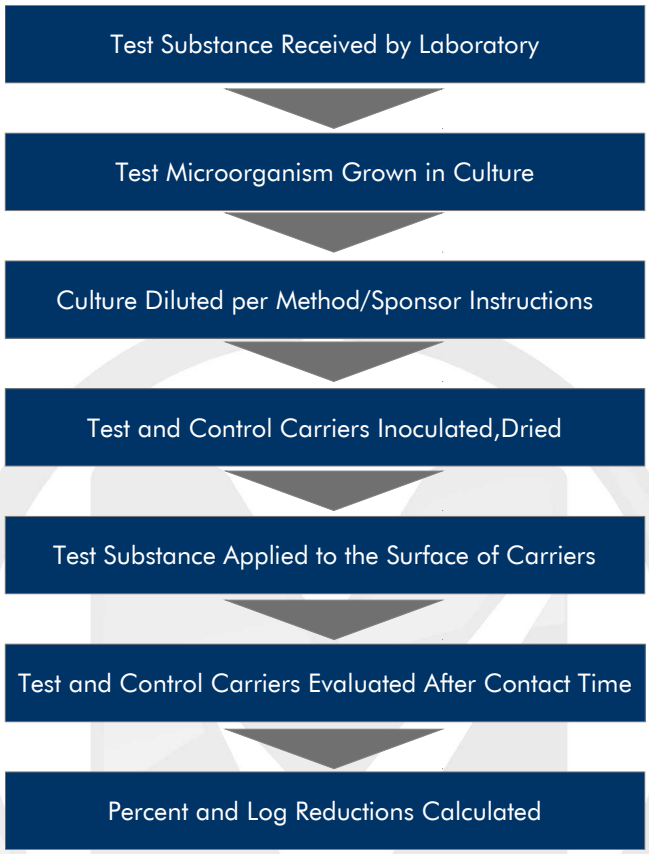
Test Microorganism Information

The test microorganism(s) selected for this test:

***Staphylococcus epidermidis* 12228**

This bacteria is a Gram-positive, cocci-shaped, facultative anaerobe. *S. epidermidis* is part of the human bacterial flora, mostly located on skin. It is not usually pathogenic, however, antibiotic resistant strains have evolved. Most *Staphylococcus* species are a hardy microorganisms capable of surviving on surfaces and under dry conditions. This bacteria, specifically, is regularly used in quality control, media testing, and pharmacuetical/personal care products testing.

Diagram of the Procedure



Summary of the Procedure

- The test microorganism is prepared, usually by growth in liquid culture medium or on an appropriate agar plate.
- 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. Only completely dried carriers are used in the test.
- Test carriers are treated with the test device and incubated for the predetermined contact time.
- Control carriers are harvested at appropriate intervals to accurately represent any reduction during the 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 a Custom Device Study

For Microchem Laboratory to consider a Device Study 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/carrier or greater.
2. Positive/Growth controls must demonstrate growth of the appropriate test microorganism.
3. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

Due to the modified nature of the study, passing criteria may be determined by the Study Sponsor.

Testing Parameters used in this Study

Test Substance:	3% Hydrogen Peroxide	Test Device:	400-420nm light
Carriers (Size):	18 x 36mm glass slides	Replicates:	Single (1)
Light Exposure Time	1 Minute	Culture Growth Time:	~24 hours
Culture Growth Media:	Tryptic Soy Broth	Culture Supplement:	N/A
Culture Dilution Media:	Phophate Buffered Saline	Inoculum Volume:	0.020 ml
Inoculum Concentration:	1.0 x 10^6 CFU/Carrier	Contact Temperature:	Ambient
Contact Time:	2 minutes	Enumeration Media:	TSA
Neutralizer (Vol.):	See Notes	Enumeration Plate Incubation Time:	~48 hours
Enumeration Plate Incubation Temperature:	36°C ± 1°C		

Study Notes

Neutralization Media

- The neutralization media used in this study was PBS with 0.1% Triton X, supplemented to contain 0.3% Catalase.

Test Substance Dilutions

- 3% H_2O_2 was used as received from study sponsor
- 0.3% H_2O_2 (2mL of 3% H_2O_2 and 18mL of Sterile DI Water)
- 3% H_2O_2 with Iron (20mL of 3% H_2O_2 with 0.5mL of 0.1N FeSO4)
- 0.3% H_2O_2 with Iron (2mL of 3% H_2O_2 with 18mL of Sterile DI Water and 0.5mL of 0.1N FeSO4)

Test Substance Exposure to Carrier

- Each test substance was treated in a glass petri dish with light for 1 minute, the applied to the carriers after the following dwell times 1 minute, 5 minutes, 10 minutes, 30 minutes, 1 hour, 12 hours, 24 hours, 2, days, 5 days, and 7 days.
- Once test substance was applied to the carrier it was allowed to sit for 2 minutes before the carrier was harvested into neutralizer.
- After 7 days of dwell time, all test substances were treated with light again for 1 minute, and a carrier was treated after 1 minute of dwell time.

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Control Results

Neutralization Method: Valid Media Sterility: Sterile
Growth Confirmation: Growth of Target Microorganism

The following is the neutralization verification results for the study.

Test Microorganism	Substance	Count 1 / Count 2	Average Count	NV Valid?
S. epidermidis ATCC 12228	Control	41/ 41	41.0	N/A
	3% H_2O_2	41/ 38	39.5	Yes
	3% H_2O_2 with Iron	45/ 34	39.5	Yes

Calculations

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

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

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

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 of the Study

Test Microorganism	Test Substance Concentration	Time After Light Exposure Substance Applied to Carrier	CFU/carrier	Percent Reduction vs. Parallel Control	Log_{10} Reduction vs. Parallel Control
S. epidermidis ATCC 12228	Control	N/A	6.04E+05	N/A	
	3% H_2O_2	1 Minute	7.00E+04	88.42%	0.94
		5 Minute	7.00E+04	88.42%	0.94
		10 Minute	3.10E+04	94.87%	1.29
		30 Minute	2.80E+04	95.37%	1.33
		1 Hour	7.10E+04	88.25%	0.93
	12 Hour Control		9.20E+04	N/A	
	3% H_2O_2	12 Hours	1.80E+04	80.43%	0.71
	24 Hour Control		1.33E+05	N/A	
	3% H_2O_2	24 Hours	2.10E+04	84.21%	0.80
	2 Day Control		3.00E+05	N/A	
	3% H_2O_2	2 Days	9.00E+04	70.00%	0.52
	5 Day Control		4.50E+04	N/A	
	3% H_2O_2	5 Days	3.29E+03	92.69%	1.14
	7 Day Control		9.80E+04	N/A	
	3% H_2O_2	7 Days	1.50E+04	84.69%	0.82
		7 Days with Reactivation	1.00E+04	89.80%	0.99

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Results of the Study (cont.)

Test Microorganism	Test Substance Concentration	Time After Light Exposure Substance Applied to Carrier	CFU/carrier	Percent Reduction vs. Parallel Control	Log_{10} Reduction vs. Parallel Control
S. epidermidis ATCC 12228	Control	N/A	6.04E+05	N/A	
	3% H_2O_2 With Iron	1 Minute	3.80E+04	93.71%	1.20
		5 Minute	4.00E+04	93.38%	1.18
		10 Minute	8.50E+04	85.93%	0.85
		30 Minute	7.10E+04	88.25%	0.93
		1 Hour	7.00E+04	88.42%	0.94
	12 Hour Control		9.20E+04	N/A	
	3% H_2O_2 With Iron	12 Hours	8.10E+04	11.96%	0.06
	24 Hour Control		1.33E+05	N/A	
	3% H_2O_2 With Iron	24 Hours	1.60E+04	87.97%	0.92
	2 Day Control		3.00E+05	N/A	
	3% H_2O_2 With Iron	2 Days	8.70E+04	71.00%	0.54
	5 Day Control		4.50E+04	N/A	
	3% H_2O_2 With Iron	5 Days	1.60E+04	64.44%	0.45
	7 Day Control		9.80E+04	N/A	
	3% H_2O_2 With Iron	7 Days	2.07E+05	No Reduction	
		7 Days with Reactivation	3.00E+04	69.39%	0.51

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Results of the Study (cont.)

Test Microorganism	Test Substance Concentration	Time After Light Exposure Substance Applied to Carrier	CFU/carrier	Percent Reduction vs. Parallel Control	Log_{10} Reduction vs. Parallel Control
S. epidermidis ATCC 12228	Control	N/A	6.04E+05	N/A	
	0.3% H_2O_2	1 Minute	1.10E+04	98.18%	1.74
		5 Minute	3.00E+04	95.04%	1.30
		10 Minute	1.98E+05	67.24%	0.48
		30 Minute	7.00E+04	88.42%	0.94
		1 Hour	7.00E+04	88.42%	0.94
	12 Hour Control		9.20E+04	N/A	
	0.3% H_2O_2	12 Hours	1.27E+05	No Reduction	
	24 Hour Control		1.33E+05	N/A	
	0.3% H_2O_2	24 Hours	1.40E+04	89.47%	0.98
	2 Day Control		3.00E+05	N/A	
	0.3% H_2O_2	2 Days	2.64E+05	12.00%	0.06
	5 Day Control		4.50E+04	N/A	
	0.3% H_2O_2	5 Days	4.40E+04	2.22%	0.01
	7 Day Control		9.80E+04	N/A	
	0.3% H_2O_2	7 Days	7.70E+04	21.43%	0.10
		7 Days with Reactivation	1.03E+05	No Reduction	

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Results of the Study (cont.)

Test Microorganism	Test Substance Concentration	Time After Light Exposure Substance Applied to Carrier	CFU/carrier	Percent Reduction vs. Parallel Control	Log_{10} Reduction vs. Parallel Control
S. epidermidis ATCC 12228	Control	N/A	6.04E+05	N/A	
	0.3% H_2O_2 With Iron	1 Minute	1.41E+05	76.67%	0.63
		5 Minute	5.38E+04	91.10%	1.05
		10 Minute	2.13E+05	64.75%	0.45
		30 Minute	6.88E+04	88.62%	0.94
		1 Hour	9.10E+04	84.94%	0.82
	12 Hour Control		9.20E+04	N/A	
	0.3% H_2O_2 With Iron	12 Hours	1.10E+05	No Reduction	
	24 Hour Control		1.33E+05	N/A	
	0.3% H_2O_2 With Iron	24 Hours	1.55E+05	No Reduction	
	2 Day Control		3.00E+05	N/A	
	0.3% H_2O_2 With Iron	2 Days	3.39E+05	No Reduction	
	5 Day Control		4.50E+04	N/A	
	0.3% H_2O_2 With Iron	5 Days	2.43E+03	94.60%	1.27
	7 Day Control		9.80E+04	N/A	
	0.3% H_2O_2 With Iron	7 Days	7.10E+04	27.55%	0.14
		7 Days with Reactivation	2.30E+04	76.53%	0.63

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Result Summary of the Study (cont.)

Test Substance Concentration	Time After Light Exposure Substance Applied to Carrier	Log_{10} Reduction vs. Parallel Control
0.3% H2O2	1 Minute	1.74
0.3% H2O2 with Iron		0.63
0.3% H2O2	5 Minutes	1.3
0.3% H2O2 with Iron		1.05
0.3% H2O2	10 Minutes	0.48
0.3% H2O2 with Iron		0.45
0.3% H2O2	30 Minutes	0.94
0.3% H2O2 with Iron		0.94
0.3% H2O2	1 Hour	0.94
0.3% H2O2 with Iron		0.82
0.3% H2O2	12 Hours	None
0.3% H2O2 with Iron		None
0.3% H2O2	24 Hours	0.98
0.3% H2O2 with Iron		None
0.3% H2O2	2 Days	0.06
0.3% H2O2 with Iron		None
0.3% H2O2	5 Days	0.01
0.3% H2O2 with Iron		1.27
0.3% H2O2	7 Days	0.10
0.3% H2O2 with Iron		0.14
0.3% H2O2	7 Days With Reactivation	None
0.3% H2O2 with Iron		0.63

Test Substance Concentration	Time After Light Exposure Substance Applied to Carrier	Log_{10} Reduction vs. Parallel Control
3% H2O2	1 Minute	0.94
3% H2O2 with Iron		1.2
3% H2O2	5 Minutes	0.94
3% H2O2 with Iron		1.18
3% H2O2	10 Minutes	1.29
3% H2O2 with Iron		0.85
3% H2O2	30 Minutes	1.33
3% H2O2 with Iron		0.93
3% H2O2	1 Hour	0.93
3% H2O2 with Iron		0.94
3% H2O2	12 Hours	0.71
3% H2O2 with Iron		0.06
3% H2O2	24 Hours	0.8
3% H2O2 with Iron		0.92
3% H2O2	2 Days	0.52
3% H2O2 with Iron		0.54
3% H2O2	5 Days	1.14
3% H2O2 with Iron		0.45
3% H2O2	7 Days	0.82
3% H2O2 with Iron		None
3% H2O2	7 Days With Reactivation	0.99
3% H2O2 with Iron		0.51

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