MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antimicrobial Activity and Efficacy of Rushlight 1.0 MOD 0 Rapid Point of Care Sterilizer Simulating Improper Loading

Test Method

Custom Device Study Based on: Modified ASTM E1153

Study Identification Number

NG19003

Study Sponsor

John-Paul Bonansinga

[redacted] LLC

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378
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Purpose of the Study

The purpose of this study was to determine the antimicrobial efficacy of [redacted] test device.

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

Devices Received	Experimental Start Date	Experimental End Date	Report Delivered
29NOV2021	02FEB2022	18FEB2022	xxMAR2022

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Test Device Information

Name of Test Device: Rushlight 1.0 MOD 0
Manufacturer: Forge Applied Science & Technology
Mode of Active: UV Light (Germicidal)

A description of how to operate the device was provided by the Study Sponsor prior to test initiation.

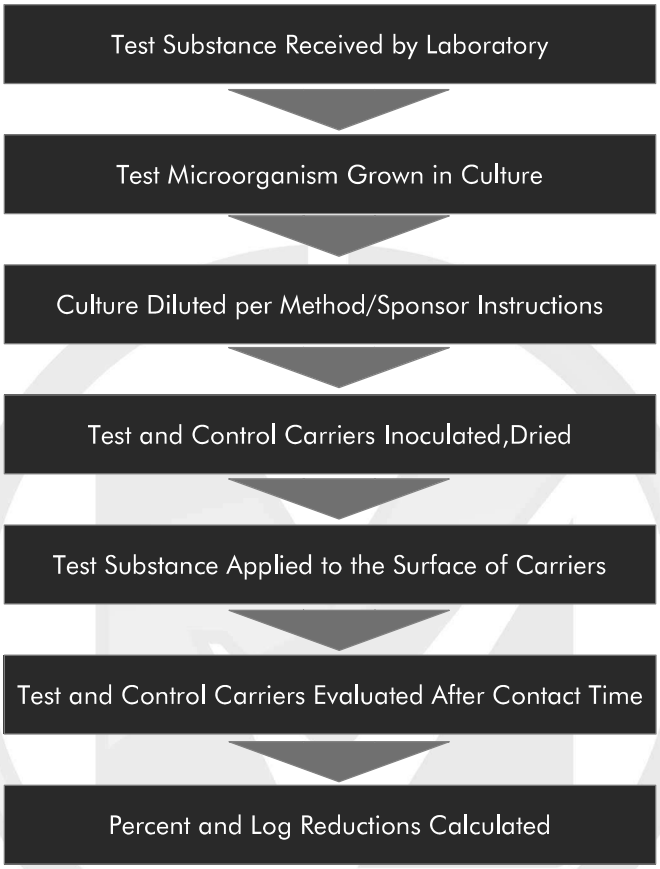
Test Microorganism Information

The test microorganism(s) selected for this test:

Staphylococcus aureus ATCC 33592, *Clostridioides difficile* ATCC 43598, *Pseudomonas aeruginosa* ATCC 15442 and MS2 Bacteriophage ATCC 15597-B1, originally received from the American Type Culture Collection (ATCC), Manassas, VA, were used in this study.

Candida auris CDC AR Bank #0385, originally received from the CDC AR Bank, Atlanta, GA, was used in this study.

Diagram of the Test Procedure



Summary of the Procedure

- Test microorganism is prepared in appropriate liquid broth.
- Test microorganism is harvested and the resulting suspension is diluted to achieve $\geq 1x10^6$ CFU/mL.
- Test and control carriers are inoculated and allowed to dry in optimal conditions for test microorganism.
- Test carriers are placed in test device for the Sponsor-determined contact time.
- Test carriers are harvested into liquid media and plated in optimal incubation conditions and time for the test microorganism.
- After incubation, microbial concentrations are determined and reductions relative to pre-treatment controls are calculated.

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 initial and final concentration of microorganisms must be significantly high enough to observe the passing criteria/log reduction.
2. The media used for testing must be sterile.
3. The target microorganism must be pure colony morphology.

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

S. aureus and *P. aeruginosa*

Culture Growth Media:	Tryptic Soy Broth	**Culture Growth Time:**	18-24 hours
Carrier Type	~1cm Steel Discs	**Inoculum Volume**	0.010 ml
Culture Diluent	N/A	**Carrier Dry Time**	20 to 40 minutes
Harvest Media (Volume)	Phosphate Buffered Saline w/ 0.1% Tween-80 (20 ml)	**Enumeration Media**	Nutrient Agar
Incubation Temperature	36°C ± 1°C	**Incubation Time**	24-48 Hours

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Testing Parameters (cont.)

C. auris

Culture Growth Media:	Sabouraud Dextrose Broth	**Culture Growth Time:**	18-24 hours
Carrier Type	~1cm Steel Discs	**Inoculum Volume**	0.010 ml
Culture Diluent	N/A	**Carrier Dry Time**	20 to 40 minutes
Harvest Media (Volume)	Phosphate Buffered Saline w/ 0.1% Tween-80 (20 ml)	**Enumeration Media**	Potato Dextrose Agar
Incubation Temperature	30°C ± 1°C	**Incubation Time**	48 – 72 Hours

MS2 Bacteriophage

Culture Growth Media:	N/A – Freezer Stock	**Culture Growth Time:**	N/A – Freezer Stock
Carrier Type	~1cm Steel Discs	**Inoculum Volume**	0.010 ml
Culture Diluent	N/A	**Carrier Dry Time**	20 to 40 minutes
Harvest Media (Volume)	Phosphate Buffered Saline w/ 0.1% Tween-80 (20 ml)	**Enumeration Media**	50% TSA
Incubation Temperature	36°C ± 1°C	**Incubation Time**	18 – 24 hours

C. difficile

Culture Growth Media:	N/A – Freezer Stock	**Culture Growth Time:**	N/A – Freezer Stock
Carrier Type	~1cm Steel Discs	**Inoculum Volume**	0.010 ml
Culture Diluent	N/A	**Carrier Dry Time**	20 to 40 minutes
Harvest Media (Volume)	Phosphate Buffered Saline w/ 0.1% Tween-80 (20 ml)	**Enumeration Media**	BHIY-HT
Incubation Temperature	36°C ± 1°C	**Incubation Time**	48 – 72 Hours

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Study Notes

A study sponsor provided hexagonal carrier holder was used to evaluate all *C. difficile, C. auris* and MS2 efficacy carriers. A study sponsor provided cuboidal carrier holder was used to evaluate the *S. aureus* and *P. aeruginosa* carriers.

The run time for cycle #1 against *S. aureus* was inadvertently not recorded. All other cycle times and ambient temperatures can be found the corresponding results section of this report.

The Study Sponsor requested a target concentration for this study of ≥1 x 10^6 CFU/Carrier. All microorganisms tested achieved this, with the exception of *C. auris.*

The following information was provided by the Study Sponsor and included per their request. Note that the information cannot be verified by Microchem Laboratory and was only edited for formatting to fit this report:

All loadouts/cycle tests in this are meant to measure suboptimal or improper loading configurations. Cycles are meant to increase minimum exposure achieved as surface area increases. Times are not consistent for same cycle runs because the sensors are reading real time output which varies depending on ambient conditions.

Staph A. (1st run)

- Top shelf, centered
- Disc facing the top of the reactor w/ no direction 1st pass UV-C
- Rectangular prism carrier surface area= 22.05 in^2 (obstruction simulation)
- Dimensions 3.64"x 1.52" x 1.06"
- Cycle #1/time not taken
- Simulates improper loading, with stages straddled/midline crossed. No significant 1st pass radiation is delivered to the surface being assessed for germicidal efficacy.

Staph A. (2nd Run)

- Bottom shelf, centered
- Disc facing the top of the reactor w/ no direction 1st pass UV-C
- Rectangular prism carrier surface area= 22.05 in^2 (obstruction simulation)
- Dimensions 3.64"x 1.52" x 1.06"
- Cycle #2/56sec
- Simulates improper loading, with stages straddled/crossing midline in the low stage configuration. Longest distance for 1st pass radiation on top surface of item(s) loaded.

Study Notes (cont.)

P. Aeruginosa
- Bottom shelf, centered
- Disc facing the top of the reactor w/ no direction 1st pass UV-C
- Rectangular prism carrier surface area= 22.05 in^2 (obstruction simulation)
- dimensions 3.64"x 1.52" x 1.06"
- Cycle #2/56sec
- Simulates improper loading, with stages straddled/crossing midline in the low stage configuration. Longest distance for 1st pass radiation on top surface of item(s) loaded.

MS2
- Bottom shelf, centered
- Disc facing the top of the reactor w/ no direction 1st pass UV-C
- Octagonal prism carrier surface area= 379.2in^2 (obstruction simulation)
- Dimensions 5.23" base edge, 2.76" hgt
- Cycle #2/56sec
- Simulates improper loading, with stages straddled/crossing midline in the low stage configuration. Longest distance for 1st pass radiation on top surface of item(s) loaded.

C. diff
- Bottom shelf, lateral treatment edge parallel with reactor at +1" from centerline
- Disc facing the R/L of the reactor
- Octagonal prism carrier surface area= 379.2in^2 (obstruction simulation)
- Dimensions 5.23" base edge, 2.76" hgt
- Cycle #3/99sec
- Simulates asymmetric loading, with stages straddled/crossing midline in the low stage configuration. Longest distance for 1st pass radiation to lateral surface of item(s) loaded.

C. Auris
- Bottom shelf, lateral treatment edge parallel with reactor at +1" from centerline
- Disc facing the R/L of the reactor
- Octagonal prism carrier surface area= 379.2in^2 (obstruction simulation) dimensions 5.23" base edge, 2.76" hgt
- Cycle #4/183sec
- Simulates asymmetric loading, with stages straddled/crossing midline in the low stage configuration. Longest distance for 1st pass radiation to lateral surface of item(s) loaded.

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Control Results

Neutralization Method: N/A
Growth Confirmation: Pure and Viable

Media Sterility: Confirmed Sterile

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 of the Study

S. aureus (MRSA)

Test Microorganism	Carrier Designation	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control	Log_{10} Reduction Compared to Control
S. aureus ATCC 33592	Control	1	8.60E+06	8.55E+06	N/A	
		2	8.50E+06			
	Cube (top shelf)	1	5.90E+03	4.89E+03	99.94%	3.24
		2	3.88E+03			

Test Microorganism	Carrier Designation	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control	Log_{10} Reduction Compared to Control
S. aureus ATCC 33592	Control	1	3.40E+06	3.40E+06	N/A	
	Cube (bottom shelf)	1	2.00E+02	2.00E+02	99.994%	4.23

Cycle	Time Elapsed	Temp. / RH
2	56 seconds	19.6°C / 44%

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Results of the Study (cont.)

C. difficile (endospores)

Test Microorganism	Carrier Designation	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control	Log_{10} Reduction Compared to Control
C. difficile	Control	1	1.43E+06	1.60E+06	N/A	
		2	1.76E+06			
	Hex (bottom shelf)	1	<1.00E+01	<1.00E+01	>99.9994%	>5.20
		2	<1.00E+01			
Note: 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 and zero in the graph.						

Cycle	Time Elapsed	Temp. / RH
3	1 minute 39 seconds	19.0°C / 47%

C. auris

Test Microorganism	Carrier Designation	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control	Log_{10} Reduction Compared to Control
C. auris CDC AR Bank #0385	Control	1	2.05E+05	1.85E+05	N/A	
		2	1.64E+05			
	Hex (bottom shelf)	1	<1.00E+01	<1.00E+01	>99.995%	>4.27
		2	<1.00E+01			
Note: 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 and zero in the graph.						

Cycle	Time Elapsed	Temp. / RH
4	3 minutes 3 seconds	19.7°C / 44%

Results of the Study (cont.)

MS2 Bacteriophage

Test Microorganism	Carrier Designation	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control	Log_{10} Reduction Compared to Control
MS2 Bacteriophage ATCC 15597-B1	Control	1	1.81E+06	1.81E+06	N/A	
	Hex (bottom shelf)	1	5.00E+01	5.00E+01	99.997%	4.56

Cycle	Time Elapsed	Temp. / RH
2	46 seconds	19.7°C / 45%

P. aeruginosa

Test Microorganism	Carrier Designation	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control	Log_{10} Reduction Compared to Control
P. aeruginosa ATCC 15442	Control	1	6.00E+06	6.00E+06	N/A	
	Cube (bottom shelf)	1	6.76E+03	6.76E+03	99.89%	2.95

Cycle	Time Elapsed	Temp. / RH
2	56 seconds	19.6°C / 44%

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

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