MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of 3B Medical Lumin CPAP UV Sanitizer Device

Test Method

Custom Device Study Based on: ASTM E1153 Method

Study Identification Number

NG14592

Study Sponsor

Alex Lucio
3B Medical, Inc.
alucio@3bproducts.com

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378
Report Author: Brady Ryan, B.S.

Purpose of the Study

The purpose of this study was to determine the antimicrobial efficacy of a UV CPAP Sanitizer device supplied by 3B Medical, Inc.

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

S. aureus ATCC 6538

Devices Received	Cultures Initiated	Carriers Inoculated	Carriers Treated	Enumeration Plates Evaluated	Report Delivered
05FEB2020	06FEB2020	10FEB2020	10FEB2020	12FEB2020	21FEB2020

K. pneumoniae ATCC 4352

Devices Received	Cultures Initiated	Carriers Inoculated	Carriers Treated	Enumeration Plates Evaluated	Report Delivered
05FEB2020	13FEB2020	17FEB2020	17FEB2020	19FEB2020	21FEB2020

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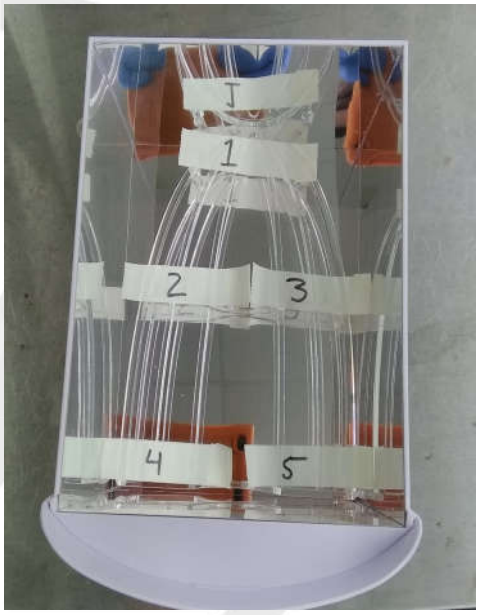
Test Device Information

Name of Test Device: Lumin UV CPAP Sanitizer
Manufacturer: 3B Medical, Inc.
Mode of Active: UV Light (Germicidal)

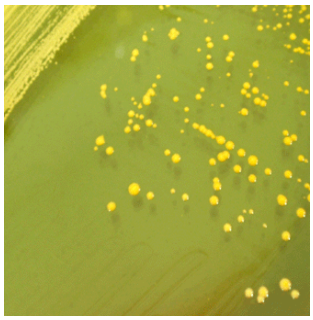
The following pictures were taken prior to testing.



Note: The numbers in the right hand picture indicate the approximate position of the carriers during testing.

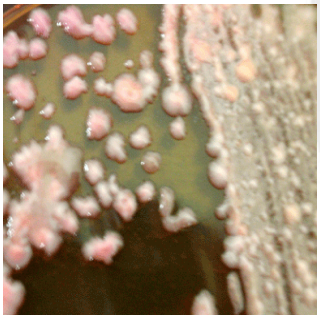
Test Microorganism Information

The test microorganism(s) selected for this test:



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.



Klebsiella pneumoniae 4352

This bacteria is a Gram-negative, rod-shaped, facultative anaerobe. *K. pneumoniae* is in the Enterobacteriaceae family which has developed resistance to carbapenem class based antibiotics. Although *K. pneumoniae* is considered normal flora of the human gastrointestinal tract, this bacterium can also cause serious diseases such as pneumonia. *K. pneumoniae* is relatively easy to disinfect and usually serves as a good representation of an antimicrobial agent's efficacy against Gram-negative bacteria.

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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.

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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 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* ATCC 6538

Culture Growth Media:	Nutrient Broth	**Culture Growth Time:**	48-54 hours
Culture Dilution Media	Nutrient Broth	**Culture Supplement**	N/A
Carrier Type	1″ x 3″ Glass Slides	**Inoculum Volume**	0.010 ml
Carrier Dry Time	≥20 minutes	**Carrier Dry Temp.**	36°C
Contact Time	5 minutes	**Contact Temperature**	Ambient
Harvest Media (Volume)	Phosphate Buffered Dilution Water	**Enumeration Media**	Tryptic Soy Agar
Incubation Temp.	36°C	**Incubation Time**	24-48 hours

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Testing Parameters *K. pneumoniae* ATCC 4352

Culture Growth Media:	Tryptic Soy Broth	**Culture Growth Time:**	48-54 hours
Culture Dilution Media	Tryptic Soy Broth	**Culture Supplement**	N/A
Carrier Type	1" x 3" Glass Slides	**Inoculum Volume**	0.020 ml
Carrier Dry Time	≥20 minutes	**Carrier Dry Temp.**	Ambient
Contact Time	5 minutes	**Contact Temperature**	Ambient
Harvest Media (Volume)	Phosphate Buffered Solution	**Enumeration Media**	Tryptic Soy Agar
Incubation Temp.	36°C	**Incubation Time**	24-48 hours

Study Notes

Due to low pre and post treatment control concentrations, the testing for *K. pneumoniae* was repeated. The retest procedure was changed in the following ways: decreased the carrier drying time, grew the culture in TSB instead of NTB, the test culture was centrifuged and resuspended in 1ml TSB and the inoculum volume was increased from 0.010 ml to 0.020 ml.

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Control Results

Neutralization Method: N/A
Growth Confirmation: Pure and Viable
Media Sterility: No Growth

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

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

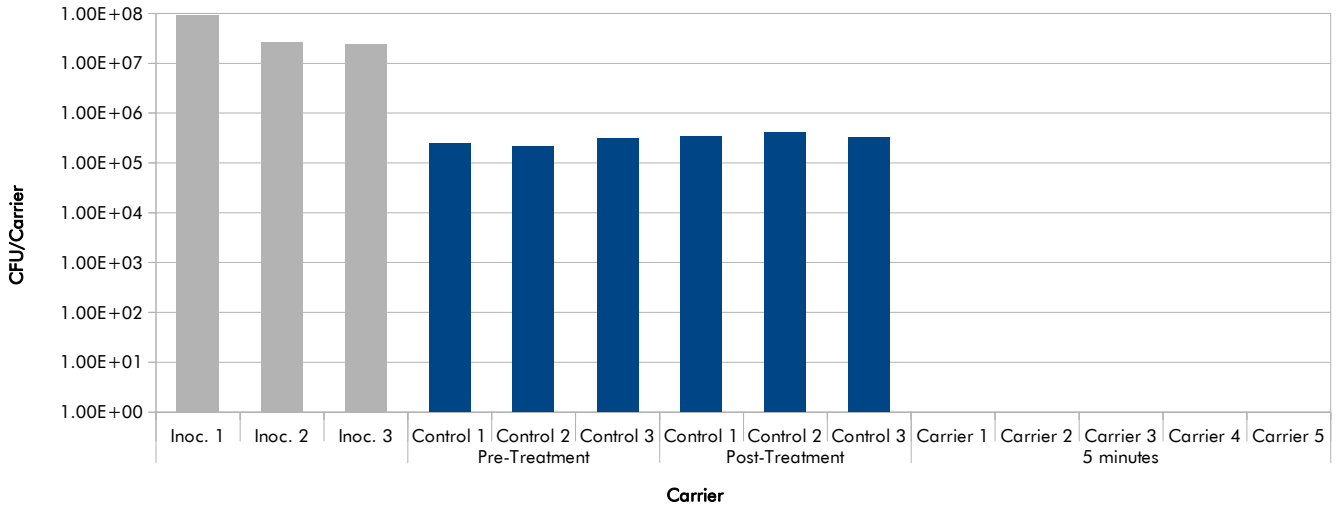
$$\text{Log}_{10}\ \text{Reduction} = \text{Log}(B/A)$$

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* ATCC 6538

Test Microorganism	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Average Percent Reduction Compared to Controls	Average Log_{10} Reduction Compared to Controls
S. aureus ATCC 6538	N/A	Inoc. 1	9.00E+07	4.69E+07	N/A	
		Inoc. 2	2.68E+07			
		Inoc. 3	2.39E+07			
	Pre-Treatment	Control 1	2.49E+05	3.06E+05		
		Control 2	2.17E+05			
		Control 3	3.14E+05			
	Post-Treatment	Control 1	3.35E+05			
		Control 2	3.98E+05			
		Control 3	3.24E+05			
	5 minutes	Carrier 1	<1.00E+00	<1.00E+00	>99.9997%	>5.49
		Carrier 2	<1.00E+00			
		Carrier 3	<1.00E+00			
		Carrier 4	<1.00E+00			
		Carrier 5	<1.00E+00			

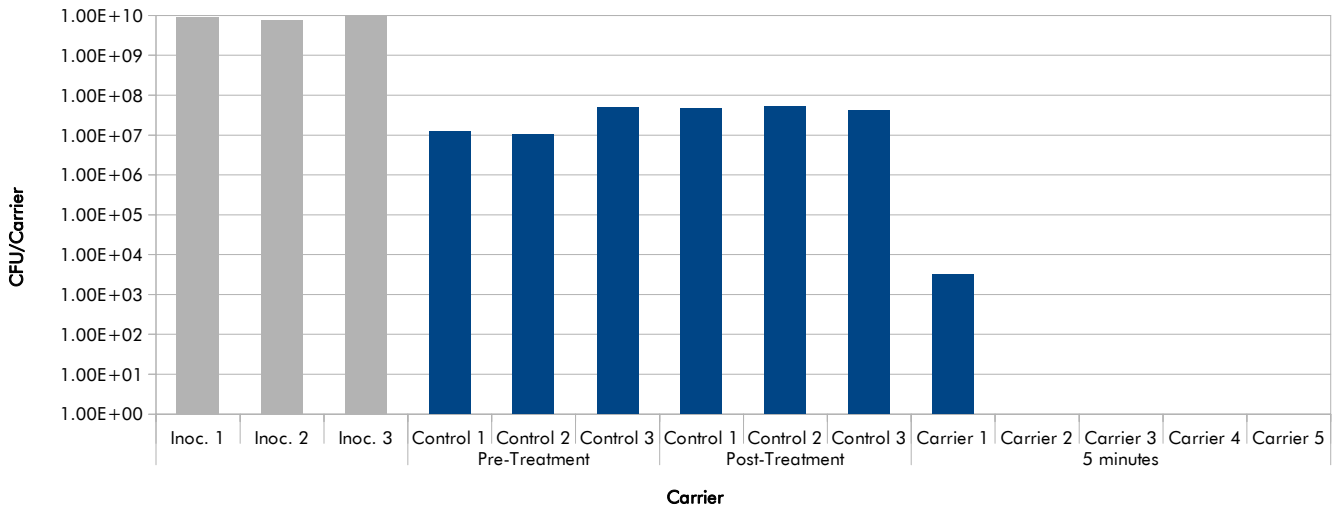
Note: Inoculum is reported as CFU/ml. All other values are CFU/Carrier. The lower limit of detection for this study was 1.00E+00 CFU/Carrier. Values observed below the limit of detection are reported as <1.00E+00 in the results table and as zero in the graph.



Results of the Study - *K. pneumoniae* ATCC 4352

Test Microorganism	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Average Percent Reduction Compared to Controls	Average Log_{10} Reduction Compared to Controls
K. pneumoniae ATCC 4352	N/A	Inoc. 1	8.89E+09	8.63E+09	N/A	
		Inoc. 2	7.50E+09			
		Inoc. 3	9.50E+09			
	Pre-Treatment	Control 1	1.20E+07	3.48E+07		
		Control 2	1.02E+07			
		Control 3	4.84E+07			
	Post-Treatment	Control 1	4.59E+07			
		Control 2	5.23E+07			
		Control 3	4.00E+07			
	5 minutes	Carrier 1	3.11E+03	<6.23E+02	>99.998%	>4.75
		Carrier 2	<1.00E+00			
		Carrier 3	1.00E+00			
		Carrier 4	<1.00E+00			
		Carrier 5	<1.00E+00			

Note: Inoculum is reported as CFU/ml. All other values are CFU/Carrier. The lower limit of detection for this study was 1.00E+00 CFU/Carrier. Values observed below the limit of detection are reported as <1.00E+00 in the results table and as zero in the graph.



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