MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antimicrobial Activity and Efficacy of C. Cretor & Co.'s Device

Test Method

Custom Device Study Based on: ASTM E1153

Study Identification Number

NG17256

Study Sponsor

Ned Vidojevic
C. Cretors & Co.
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Test Facility

Microchem Laboratory
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Report Author: Brady Ryan, B.S.
Testing Performed By: Brady Ryan, B.S.

Purpose of the Study

The purpose of this study was to determine the antimicrobial efficacy of C. Cretor & Co.'s submitted 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	Cultures Initiated	Carriers Inoculated	Carriers Treated	Enumeration Plates Evaluated	Report Delivered
		MS2 15597-B1			16 APR 2021
09 MAR 2021	Freezer Stock	01 APR 2021	01 APR 2021	02 APR 2021	
		S. aureus 6538 and *E. coli* 8739			
	31 MAR 2021	01 APR 2021	01 APR 2021	05 APR 2021	

Test Device Information

Name of Test Device: UV-C-A1-X
Manufacturer: C. Cretor's & Co.
Mode of Active: UV (Germicidal)

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

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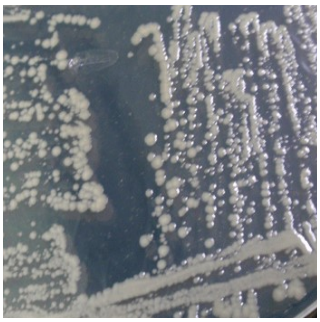
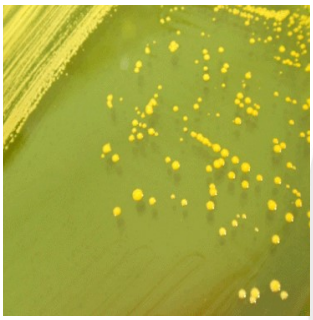
Test Microorganism Information

The test microorganism(s) selected for this test:



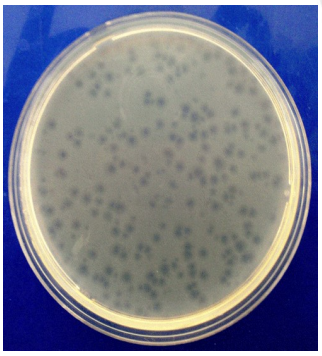
Escherichia coli

This bacteria is a Gram-negative, rod shaped, facultative anaerobe commonly found in the gastrointestinal tract of mammals. Although most serotypes of this microorganism are harmless there are pathogenic groups of *E. coli* such as enterohemorrhagic (EHEC), verocytotoxin producing (VTEC) and Shiga-like toxin producing (STEC) that can cause a multitude of illnesses. *E. coli* is relatively susceptible to disinfection when dried on a surface, yet it can be a challenging microorganism to mitigate in solution.



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.



MS2 Bacteriophage (MS2), ATCC 15597-B1

This virus is a non-enveloped positive-stranded RNA virus of the bacteriophage family Leviviridae. Bacterial cells are the hosts for bacteriophages, and *E. coli* 15597 serves this purpose for MS2 bacteriophage. Its small size, icosohedral structure, and environmental resistance has made MS2 ideal for use as a surrogate virus (particularly in place of picornaviruses such as poliovirus and human norovirus) in water quality and disinfectant studies.

Permissive Host Cell System for MS2: *Escherichia coli*, 15597

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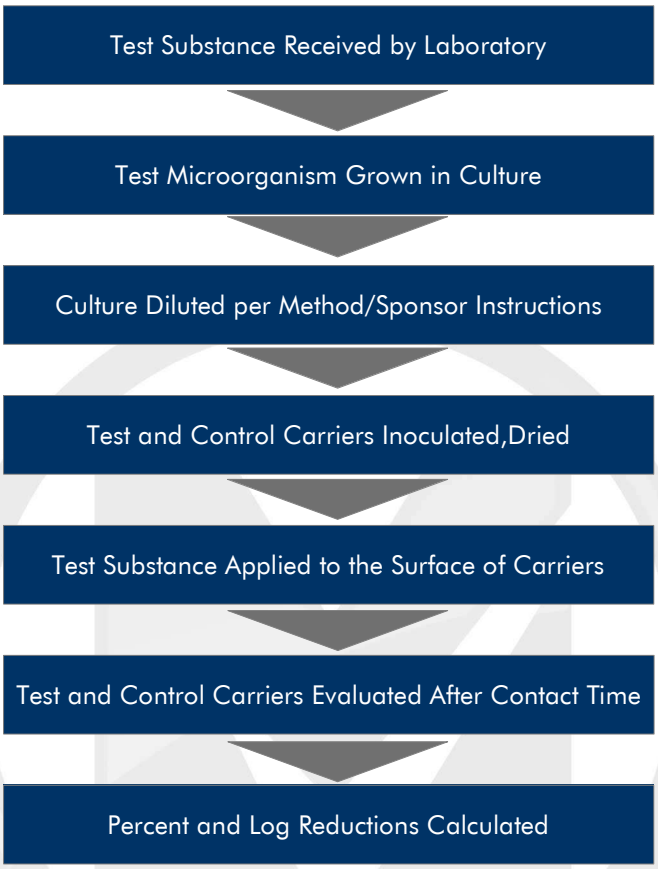
Diagram of the Test Procedure



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.

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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

SA6538 and EC8739

Culture Growth Media:	Tryptic Soy Broth	**Culture Growth Time:**	18-24 hours
Carrier Type	1"x 3" Glass Slides	**Inoculum Volume**	0.020 ml
Carrier Dry Time	20 to 40 minutes	**Carrier Dry Temp. and Humidity**	Ambient
Contact Time	1, 2, 3 and 5 minutes	**Contact Temperature**	Ambient
Harvest Media (Volume)	PBS with 0.1% Tween-80 (20.0 ml)	**Enumeration Media**	TSA (SA6538 and EC8739) 50% TSA (MS2)
Incubation Temperature	36°C ± 1°C	**Incubation Time**	24 to 48 hours

PARAMETERS

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Study Notes

For all contact times and microorganisms tested, replicate 1 was placed in the back left of the test chamber, replicate 2 was placed in front of the center lamp and replicate 3 was placed in the front right.

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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

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

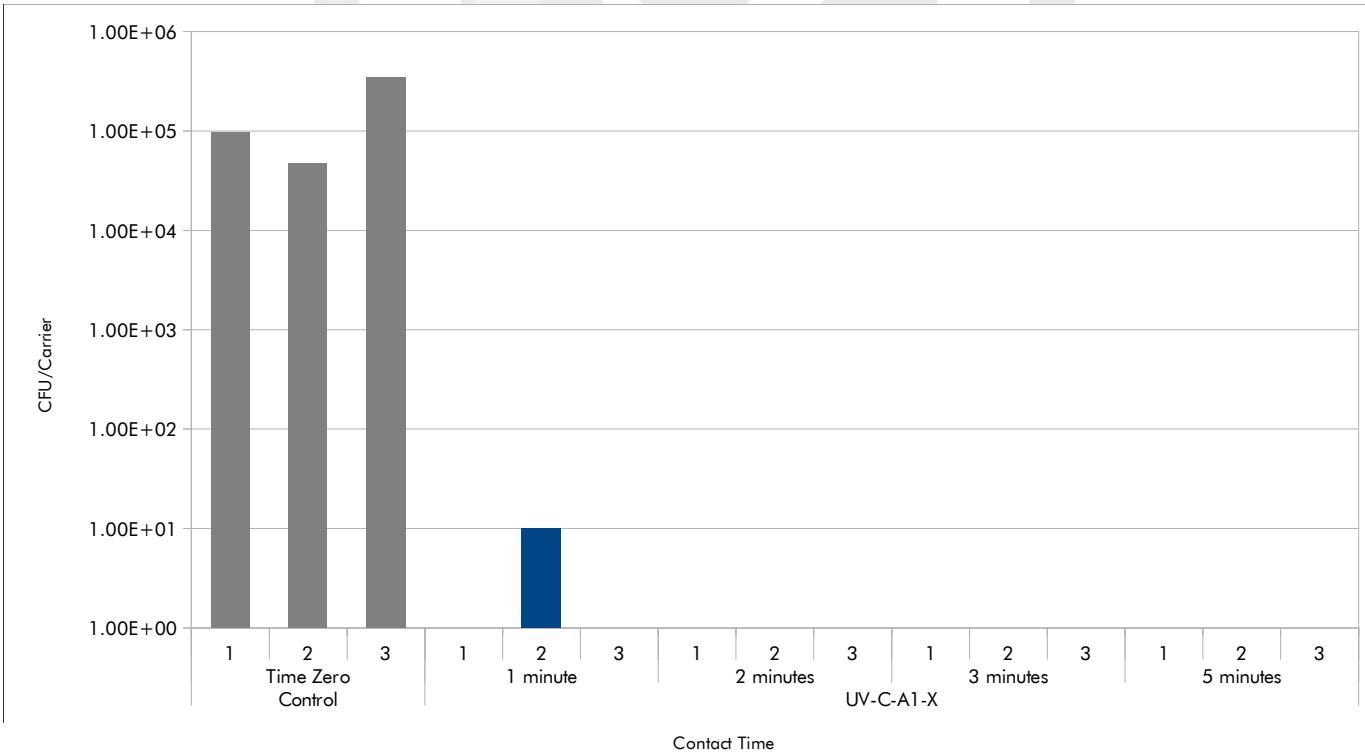
$$\text{Log}_{10}\ \text{Reduction} = \text{Log}(B/A)$$

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 – *E. coli* ATCC 8739

Test Microorganism	Device	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
E. coli ATCC 8739	Control	Time Zero	1	1.29E+05	1.89E+05	N/A	
			2	7.90E+04			
			3	3.60E+05			
	UV-C-A1-X	1 minute	1	<1.00E+01	<1.00E+01	>99.994%	>4.21
			2	1.00E+01			
			3	<1.00E+01			
		2 minutes	1	<1.00E+01	<1.00E+01	>99.994%	>4.21
			2	<1.00E+01			
			3	<1.00E+01			
		3 minutes	1	<1.00E+01	<1.00E+01	>99.994%	>4.21
			2	<1.00E+01			
			3	<1.00E+01			
		5 minutes	1	<1.00E+01	<1.00E+01	>99.994%	>4.21
			2	<1.00E+01			
			3	<1.00E+01			

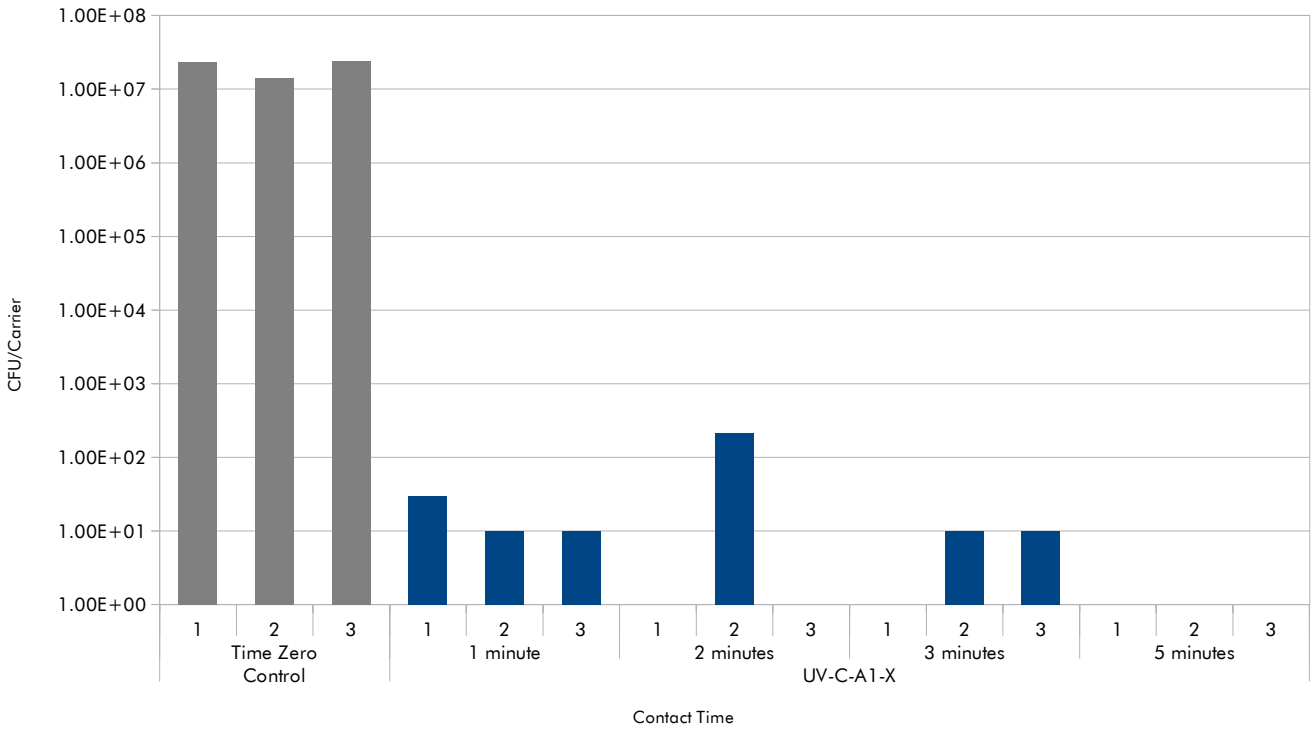
Note: The lower limit of detection for this study was 1.00E+01 CFU/mL. Values observed less than the limit are reported as "<1.00E+01" in the results table and zero in the graph.



Results of the Study – *S. aureus* ATCC 6538

Test Microorganism	Device	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
S. aureus ATCC 6538	Control	Time Zero	1	2.30E+07	2.02E+07	N/A	
			2	1.41E+07			
			3	2.35E+07			
	UV-C-A1-X	1 minute	1	3.00E+01	1.67E+01	99.99992%	6.08
			2	1.00E+01			
			3	1.00E+01			
		2 minutes	1	<1.00E+01	<7.67E+01	>99.9996%	>5.42
			2	2.10E+02			
			3	<1.00E+01			
		3 minutes	1	<1.00E+01	<1.00E+01	>99.99995%	>6.31
			2	1.00E+01			
			3	1.00E+01			
		5 minutes	1	<1.00E+01	<1.00E+01	>99.99995%	>6.31
			2	<1.00E+01			
			3	<1.00E+01			

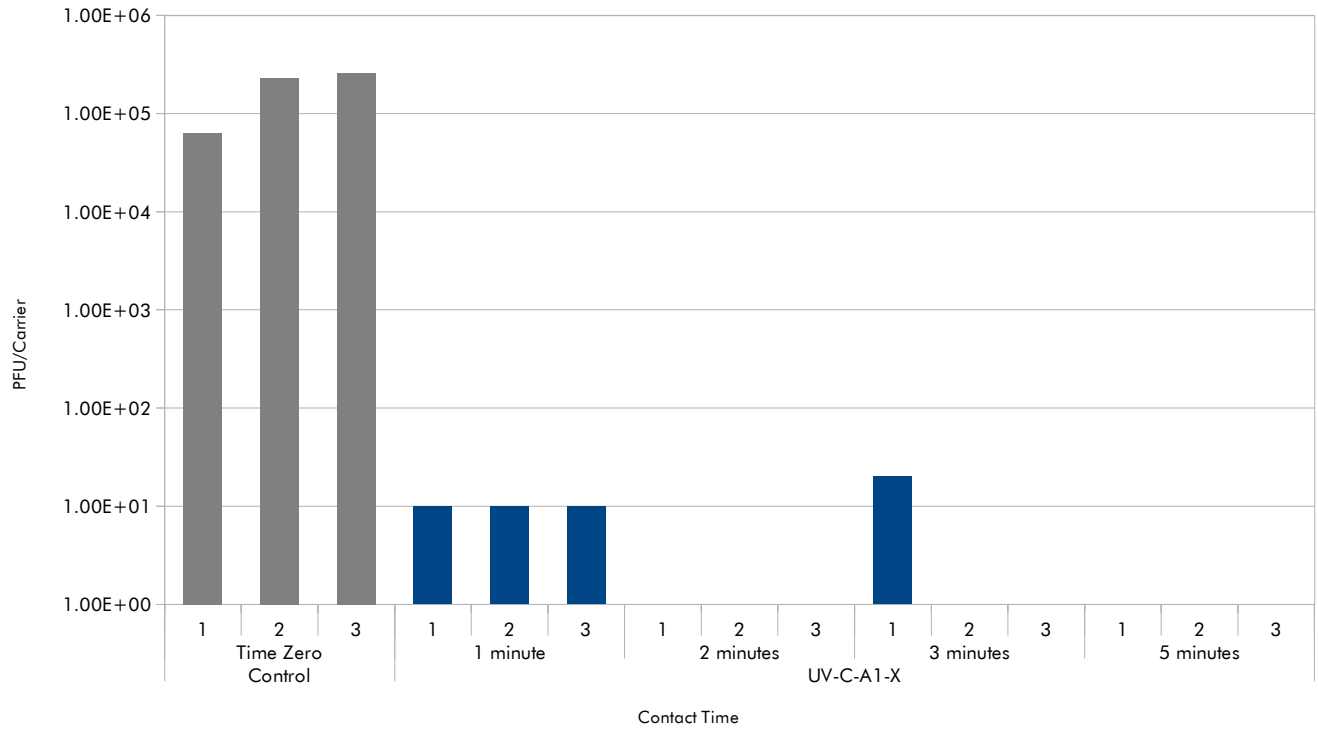
Note: The lower limit of detection for this study was 1.00E+01 CFU/mL. Values observed less than the limit are reported as "<1.00E+01" in the results table and zero in the graph.



Results of the Study – MS2 Bacteriophage ATCC 15597-B1

Test Microorganism	Device	Contact Time	Replicate	PFU/Carrier	Average PFU/Carrier	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
MS2 Bacteriophage ATCC 15597-B1	Control	Time Zero	1	6.30E+04	1.84E+05	N/A	
			2	2.30E+05			
			3	2.60E+05			
	UV-C-A1-X	1 minute	1	1.00E+01	1.00E+01	99.995%	4.27
			2	1.00E+01			
			3	1.00E+01			
		2 minutes	1	<1.00E+01	<1.00E+01	>99.995%	>4.27
			2	<1.00E+01			
			3	<1.00E+01			
		3 minutes	1	2.00E+01	2.00E+01	99.989%	3.96
			2	<1.00E+01			
			3	<1.00E+01			
		5 minutes	1	<1.00E+01	<1.00E+01	>99.995%	>4.27
			2	<1.00E+01			
			3	<1.00E+01			

Note: The lower limit of detection for this study was 1.00E+01 PFU/mL. Values observed less than the limit are reported as "<1.00E+01" in the results table and zero in the graph.



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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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