FINAL REPORT

PROTOCOL
Efficacy Testing of ELIM Sterilizer
ASTM E2315

PRODUCT TESTED
ELIM Sterilizer

EMSL ORDER NUMBER
152003212

TESTING LABORATORY
EMSL Analytical, Inc.
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Houston TX 77040
Phone: (713) 686-3635
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SPONSOR
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STUDY START DATE
April 21, 2020

STUDY COMPLETION DATE
June 15, 2020

Test Summary

Project Title: Efficacy Testing of ELIM Sterilizer water against *S. aureus*.

Study Methods: ASTM E2315 Time Kill Study

Product Tested: Water produced by ELIM Sterilizer

Sponsor: ELIM, Inc.

Test Conditions: 25°C and 30°C - Water Temperature

Challenge Organisms: *Staphylococcus aureus* (*S. aureus*) - ATCC 6538

Exposure Times: 0, 1, 5, 15 minutes and 1, 8, 24, 48 hours

Study Dates and Facilities

All analytical testing was performed at EMSL Analytical, Inc. in Houston, Texas from date 04/21/2020 to 6/15/2020.

Record Retention

All raw data and a copy of the final report will be archived and stored by EMSL Analytical, Inc. for 5 years.

Objectives

To determine the antibacterial efficacy of water produced by ELIM Sterilizer against *S. aureus*.

Test Method

Inoculum Preparation

Test bacteria was grown from a pure stock onto tryptic soy agar supplemented with 5% sheep blood (TSAB) and incubated at 36±1°C for 24 hours. A well-isolated colony was suspended into 10 mL of tryptic soy broth (TSB) and incubated at 36±1°C for 24 hours. This 24 hour culture was shaken and allowed to stand at room temperature for 15-20 minutes before preparing the inoculum. This culture was diluted 1:10 in PBS and used to inoculate the test water samples.

Procedure

The ionizer was hooked up to local tap water and with the tap open fully, for maximum pressure, ionized water was collected in a sterile vessel. Water was a collected at both 25ºC and 30ºC. Test containers were prepared in triplicate with ionized water inoculated with 1 mL of the bacterial suspension.

After inoculation, the water remained at room temperature for the duration of the testing period. At each time point, water was removed and placed into tubes containing 10 mL of D/E neutralizing broth and then vortexed for 1 minute to recover any remaining microbes. The recovered samples were serially diluted, plated onto Petrifilm AC plates and incubated for 48 h at 37±2 °C. All tests were performed in triplicate.

Experimental Results:

Table 1: Quantitative Counts for *S. aureus* Exposed to Ionized Water at 25°C.

Sample	Treatment	Time Point	Bacterial Recovery CFU/Test Surface (average of 3 surfaces)	Log Reduction	Percent Reduction
Control	Ionized water	0	3,600,000		
Test 1	Ionized water	1 min	7,400,000	None	None
Test 2	Ionized water	5 min	4,600	2.90	99.87
Test 3	Ionized water	15 min	<10	>5.56	>99.9997
Test 4	Ionized water	1 hour	<10	>5.56	>99.9997
Test 5	Ionized water	8 hour	<1	>6.56	>99.99997
Test 6	Ionized water	24 hour	<10	>5.56	>99.9997
Test 7	Ionized water	48 hour	<1	>6.56	>99.99997

Log Reduction = difference between Log of Control (untreated inoculated test specimen immediately after inoculation (at "0" contact time)) and Log of inoculated treated test specimen.
Detection limit was 1 or 10 CFU/mL.

Table 2: Quantitative Counts for *S. aureus* Exposed to Ionized Water at 30°C.

Sample	Treatment	Time Point	Bacterial Recovery CFU/Test Surface (average of 3 surfaces)	Log Reduction	Percent Reduction
Control	Ionized water	0	3,600,000		
Test 1	Ionized water	1 min	18,100,000	None	None
Test 2	Ionized water	5 min	6,100	2.78	99.8
Test 3	Ionized water	15 min	<10	>5.56	>99.9997
Test 4	Ionized water	1 hour	<10	>5.56	>99.9997
Test 5	Ionized water	8 hour	<1	>6.56	>99.99997
Test 6	Ionized water	24 hour	<1	>6.56	>99.99997
Test 7	Ionized water	48 hour	<1	>6.56	>99.99997

Log Reduction = difference between Log of Control (untreated inoculated test specimen immediately after inoculation (at "0" contact time)) and Log of inoculated treated test specimen.
Detection limit was 1 or 10 CFU/mL.

Signatures

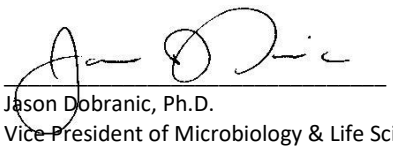
Study Performed by:



Mona Ramadi, Ph.D.
Microbiologist

6/15/2020
Date

Report Issued by:



Jason Dobranic, Ph.D.
Vice President of Microbiology & Life Sciences
Study Director

6/15/2020
Date