FINAL REPORT

Germicidal Spray Efficacy of
Arirangion Multi-Ionizer

PROTOCOL
Modified AOAC 961.02

ORDER Number
151501967

PREPARED FOR:

Arirangion CO.,LTD
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Note: Any test results under the name 'Arirangion' refer to ELIM Sterilizer products.

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Certificate of Analysis

Client: Arirangion CO, LTD.

Contact: Robert F. Chen
Project: Efficacy testing of germicidal spray produced from Arirangion ionizer.

Product: Arirangion Multi-Ionizer
EMSL NO: 151501967
Note: Any test results under the name 'Arirangion' refer to ELIM Sterilizer products.
Sample received: 3/19/2015
Start date: 3/20/2015 **Repor**
t date: 4/13/2015

Challenge Bacteria: *Escherichia coli* O157:H7 **ATCC** 43888 *Salmon ella enterica* serovar Abony **ATCC** 6017 *Staphyl ococcus aureus* **ATCC** 25923 *Escherichia coli* **ATCC** 8739
Pseudomonas aeruginosa **ATCC** 27853
*Staphylococcus aureus (*MRSA) **ATCC** 33592
Listeria monocytogenes **ATCC** 15313 *Entero coccus feacalis* (VRE) **ATCC** 700221

Water Temperatures: 20°C, 25°C and 30°C

Experimental Summary: The testing procedure was designed after discussi ons between EMSL Analytical, the testing company, and the client, Premier Living Essentials, Inc. The testing procedure is based on AOAC 961.02, with the testing conducted on a multi-ionizer attachment to a sink head for its ability to sterilize a nd disinfect bacteria. The testing was conducted in our Houston Microbiology Lab oratory.

Procedure:

The testing was done to determine the effectiveness of a multi-ionizer against the challenge bacteria *Salmonella enterica (S. enterica), Escherichia coli* O157:H7 (*E. coli* O157:H7), *Staphylococcus aureus* (*S. aureus), Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli* (*E. coli*), Methicillin Resistant *Staphylococcus aureu s* (MRSA*), Listeria monocytogenes* (*L. monocytogenes*), and Vancomycin Resista nt *Enterococcus feacalis* (VRE). Two test methods were used; one being qualitati ve and the other quantitative. All bacteria were first grown on tryptic soy agar plat es at 35°C for 24 h, then transferred into 9 mL of Tryptic soy broth (TSB) and incu bated for an additional 24 h. 50 µL of this TSB solution was then

inoculated onto individual sterile glass slides. Each slide was placed in an empty Petri dish and allowed to dry at room temperature inside a biological safety cabinet (~60 min).

Qualitative Test:

As per a modified AOAC 961.02 method, the presence/absence test was completed using 10 carriers inoculated with the challenge bacteria. The bacteria were allowed to dry on the pre-sterilized glass carriers as described above.

Viability Control: Two inoculated carriers not exposed to the testing water were immediately placed into 20 mL of Tryptic Soy Broth (TSB). Each tube was then incubated at 35°C for 24 h. Quality control of the 20 mL TSB tubes was conducted by incubating two un-inoculated tubes at the same conditions.

Test Procedure: The multi-ionizer was attached to a sink head, as per client's instructions, before testing took place. The sink was then turned on and the temperature was determined first before contact with the inoculated carriers. Once the correct temperature was achieved the inoculated glass carrier was sprayed with the multi-ionizer for 10 seconds and then placed into 20 mL of TSB and vortexed for 30 seconds. This was repeated 10 times. Each tube of TSB was incubated at 35°C for 24 h. Following incubation the tubes were observed for presence or absence of growth.

Quantitative Test:

In conjunction with the presence/absence test, triplicate carriers were used to determine the log reduction and percent reduction. To determine the starting population three glass carriers were placed into 10 mL of phosphate buffer solution (PBS) and vortexed for 30 seconds. 1 mL aliquots were then taken, serially diluted, and plated on aerobic count plates (Petrifilm). Simultaneously, three glass carriers were individually sprayed with the multi-ionizer for 10 sec. Following exposure the carriers were placed into 10 mL of PBS and similarly diluted and plated onto aerobic count plates. All plates were incubated at 35°C for 24 h. All tests were performed in triplicate.





Photos 1 & 2. Arirangion Multi-Ionizer set-up for testing at EMSL Analytical, Inc.

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Photo 3. Example of test carriers air drying under a biosafety cabinet in preparation of testing against the ionized water.

Experimental Results:

Table 1.1 Presence/Absence of Bacteria Exposed to Ionized Water

Organism	20°C		25°C		30°C	
	Presence	Absence	Presence	Absence	Presence	Absence
***E. coli* O157:H7**	10*	0	10	0	10	0
S. enterica	10	0	10	0	10	0
S. aureus	10	0	10	0	10	0
E. coli	10	0	10	0	10	0
P. aeruginosa	10	0	10	0	10	0
MRSA	10	0	10	0	10	0
L. monocytogenes	10	0	10	0	10	0
VRE	10	0	10	0	10	0

* Numbers represent the number of tubes (out of 10) that were present or absent for growth after exposure to ionized water.

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Table 1.2 Quantitative Counts for *E. coli* O157:H7 Exposed Ionized Water

Temperature	Control Avg CFU	Test Avg CFU	LR	%Reduction
20°C	4.73×10^7	1.00×10^2	5.67	99.9998%
25°C	1.39×10^7	1.00×10^2	5.14	99.9993%
30°C	7.70×10^6	1.00×10^2	4.88	99.998%

LR – Log Reduction; Log difference between starting population and test carrier.
%Reduction – percent difference between starting population and exposed population.
Detection Limit= 100 CFU

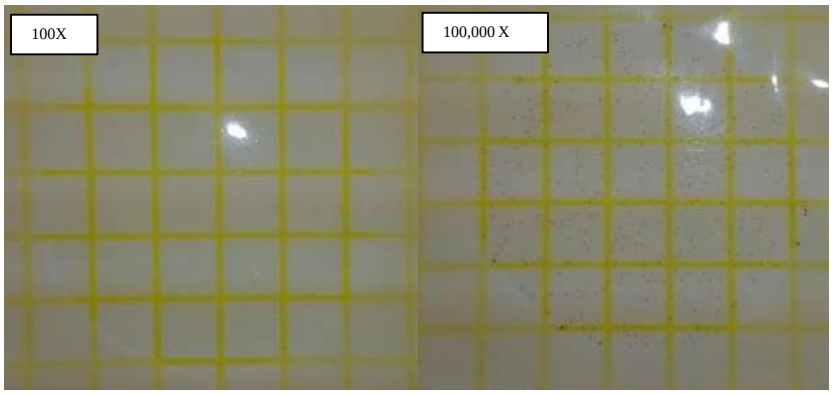


Photo 4. Example culture plates of *E. coli* O157:H7, left side (no growth) was from a carrier treated with ionized water and right side an untreated test carrier.

Table 1.3 Quantitative Counts of *S. enterica* Exposed to Ionized Water

Temperature	Control Avg CFU	Test Avg CFU	LR	%Reduction
20°C	1.00×10^7	1.20×10^3	4.16	99.993%
25°C	6.63×10^6	1.67×10^2	4.65	99.998%
30°C	1.32×10^7	1.00×10^2	5.12	99.9992%

LR – Log Reduction; Log difference between starting population and test carrier.
%Reduction – percent difference between starting population and exposed population.
Detection Limit= 100 CFU

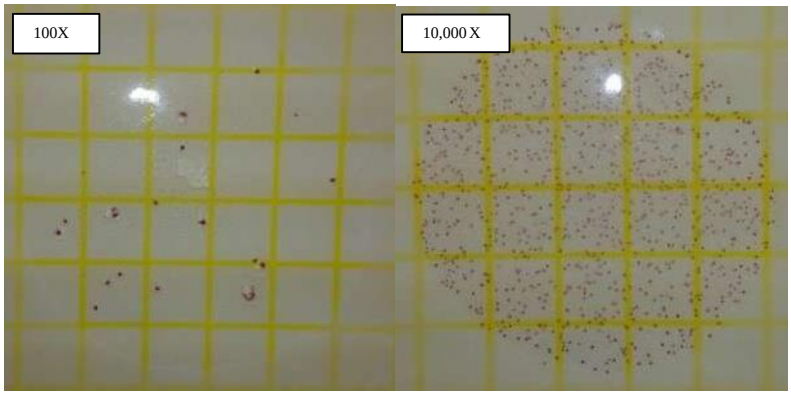


Photo 5. Example culture plates of *S. enterica*, left side was from a test carrier treated with ionized water and right side is a control, untreated test carrier.

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Table 1.4 Quantitative Counts for *S. aureus* Exposed to Ionized Water

Temperature	Control Avg CFU	Test Avg CFU	LR	%Reduction
20°C	1.04×10^7	1.10×10^5	2.03	99.057%
25°C	2.93×10^7	1.00×10^2	5.46	99.9997%
30°C	1.95×10^7	2.30×10^4	3.60	99.975%

LR – Log Reduction; Log difference between starting population and test carrier.
%Reduction – percent difference between starting population and exposed population.
Detection Limit= 100 CFU

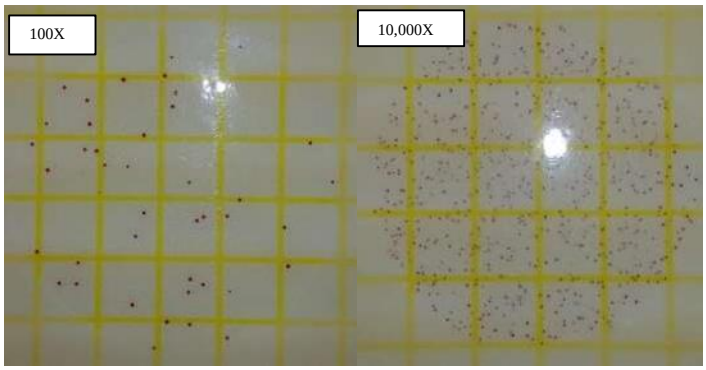


Photo 6. Example culture plates of *S. aureus*, left side was from a test carrier treated with ionized water and right side is a control, untreated test carrier.

Table 1.5 Quantitative Counts for *E. coli* Exposed Ionized Water

Temperature	Control Avg CFU	Test Avg CFU	LR	%Reduction
20°C	6.13×10^7	1.00×10^2	5.77	99.9998%
25°C	3.33×10^6	1.00×10^2	4.51	99.997%
30°C	8.23×10^6	1.00×10^2	4.90	99.9987%

LR – Log Reduction; Log difference between starting population and test carrier.
%Reduction – percent difference between starting population and exposed population.
Detection Limit= 100 CFU

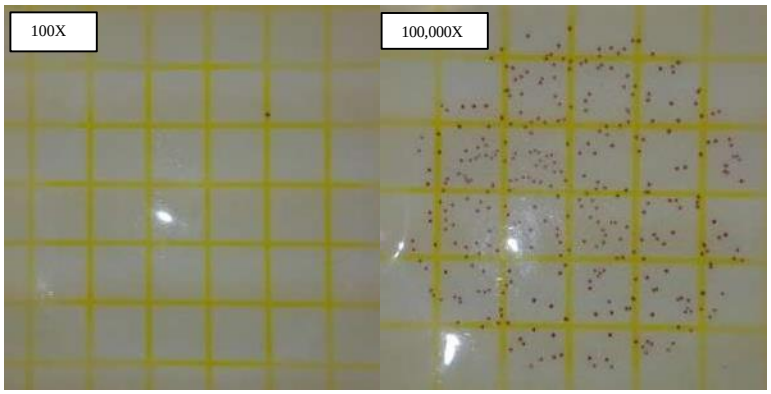


Photo 7. Example culture plates of *E. coli*, left side was from a test carrier treated with ionized water and right side is a control, untreated test carrier.

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Table 1.6 Quantitative Counts of *P. aeruginosa* Exposed to Ionized Water

Temperature	Control Avg CFU	Test Avg CFU	LR	%Reduction
20°C	1.07×10^8	1.00×10^2	6.01	99.9999%
25°C	5.70×10^6	2.47×10^4	2.37	99.575%
30°C	3.70×10^6	1.00×10^2	4.56	99.997%

LR – Log Reduction; Log difference between starting population and test carrier.
%Reduction – percent difference between starting population and exposed population.
Detection Limit= 100 CFU

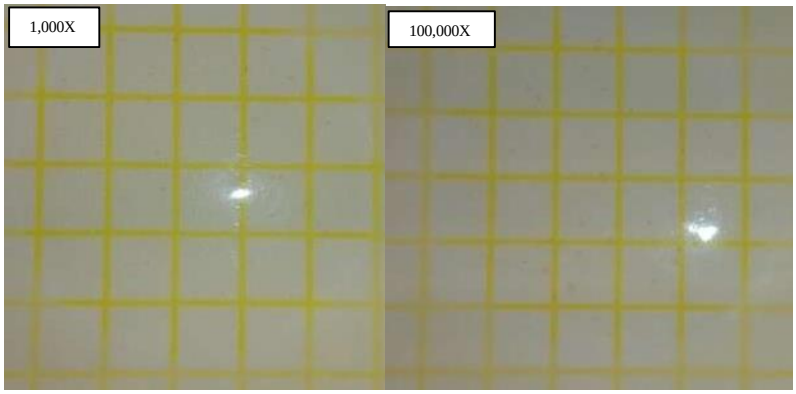


Photo 8. Example culture plates of *P. aeruginosa*, left side was from a test carrier treated with ionized water and right side is a control, untreated test carrier.

Table 1.7 Quantitative Counts for MRSA Exposed to Ionized Water

Temperature	Control Avg CFU	Test Avg CFU	LR	%Reduction
20°C	3.87×10^7	2.50×10^5	2.24	99.426%
25°C	9.63×10^6	4.33×10^2	4.61	99.998%
30°C	1.07×10^7	3.33×10^2	4.73	99.998%

LR – Log Reduction; Log difference between starting population and test carrier.
%Reduction – percent difference between starting population and exposed population.
Detection Limit= 100 CFU

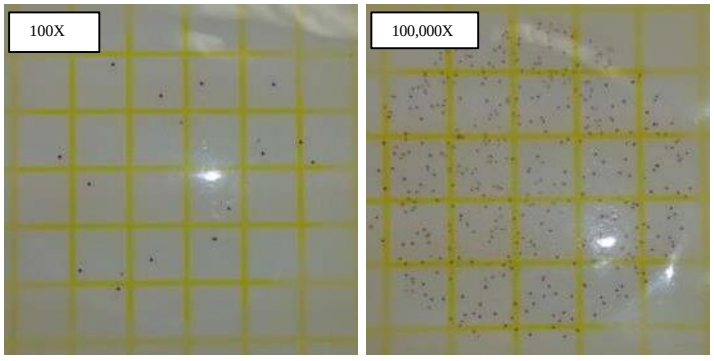


Photo 9. Example culture plates of MRSA, left side was from a test carrier treated with ionized water and right side is a control, untreated test carrier.

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Table 1.8 Quantitative Counts for VRE Exposed to Ionized Water

Temperature	Control Avg CFU	Test Avg CFU	LR	%Reduction
20°C	1.06×10^7	2.77×10^3	3.67	99.979%
25°C	1.29×10^7	2.33×10^2	4.86	99.998%
30°C	3.00×10^7	1.00×10^2	5.47	99.9997%

LR – Log Reduction; Log difference between starting population and test carrier.
%Reduction – percent difference between starting population and exposed population.
Detection Limit= 100 CFU

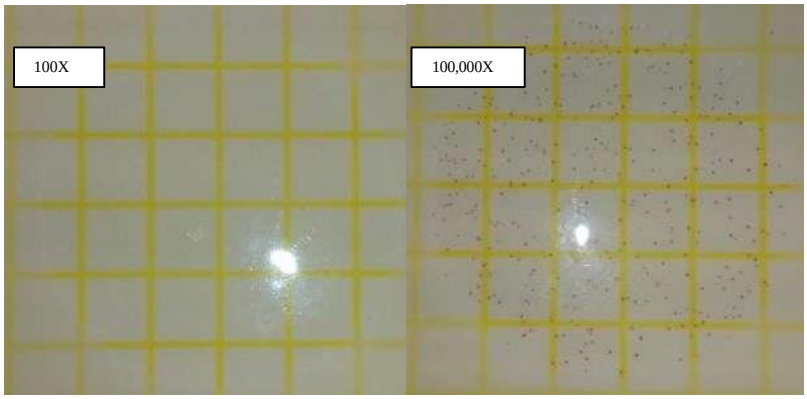


Photo 10. Example culture plates of VRE, left side (no growth) was from a test ca rrier treated with ionized water and right side is a control, untreated test carrier.

Table 1.9 Quantitative Counts for *L. monocytogenes* Exposed to Ionized Water

Temperature	Control Avg CFU	Test Avg CFU	LR	%Reduction
20°C	1.77×10^6	1.00×10^2	4.24	99.994%
25°C	1.03×10^5	1.00×10^2	3.01	99.903%
30°C	2.33×10^5	1.00×10^2	3.37	99.957%

LR – Log Reduction; Log difference between starting population and test carrier.
%Reduction – percent difference between starting population and exposed population.
Detection Limit= 100 CFU

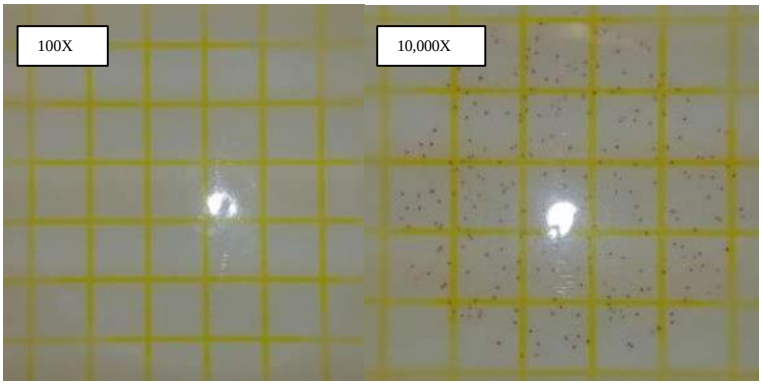


Photo 11. Example culture plates of *L. monocytogenes*, left side (no growth) was from a test carrier treated with ionized water and right side is a control, untreated test carrier.

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Conclusions/Observations:

The Arirangion multi-ionizer provided by Premier Living Essentials, Inc. was tested using a qualitative and quantitative method at three different water temperatures (20°C, 25°C and 30°C). The qualitative test demonstrated that a contact time of 10 seconds was not sufficient to sterilize (kill) all the bacteria tested (10 glass carriers Table 1.1). Blank media was tested to confirm sterility of media used during testing. Viability controls for each bacteria was tested to ensure growth performance.

In addition, a quantitative test was performed, tables 1.2 – 1.9, with results highlighted in cells where the desired 99.99% reduction was acquired. The results demonstrate that at all water temperatures tested the multi-ionizer was effective against some of the bacteria tested. At all three temperatures, *E. coli* O157:H7, *S. enterica,* and *E. coli* were killed by the ionized water by >99.99%. MRSA and VRE were killed at >99.99% when the ionized water was at 25 or 30°C. The other bacteria were also killed at >99.99% but a varying water temperatures.

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