FINAL REPORT

Assessment of Antimicrobial Activity

Using a Time-Kill Procedure

Order Number: 551710040

PREPARED FOR
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CERTIFICATE OF ANALYSIS

CLIENT: FRANKE KINDRED CANADA

CONTACT: CORY MACEY

PROJECT:

ASSESSMENT OF ANTIMICROBIAL ACTIVITY USING ASTM METHOD E2315

PRODUCT: OZONATED TAP WATER

SAMPLE RECEIVED: 09/27/2017

REPORT DATE: 09/27/2017

CHALLENGE BACTERIA:

P.AERUGINOSA, S.AUREUS (MRSA)

I. EXPERIMENTAL SUMMARY

The testing procedure was designed after discussions between EMSL Canada Inc. and Franke Kindred Canada. The procedure is based on ASTM E2315 method guidelines and conducted on two ozonated water samples of concentrations 0.5 ppm and 5 ppm, to demonstrate its effectiveness at killing *Staphylococcus aureus* MRSA (ATCC 43300) and *Pseudomonas aeruginosa* (ATCC 33988). The testing was conducted in the Mississauga Microbiology Laboratory.

II. PROCEDURE

The testing was done to determine the effectiveness of ozonated water samples of concentrations 0.5 ppm and 5 ppm (provided by Cory Macey), to demonstrate effectiveness at killing *Staphylococcus aureus* MRSA (ATCC 43300) and *Pseudomonas aeruginosa* (ATCC 33988) for 30 seconds, 5-minute and 15 minutes exposure times.

Culture preparation:

Staphylococcus aureus (MRSA) and *Pseudomonas aeruginosa* were plated onto Tryptic Soy Agar with 5% sheep blood (TSAB), and incubated at 35°C for 24-h. Then a single isolated colony of each was taken and inoculated in 100 mL of Tryptic Soy Broth at 35°C for 24-h before testing was conducted.

The control suspension of each test microorganism was standardized to a minimum concentration of 1.0 x 10^6 CFU/mL, by dilution in a buffered saline solution.
Test and control substances were dispensed in identical volumes to sterile test tubes.
Independently, Test and Control substances were inoculated with each test microorganism,

mixed and incubated.
Control suspensions were immediately plated to represent the concentration present at the start of the test, or time zero.
At the conclusion of each contact time, a volume of the liquid test solution was neutralized.
Dilutions of the neutralized test solution were plated on TSA plates and incubated at 35°C for 24-h to 48 hours to determine the surviving microorganisms at the respective contact times.
Reductions of microorganisms were calculated by comparing initial microbial concentrations to surviving microbial concentrations.
All tests were performed in duplicates and counts averaged.

Calculations:

Calculations were based on the following:

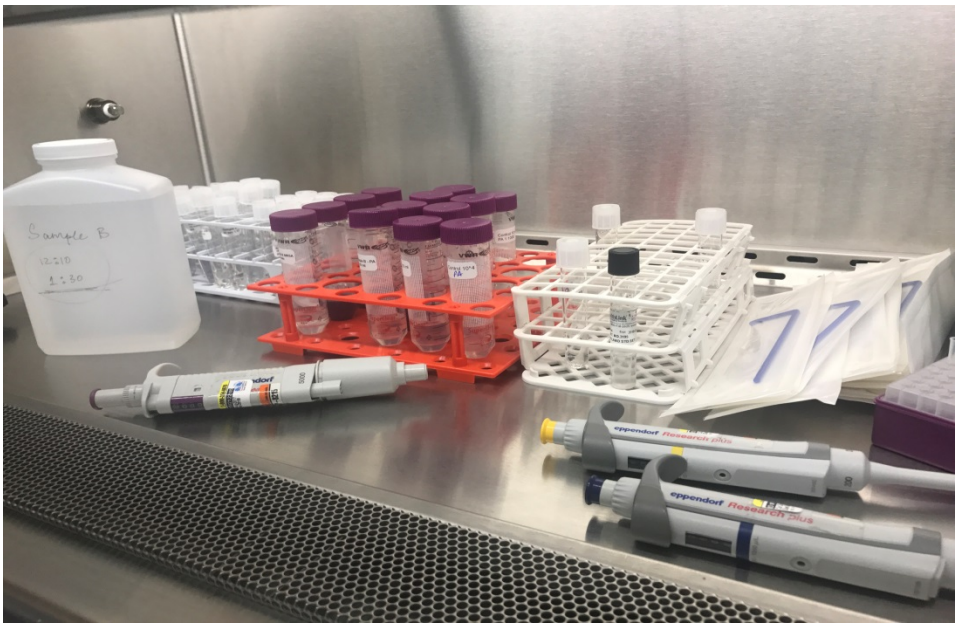
Percentage reduction = (B-A/B) x 100

Log 10 Reduction = Log (B/A)

Where:

B = Number of viable test microorganisms in the control substance immediately after inoculation

A = Number of viable test microorganisms in the test substance after the contact time



III. EXPERIMENTAL RESULTS

Test Microorganism	Test Substance	Contact Time	CFUs/mL	Percent Reduction Compared to Control at Time Zero	Log10 Reduction compared to Control at Time Zero
P.aeruginosa	CONTROL	Time Zero	2.10E+07	n/a	
	SAMPLE A: 0.5 PPM CONC.	30 seconds	1.2E+05	99.42%	2.24
		15 mins	7.3E+04	99.65%	2.45

Test Microorganism	Test Substance	Contact Time	CFUs/mL	Percent Reduction Compared to Control at Time Zero	Log10 Reduction compared to Control at Time Zero
P.aeruginosa	CONTROL	Time Zero	1.33E+06	n/a	
	SAMPLE B 5.0 PPM CONC.	30 seconds	<1.00E+01	>99.98%	>4.90
		5 mins	<1.00E+01	>99.98%	>4.90
		15 mins	<1.00E+01	>99.98%	>4.90

Test Microorganism	Test Substance	Contact Time	CFUs/mL	Percent Reduction Compared to Control at Time Zero	Log10 Reduction compared to Control at Time Zero
S.aureus (MRSA)	CONTROL	Time Zero	5.8E+06	n/a	
	SAMPLE A 0.5 PPM CONC.	30 seconds	7.7E+04	98.67%	1.90
		5 mins	6.6E+04	98.86%	1.94
		15 mins	5.1E+04	99.12%	2.05

Test Microorganism	Test Substance	Contact Time	CFUs/mL	Percent Reduction Compared to Control at Time Zero	Log10 Reduction compared to Control at Time Zero
S.aureus (MRSA)	CONTROL	Time Zero	5.8E+06	n/a	
	SAMPLE B 5.0 PPM CONC.	30 seconds	<1.00E+01	>99.98%	>5.10
		5 mins	<1.00E+01	>99.98%	>5.10
		15 mins	<1.00E+01	>99.98%	>5.10

IV. CONCLUSIONS/OBSERVATIONS

Sample B (concentration 5.0 ppm) was more effective on both the test microorganisms *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* with more than 99.98% reduction for all time points. While Sample A (concentration 0.5 ppm) proved to be more effective on P.aeruginosa for both 30 seconds (99.42% reduction) and 15 minutes (99.65% reduction) as compared to S.aureus (MRSA) for the same time points.

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