FINAL REPORT

Assessment of Antimicrobial Activity

Using a Time-Kill Procedure

Order Number: 551801924

PREPARED FOR
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2/28/2018

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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: 02/01/2018

REPORT DATE: 02/28/2018

CHALLENGE BACTERIA:

Clostridium difficile spores

Legionella pneumophila

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 ozonated water samples of concentrations 2.0 ppm to demonstrate its effectiveness at killing *Clostridium difficile* (ATCC 9689) spores and *Legionella pneumophila*. The testing was conducted in the Mississauga Microbiology Laboratory.

II. PROCEDURE

The testing was done to determine the effectiveness of ozonated water sample of concentration 2.0 ppm (provided by Cory Macey) at killing *Clostridium difficile* (ATCC 9689) spores for 20 seconds exposure time and *Legionella pneumophila* for 5 minutes exposure time.

Culture preparation:

Clostridium difficile spores: A pure culture of *Clostridium difficile* (ATCC 9689) was streaked on to Healthlink Anaerobic Blood Agar plates and incubated at 35°C for up to 10 days under anaerobic conditions. Following incubation, the surface of agar plate was scraped and spores were harvested. The control spore suspension was adjusted to a concentration of >90%.

Legionella pneumophila: A pure culture of *Legionella pneumophila* was streaked onto Buffered Charcoal Yeast Extract agar for 48 hours at 35°C. The control suspension of the test microorganism was standardized to a minimum concentration of 1.0 x 10^6 CFU/mL.

Test and control substances were dispensed in identical volumes to sterile test tubes.
Independently, Test and Control substances were inoculated with the 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 to appropriate agar plates and incubation temperatures 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	Log10Reduction compared to Control at Time Zero
Legionella pneumophila	CONTROL	Time Zero	2.0E+06	n/a	
	SAMPLE A: 2.0 PPM	5 minutes	<1.00E+01	99.99%	5.3

Test Microorganism	Test Substance	Contact Time	CFUs/mL	Percent Reduction Compared to Control at Time Zero	Log10 Reduction compared to Control at Time Zero
C.difficile spores	CONTROL	Time Zero	3.9E+03	n/a	
	SAMPLE A: 2.0 PPM CONC.	20 seconds	1.4E+03	64.10%	0.44

IV. CONCLUSIONS/OBSERVATIONS

Sample A (concentration 2.0 ppm) caused a 99.99% reduction on *L.pneumophila* upon 5 minutes exposure.

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