FINAL REPORT

Assessment of Antimicrobial Activity

Using a Time-Kill Procedure

Order Number: 551903868

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: 03/15/2019

REPORT DATE: 04/05/2019

CHALLENGE BACTERIA:

Klebsiella pneumonia ATCC BAA-1705

I. EXPERIMENTAL SUMMARY

The testing procedure was designed after discussions between EMSL Canada Inc. and Cory Macey, Franke Kindred Canada. The procedure is based on ASTM E2315 method guidelines and conducted on two ozonated water samples as below, to demonstrate its effectiveness at killing *Klebsiella pneumoniae* ATCC BAA-1705. The testing was conducted in the Mississauga Microbiology Laboratory.

Sample Concentration A: 0.15 ppm (exposure times 15 mins and 60 minutes) and,

Sample Concentration B: 1.5 ppm (exposure times 30 seconds and 15 minutes)

II. PROCEDURE

Culture preparation:

A pure culture of *Klebsiella pneumoniae* ATCC BAA-1705 was streaked on to TSA w/5% Sheep Blood Agar plates and incubated at 35°C for up to 24 to 48 hours. A single isolated colony of each was taken and inoculated in 10 mL of Tryptic Soy Broth at 35°C for 24 hours before testing was conducted. The control suspension of the 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 stated exposure time, a volume of the liquid test solution was neutralized. Dilutions of the neutralized test solutions were plated on 3M Petrifilm plates and incubated at 35°C for 24- 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	Averaged CFUs/mL	Percent Reduction Compared to Control at Time Zero	Log10 Reduction compared to Control at Time Zero
K.pneumoniae ATCC BAA 1705	CONTROL	Time Zero	1.15E+06	n/a	
	SAMPLE A: 0.15 PPM	15 mins	<1.00E+01	99.99%	5.06
		60 mins	<1.00E+01	99.99%	5.06
	SAMPLE B: 1.5 ppm	30 sec	<1.00E+01	99.99%	5.06
		15 mins	<1.00E+01	99.99%	5.06

IV. CONCLUSIONS/OBSERVATIONS

Both the samples - 0.15 ppm (exposure times 15 and 60 mins) and 1.5 ppm (exposure times 30 seconds and 15 mins) were able to demonstrate a strong efficacy against *Klebsiella pneumoniae* ATCC BAA-1705 causing a 99.99% reduction.

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