



TEST REPORT

Antimicrobial Efficacy Testing of a Portable Water Purifier Against *Vibrio cholerae*

Client: Del Industries
Client Number: 12830
NSF Program: 0025
Product: Portable Water Purifier
Job Number: J-00095570
Date of Experimentation: December 17, 2010 – January 6, 2011
Overall Study Result: COMPLETE

Study Director: Robert Donofrio **Date:** 1-17-11
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STUDY PERSONNEL

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METHODOLOGY

Antimicrobial Efficacy Evaluation

The Client's product, a portable water purifier, was evaluated for its antimicrobial efficacy against a consortium of multiple strains of *Vibrio cholerae* at varying exposure times for multiple test water volumes. ASTM E 2315: Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure was followed with the modifications described below.

Organism Growth: Three strains of *Vibrio cholerae* were used in this study. The strains used were as follows: ATCC 14100, ATCC 14104, and ATCC 14366. The organism stock suspensions were prepared by growing the strains individually in Tryptic Soy Broth and incubated for 24 hours at 35°C. For all of the viability studies, aliquots of the exposure samples were spread plated on Tryptic Soy Agar and incubated for 24 hours at 35°C.

Test Water Selection: In order to select the challenge water, an initial study was performed to determine the viability of *V. cholerae* in three water types: dechlorinated tap water, phosphate buffer and 0.8% saline solution. The organism stock suspensions were centrifuged, washed three times and resuspended in demand-free phosphate buffer water prior to inoculation into the test water. The three organism strains were combined and then added to the test flasks to achieve a target concentration of 1×10^6 CFU/mL. The suspensions were assayed for *V. cholerae* viability at the following exposure times: 0 hours, 0.5 hours, 1 hour and 24 hours.

Efficacy Study: Based on the results of the preliminary viability study, dechlorinated tap water was selected as the test water. Four different volumes of test water were evaluated: 1 liter, 1 gallon, 3 gallons and 5 gallons. Triplicate flasks were set up for each test volume. The organism stock



suspensions were centrifuged, washed three times and resuspended in demand-free phosphate buffer water prior to inoculation into the test water. The three organism strains were combined and then added to the test flasks to achieve a target concentration of 1×10^6 CFU/mL. Exposure times for each test volume were as follows:

- 1 liter – 0, 0.5, 2, and 5 minutes
- 1 gallon – 0, 1, 3, and 5 minutes
- 3 gallons – 0, 10, 15, and 20 minutes
- 5 gallons – 0, 20, 25 and 30 minutes

Time 0 samples were collected following addition of the challenge organism to the triplicate flasks. Following the Time 0 sample collection, the Client's water purifier was introduced to the test flask. At each exposure time, a sample aliquot was removed from each triplicate flask and placed in a neutralizer solution. The neutralized, exposed sample was then processed for viable *V. cholerae* viability utilizing the growth procedure described previously. The log reduction of the challenge organisms was calculated by comparing the viable concentration of challenge organisms at the specified exposure times against an untreated numbers control (Time 0). Additionally, the oxidation / reduction potential (ORP) was measured for each test flask at each sampling point.



RESULTS

The data obtained from the efficacy studies is presented in Appendix A. The Client's product achieved a greater than a 3-log reduction (>99.9% reduction) for the following volumes and exposure times:

- **5 gallon – 20, 25 and 30 minutes**
- **3 gallon – 10, 15 and 20 minutes**
- **1 gallon – 5 minutes**
- **1 liter – 2 and 5 minutes**



Table 1. Log reduction in *Vibrio cholerae* viability due to antimicrobial activity of Client’s portable water purifier – 5 gallon test water volume. Bacterial concentrations are presented in CFU/mL and are the geometric mean of three replicates. Standard deviations are presented in parentheses. The detection limit is 10 CFU/mL. ORP values are presented in mV and are averages of three replicates.

Exposure Time	<i>V. cholerae</i> Concentration	Log Reduction	ORP
0 minutes	4.82 x 10 ⁶ (2.95 x 10 ⁵)	NA	297.7
20 minutes	7.15 x 10 ¹ (3.33 x 10 ²)	4.83	297.0
25 minutes	6.60 x 10 ¹ (1.15 x 10 ²)	4.86	297.0
30 minutes	2.29 x 10 ¹ (6.35 x 10 ¹)	5.32	297.3



Table 2. Log reduction in *Vibrio cholerae* viability due to antimicrobial activity of Client’s portable water purifier – 3 gallon test water volume. Bacterial concentrations are presented in CFU/mL and are the geometric mean of three replicates. Standard deviations are presented in parentheses. The detection limit is 10 CFU/mL. ORP values are presented in mV and are averages of three replicates.

Exposure Time	<i>V. cholerae</i> Concentration	Log Reduction	ORP
0 minutes	7.11 x 10 ⁶ (1.40 x 10 ⁶)	NA	293.7
10 minutes	1.59 x 10 ¹ (1.73 x 10 ¹)	5.65	289.7
15 minutes	<1.00 x 10 ¹ (NA)	>5.85	288.7
20 minutes	1.44 x 10 ¹ (1.15 x 10 ¹)	5.69	288.0



Table 3. Log reduction in *Vibrio cholerae* viability due to antimicrobial activity of Client’s portable water purifier – 1 gallon test water volume. Bacterial concentrations are presented in CFU/mL and are the geometric mean of three replicates. Standard deviations are presented in parentheses. The detection limit is 10 CFU/mL. ORP values are presented in mV and are averages of three replicates.

Exposure Time	<i>V. cholerae</i> Concentration	Log Reduction	ORP
0 minutes	3.68 x 10 ⁶ (7.64 x 10 ⁵)	NA	303.3
1 minute	1.01 x 10 ⁶ (6.24 x 10 ⁵)	0.56	303.3
3 minutes	1.95 x 10 ⁵ (4.88 x 10 ⁵)	1.28	302.7
5 minutes	2.97 x 10 ³ (5.51 x 10 ⁴)	3.09	302.7



Table 4. Log reduction in *Vibrio cholerae* viability due to antimicrobial activity of Client’s portable water purifier – 1 liter test water volume. Bacterial concentrations are presented in CFU/mL and are the geometric mean of three replicates. Standard deviations are presented in parentheses. The detection limit is 10 CFU/mL. ORP values are presented in mV and are averages of three replicates.

Exposure Time	<i>V. cholerae</i> Concentration	Log Reduction	ORP
0 minutes	1.51 x 10 ⁶ (1.46 x 10 ⁵)	NA	301.0
0.5 minutes	3.44 x 10 ⁴ (8.38 x 10 ⁴)	1.64	299.7
2 minutes	1.44 x 10 ¹ (1.15 x 10 ¹)	5.02	299.3
5 minutes	1.44 x 10 ¹ (1.15 x 10 ¹)	5.02	299.7



REFERENCES

ASTM E 2315: Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure
Standard Methods for the Examination of Water and Wastewater, 20th edition.