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Client: LARQ

Invoice Number: IN2024020047 - V2 (Updated the wording for this report)

Sample Number: HR20240290135, HR20240290136

Study: Antimicrobial efficacy of the LARQ Bottles against *Escherichia coli*

Method: ASTM E2315

Report Date: 02/14/2024

Certificate of Analysis

Experimental Summary:

The objective of this procedure was to test the antimicrobial efficacy of LARQ's UV-C LED technology within the LARQ Bottle against water samples enriched with *E. coli*. The testing procedure was designed after discussions between LARQ and Harrens Lab Inc. and based on ASTM E2315 ("Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure") testing guidelines. The testing procedure was conducted at Harrens Lab Inc. in Hayward, CA.

Methods and Materials:

LARQ provided 2 stainless steel bottles of two different volumes (680mL, 1000mL) and one UV-C LED cap for the testing. All bottles were washed with sterile deionized water prior to testing. *Escherichia coli* (ATCC 25922) was used as the testing organism in this experiment and spiked into DI water to create a starting solution of about $1.0E+07$ CFU/mL to be treated. Testing was done in 3 replicates for 1-min (Normal Activation Mode), 2-min (Maintenance Mode) and 3-min (Adventure Mode) tests. Inoculated volumes for each run was 600mL to be tested in the 680mL stainless steel bottle with the UV-C cap and 900mL to be tested in the 1000mL stainless steel bottle with the UV-C cap. Pre and post treatment aliquots of the culture suspensions were plated in serial dilutions ranging from 10^{-1} to 10^{-8} on APC media. Plates were incubated for 48 hours at 30-35°C.

Figure 1 and 2: LARQ Bottles with cap on and off



Results:

Table 1: Experimental results using 1-min (Normal Activation Mode) UV-C treatment against *E.coli*

Bottle	Initial Population (CFU/mL)	T-1 min (CFU/mL)	Log Reduction (T1)	% Reduction (T1)
680 mL	7.0E+07	1.0E+01	6.845	99.9999%
	6.5E+07	1.0E+01	6.813	99.9999%
	7.2E+07	1.0E+01	6.857	99.9999%
1000 mL	5.0E+07	8.5E+02	4.77	99.998%
	5.5E+07	6.9E+02	4.902	99.999%
	6.0E+07	7.0E+02	4.933	99.999%

Comment: No growth was detected on 680 mL plates so a value of 10 was used to indicate the detection limit (< 10 CFU).

Table 2: Experimental Results using 2-min (Maintenance Mode) UV-C treatment against *E.coli*

Bottle	Initial Population (CFU/mL)	T-2 min (CFU/mL)	Log Reduction (T2)	% Reduction (T2)
680 mL	6.4E+07	1.0E+01	6.806	99.9999%
	7.1E+07	1.0E+01	6.851	99.9999%
	6.2E+07	1.0E+01	6.792	99.9999%
1000 mL	6.0E+07	1.0E+01	6.778	99.9999%
	5.3E+07	1.0E+01	6.724	99.9999%
	5.7E+07	1.0E+01	6.756	99.9999%

Comment: No growth was detected on 2-min (Maintenance Mode) treated plates so a value of 10 was used to indicate the detection limit (< 10 CFU).

Table 3: Experimental Results using 3-min (Adventure Mode) UV-C treatment against *E.coli*

Bottle	Initial Population (CFU/mL)	T-3 min (CFU/mL)	Log Reduction (T3)	% Reduction (T3)
680 mL	6.7E+07	1.0E+01	6.806	99.9999%
	7.0E+07	1.0E+01	6.813	99.9999%
	8.0E+07	1.0E+01	6.857	99.9999%
1000 mL	8.1E+07	1.0E+01	6.908	99.9999%
	8.5E+07	1.0E+01	6.93	99.9999%
	7.9E+07	1.0E+01	6.898	99.9999%

Comment: No growth was detected on 3-min (Adventure Mode) treated plates so a value of 10 was used to indicate the detection limit (< 10 CFU).

Conclusion:

The purpose of this study was to determine how effective a LARQ Bottle was at killing *E.coli* at 1-min (Normal Activation Mode), 2-min (Maintenance Mode) and 3-min (Adventure Mode) treatments. Tables 1, 2 and 3 show that the LARQ bottles produced detectable log reductions of *E.coli* at 1-min (Normal Activation Mode), 2-min (Maintenance Mode), and 3-min (Adventure Mode) treatments. Table 1 shows that at 1-min (Normal Activation Mode) treatments, the LARQ bottles yielded a common log reduction of 4.87, 6.83, and killed 99.999% of *E.coli*. Table 2 shows that at 2-min (Maintenance Mode) treatments, the LARQ bottles yielded a common log reduction of 6.82, 6.75 and killed 99.9999% of *E.coli*. Table 3 shows that at 3-min (Adventure Mode) treatments, the LARQ bottles yielded a common log reduction of 6.82, 6.91 and killed 99.9999% of *E.coli*. The 3-min (Adventure Mode) treatment produced the greatest log reduction and percent reduction against *E.coli*.

Respectfully Submitted,



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