3980 Trust Way Hayward, Ca 94545
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Client: LARQ Inc.
Job Number: JIV20190723-1
Sample Number: SN20190723-1A, SN20190723-1B, SN20190723-1C, SN20190723-1D, SN20190723-1E, SN20190723-1F, SN20190723-1G, SN20190723-1H

Study: Antibacterial efficacy of LARQ UV-C cap with various bottle designs, against *Escherichia coli*
Method: ASTM E2315

Report Date: 7/23/2019

# Certificate of Analysis

**Experimental Summary:**
The purpose of this procedure was to test the effectiveness of LARQs UVC cap at deactivating e. coli in water samples. The testing procedure was designed after discussions between LARQ and RayVio Inc. and is based on ASTM E2315 ("Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure") testing guidelines and was conducted at RayVio Inc. in Hayward, CA.

**Methods and Materials:**
One LARQ UVC cap (marked LX270) and four stainless steel bottles (marked A, B, C, D) were presented for testing. RayVio prepared a working solution of e. coli (ATCC 25922) at a target concentration of 10,000 CFU/ml. Before each test the bottles were cleaned with detergent and a bottle brush, and thoroughly rinsed with DI water. For each of the tests below, 350 ml of test solution was poured into a test bottle, the UV cap was placed on the bottle, and the UV cycle was activated for the planed time. The bottles were not agitated during the UV exposure. Post treatment aliquots were plated in serial dilutions ranging from $10^0$ to $10^{-3}$ on APC Media plates. The plates were incubated at 37 $^oC$ for at least 24 hours before counting CFUs

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**Results:**

Test 1: Using cap LX270 and bottle-A; Double Wall Medium

| Replicate | Initial CFU/ml | UV exposure time (sec) | Log Reduction | % Reduction |
|---|---|---|---|---|
| Avg. | 9,295 | 10 | 2.18 | 99.33 |
| Avg. | 9,295 | 20 | 2.95 | 99.89 |

Test 2: Using cap LX270 and bottle-B; Double Wall Large

| Replicate | Initial CFU/ml | UV exposure time (sec) | Log Reduction | % Reduction |
|---|---|---|---|---|
| Avg. | 9,295 | 10 | 2.30 | 99.49 |
| Avg. | 9,295 | 20 | 3.07 | 99.91 |

Test 3: Using cap LX270 and bottle-D; Single Wall Medium

| Replicate | Initial CFU/ml | UV exposure time (sec) | Log Reduction | % Reduction |
|---|---|---|---|---|
| Avg. | 9,295 | 10 | 2.05 | 99.10 |
| Avg. | 9,295 | 20 | 3.37 | 99.96 |

Test 4: Using cap LX270 and bottle-D; Single Wall Large

| Replicate | Initial CFU/ml | UV exposure time (sec) | Log Reduction | % Reduction |
|---|---|---|---|---|
| Avg. | 9,295 | 10 | 2.15 | 99.29 |
| Avg. | 9,295 | 20 | 2.82 | 99.85 |

**Conclusions:**

This study was intended to measure the effectiveness of LARQs UVC cap at deactivating e. coli at different times, and with different bottle designs. The tables show the LARQ product achieved detectable reductions of e. coli for all exposure times, and for all bottle designs.

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**Client:** LARQ, 3980 Trust Way, Hayward, CA 94545
**Order Number:** IN201804033
**Sample Number:** HR20180490108

**Study:** Antimicrobial efficacy of the LARQ Bottle against *Escherichia coli*
**Method:** ASTM E2315

**Report Date:** 5/30/2018

# Certificate of Analysis

**Experimental Summary:**

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

**Materials and Methods:**

LARQ provided 4 stainless steel bottles and one UV-C LED cap for the testing. LARQ provided Sterile Deionized water that was used to spike the samples and treat. *E. coli* (ATCC 25922) was used as the testing organism in this experiment with a starting solution of about $1.00 \times 10^7$ CFU/mL. Testing was done in 3 replicates for 2-min tests, and 6 replicates for 1-min and 3-min tests. Inoculated volumes for each run was 500 mL, out of which 50 mL was collected for LARQ internal purposes, and 450 mL was tested in a stainless bottle with UV cap (provided by LARQ) for designated run-times. Pre and post treatment aliquots were plated in serial dilutions ranging from $10^{-1}$ to $10^{-9}$ on APC Media using a pour plate technique. Plates were incubated for 48-hr at 35°C.

**Figure 1: LARQ Bottle with cap on and off**

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**Results:**

**Table 1: Experimental results using 1-min light treatment against *E. coli***

| Replicate | Initial Population | T-1 min | Log Reduction (T1) | % Reduction (T1) |
|---|---|---|---|---|
| 1 | 1.60E+07 | 2.50E+03 | 3.81 | 99.9844 |
| 2 | 6.00E+06 | 6.80E+02 | 3.95 | 99.9887 |
| 3 | 6.00E+06 | 7.20E+02 | 3.92 | 99.9880 |
| 4 | 4.00E+06 | 2.12E+03 | 3.28 | 99.9470 |
| 5 | 5.20E+06 | 8.80E+02 | 3.77 | 99.9831 |
| 6 | 6.50E+06 | 1.68E+03 | 3.59 | 99.9742 |
| **Average** | 7.28E+06 | 1.43E+03 | 3.72 | 99.9775 |

**Table 2: Experimental results using 2-min light treatment against *E. coli***

| Replicate | Initial Population | T-2 min | Log Reduction (T2) | % Reduction (T2) |
|---|---|---|---|---|
| 1 | 5.80E+06 | 1.00E+01 | 5.76 | 99.9998 |
| 2 | 2.90E+06 | 1.00E+01 | 5.46 | 99.9997 |
| 3 | 5.60E+06 | 1.00E+01 | 5.75 | 99.9998 |
| **Average** | 4.77E+06 | 1.00E+01 | 5.66 | 99.9998 |

**Table 3: Experimental results using 3-min light treatment against *E. coli***

| Replicate | Initial Population | T-3 min | Log Reduction (T3) | % Reduction (T3) |
|---|---|---|---|---|
| 1 | 1.10E+08 | 1.00E+01 | 7.04 | 99.9999 |
| 2 | 4.70E+06 | 1.00E+01 | 5.67 | 99.9998 |
| 3 | 7.60E+06 | 1.00E+01 | 5.88 | 99.9999 |
| 4 | 1.20E+07 | 1.00E+01 | 6.08 | 99.9999 |
| 5 | 8.40E+06 | 1.00E+01 | 5.92 | 99.9999 |
| 6 | 7.20E+06 | 1.00E+01 | 5.86 | 99.9999 |
| **Average** | 2.50E+07 | 1.00E+01 | 6.08 | 99.9999 |

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

**Conclusions:**

This purpose of this study was to determine how effective a LARQ Bottle was at killing *E. coli* at 1-min, 2-min, and 3-min treatments. Tables 1, 2, and 3 shows that the LARQ Bottle produced detectable log reductions of *E. coli* at 1-min, 2-min and 3-min treatments. Table 1 shows that at 1-min treatments, the LARQ Bottle yielded a log reduction of 3.72 and killed 99.9775% of *E. coli*. Table 2 shows that at 2-min treatments, the LARQ Bottle

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yielded a log reduction of 5.66 and killed 99.9998% of *E. coli*. Table 3 shows that at 3-min treatments, the LARQ Bottle yielded a log reduction of 6.08 and killed 99.9999% of *E. coli*. The 3-min treatment produced the greatest log reduction and percent reduction against *E. coli*.

Respectfully Submitted,

Ming Li
General Manager
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