Summary and analysis of LARQ Bottle PureVis™ 2 laboratory testing against T1UV bacteriophage

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I. Introduction

The LARQ Bottle PureVis™ 2 is a water bottle that purifies and filters water on the go using its built-in UV-C LED light and water filter. The LARQ Bottle produces ultraviolet (UV) energy that destroys microorganisms, without chemicals. UV light is emitted from the LARQ Bottle cap and dosed into the volume of water in the water bottle, where microorganisms in the water and the bottle surface are exposed to a dose of 275nm UV light. UV light in this wavelength inactivates a wide range of microorganisms, including bacteria, viruses, and protozoan cysts. This inactivation occurs as the UV disrupts the organism's DNA structure, making reproduction impossible. The intensity of the UV light and the microorganism's exposure time to the light influence which microorganisms are inactivated, as shown in the compilation issued by the team at the University of British Columbia (UBC)[1].

Our study aims to determine how effective a LARQ Bottle PureVis™ 2 is at deactivating a range of microorganisms, including protozoan cysts, viruses, and bacteria, at 6-min treatments (Apocalypse Mode).

The study is focused on examining **Cryptosporidium** and **Giardia lamblia**, due to their resistance to chlorine, and based on the high prevalence of cryptosporidiosis cases in the United States, estimated at 823,000 per year. Giardia is the most common gut parasite causing illness in the United States, with over 1 million people falling ill from it annually. The study also compares the measured UV power output.

II. Test organism

The selection of the **T1UV virus** as a challenge organism for this study was based on referencing the Ultraviolet Disinfection Guidance Manual (UVDGM) guidelines established by the U.S. Environmental Protection Agency (EPA).

The Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) and UVDGM are documents published by the EPA in 2006. The LT2ESWTR requires public water systems (PWSs) to monitor source water for microbial pathogens, such as Cryptosporidium, Escherichia coli (E. coli), and turbidity. The UVDGM provides technical guidance for PWSs to implement UV disinfection in compliance with the Safe Drinking Water Act (SDWA); a treatment technique that can inactivate waterborne pathogens and reduce the risk of illness. The UVDGM's contents meet the requirements of the LT2ESWTR.

In 2020, the EPA released a supplementary update to the UVDGM, which included guidelines for challenge testing using surrogate organisms. The document recommended using T1UV bacteriophage as a surrogate microorganism for Giardia and Cryptosporidium due to its UV sensitivity closely matching that of these pathogens, as opposed to other common proxy microorganisms.

T1UV is a benign bacteriophage that infects only certain strains of E. coli. It is commonly used in the UV disinfection industry to measure the efficiency of UV disinfection units. The UVDGM recommends it as a challenge microorganism to validate UV disinfection systems according to the LT2ESWTR. The LT2ESWTR defines drinking water disinfection standards established under the SDWA.

III. Test procedure

The UVDGM and other UV testing procedures, such as NSF 55, are designed for flow-through systems in municipal point-of-use, and point-of-entry water disinfection systems. As such, the method was modified for batch treatment instead of a flow-through system. The protocol and analysis were adjusted for the LARQ Bottle PureVis™ 2 for 680mL and 1L sizes.

The test setup included two UV LED bottle caps, two bottles (680mL and 1L), and exposure times of 6 or 9 minutes. The test water was dechlorinated municipal tap water (City of London) spiked with T1UV bacteriophage to about 107.2 pfu/mL. A 10-liter batch tank with dechlorinated municipal tap water (City of London) was spiked with T1UV bacteriophage to ~107.2 pfu/mL.

Caps were charged fully using the provided USB adapter. Various water volumes and cap/bottle combinations were used. For testing, the bottle was filled (600mL for the 680mL bottle, 900mL for the 1L bottle), capped, and started with a 10-minute cycle. The bottle was moved in a circular motion every 30 seconds during the test. Afterwards, the cap was powered off, and

1 Mahsa Masjoudil, Madjid Mohsenil, and James R. Bolton, Sensitivity of Bacteria, Protozoa, Viruses, and Other Microorganisms to Ultraviolet Radiation (Journal of Research of the National Institute of Standards and Technology, Volume 126, Article No. 126021 (2021))

2 EPA Regulation 40 CFR 141.720(d)(1)

treated water was collected. Untreated water was sampled at the start, midpoint, and end of the test series, with the geometric means, used for comparison.

IV. Summary and analysis of results

The experiment involved conducting three trials for each test scenario to determine the average log reduction of T1UV, which can be used to calculate the UV doses (Table 2: UV Dose Requirements), yielding an average range from approximately 31.1 mJ/cm2 to 43.5 mJ/cm2. Assessing the effectiveness against Cryptosporidium and Giardia, the average UV dose across all trials exceeded the required 4-log reduction dose of 22 mJ/cm2, as specified by the LT2ESWTR and EPA Regulation[2].

Furthermore, by referencing the comprehensive compilation from the UBC[1], the study estimated the necessary UV dosage to inactivate a variety of microorganisms, such as Coronaviruses, Hepatitis A, H1N1, Polio, Rotavirus, Legionella, and Staphylococcus, showcasing varying log reductions at distinct UV doses.

Overall, computing the average measured logarithmic reduction over three trials for each scenario resulted in the following calculated UV doses.

Table 1: T1UV Test Results

	6 min/680mL	**6 min/ 1000mL**	**9 min/680mL**	**9 min/1000mL**
Avg Log Reduction	7.60	5.75	7.64	7.24
Average Dose	43.51093	31.14156	73.79101	41.01427

Evaluation of effectiveness against Cryptosporidium and Giardia

To assess the efficient UV dose against Cryptosporidium and Giardia, we referred to the UVDGM for a 4-log reduction of these microorganisms, per the EPA regulation[2].

The average UV dose in all test cases exceeded the UVDGM requirement for a 4-log reduction of Cryptosporidium and Giardia, set at 22 mJ/cm2 by the EPA for PWSs utilizing surface water.

Table 2: UV Dose Requirements[2]

	UV Dose (mJ/cm2) for an inactivation of:							
	0.5 log	**1.0 log**	**1.5 log**	**2.0 log**	**2.5 log**	**3.0 log**	**3.5 log**	**4.0 log**
Cryptosporidium	1.6	2.5	3.9	5.8	8.5	12	15	22
Giardia lamblia	1.5	2.1	3.0	5.2	7.7	11	15	22

Assessment of effectiveness against other microorganisms

To gauge the product's efficiency of the recorded UV dose against various other microorganisms, the compilation issued by the team at UBC[2] was consulted. This research employs a meta-analysis to estimate the required UV dosage for inactivating different microorganisms. The results from the study are summarized below:

- Coronavirus (HcoV-229E): 3-log @ 5.6mJ/cm2
- Coronavirus (SARS-CoV-2): 3-log @ 32mJ/cm2
- Hepatitis A: 5-log @ 30mJ/cm2
- H1N1: 4-log @ 27mJ/cm2
- Polio: 4-log @ 9.2mJ/cm2
- Rotavirus: 4-log @ 40mJ/cm2
- Legionella: 5-log @ 8mJ/cm2
- Staphylococcus: 2-log @ 3.2mJ/cm2

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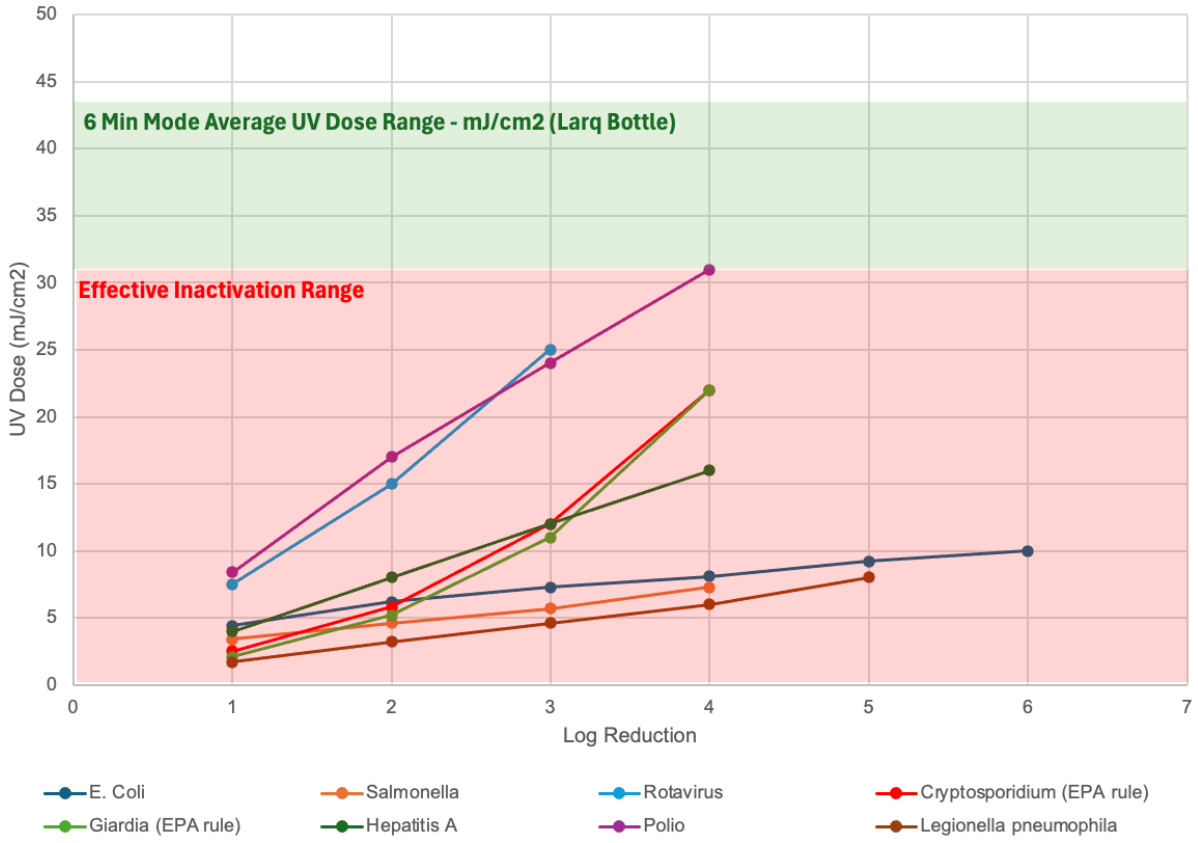
We compared the log reduction at different UV doses for various microbes (Table 3). This includes both EPA's UVDGM requirements[2] for Cryptosporidium and Giardia, and UBC Complication[1] estimates for other microorganisms.

We overlay this data with the range of measured UV dosage from the LARQ Bottle below (Table 3: Comparison of log reductions).

Table 3: Comparison of log reductions required for various microorganisms against the LARQ Bottle PureVis™ 2



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July 23, 2024

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Introduction

Larq delivered 2 UV LED batch treatment units (Figure 1) to GAP EnviroMicrobial Services Ltd. for testing in various bottle/lid combinations, water volumes and exposure times. The bioassay was completed using T1UV bacteriophage on June 6, 2024. The test array is outlined in Table 1 and consisted of two UV LED bottle caps, two bottles, sized 1L and 680mL, and exposure times of 6 or 9 minutes. The test water consisted of dechlorinated municipal tap water (City of London) and spiked with T1UV bacteriophage to approximately $10^{7.2}$ pfu/mL. T1UV is a benign bacteriophage that infects only certain strains *of Escherichia coli* (*E. coli*). It is commonly used in the UV disinfection industry for measuring the efficiency of UV disinfection units. It is used in validations following the US EPA Ultraviolet Disinfection Guidance Manual as a challenge microorganism for validating UV disinfection systems according to the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). The LT2ESWTR defines drinking water disinfection standards established under the Safe Drinking Water Act (SDWA).

Stock Preparation

T1UV Bacteriophage – University of Laval Phage Culture Collection HER 468

The T1UV Bacteriophage was prepared at GAP using a proprietary procedure, identified internally as SOP #40: Preparation and Storage of Concentrated Bacteriophage. In short, a host *E. coli* was grown in Tryptic Soy Broth to log phase in a shaking incubator. A small volume of bacteriophage (1 transfer removed from the culture collection) was added at this time, and the broth mixture remained shaking overnight. The *E. coli* was subsequently removed via centrifugation, leaving the bacteriophage in solution.

Sample Enumeration

Enumeration of the surviving microbial population was achieved using GAP's ISO 17025 accredited method BACTPHAGE-0001: Quantitative Recovery of Bacteriophage Used for Disinfection Equipment Validation. GAP holds accreditation to ISO 17025 for the enumeration of bacteriophage in spiked samples from the Canadian Association for Laboratory Accreditation (CALA). The media

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used was Tryptone Yeast Extract Glucose agar (TYGA) containing triphenyl tetrazolium chloride (TTC) and incubation was 35±0.5°C.

Test Procedure

Testing was performed using a modified version of the NSF 55 protocol. A 10-litre batch tank was prepared using dechlorinated municipal tap water (City of London). T1UV bacteriophage was added to the batch at a concentration of approximately $10^{7.2}$ pfu/mL. Residual chlorine was verified to be non-detectable before testing began, and the UV transmittance at 254nm (UVT_{254nm}) was verified after testing was completed using a Biochrom Ultrospec 3300 Pro spectrophotometer.

Before any testing was completed, the caps were fully charged using the provided USB adapter until the top light turned a solid green. During testing a variety of water volumes and cap/bottle combinations were used (Figure 1) with each cap being charged for a minimum of 10 minutes immediately before conducting a given test. To start the test the bottle was filled with an appropriate volume of the test water; 600mL for the 680mL bottle and 900mL for the 1L bottle. The cap was then placed on the bottle and tightened. The button on the cap was then pressed 2x to start a 10-minute cycle; proper operation was verified by the button emitting a dark blue pulse every 1 second (Figure 2). Since tests were to be conducted for either 6 minutes or 9 minutes a calibrated timer was also started simultaneously with pressing the button on the cap. The bottle was left sitting upright on the counter and moved in a circular motion at a rate of approximately 2 revolutions per second for 5 seconds every 30 seconds for the duration of the test (Figure 2). After the appropriate time had elapsed for the test, the cap was powered off by single pressing the button on the cap. Treated water was collected at the conclusion of the test point. Untreated water was also collected from the batch at the beginning, approximately halfway through and at the conclusion of the test series. It was shown through the bioassay that no major variation existed between the untreated samples, so the geometric mean of all untreated samples was used for comparison to the treated samples.

Results

The effectiveness of the devices at inactivating T1UV was calculated for each time and water volume combination in two ways. One was using the typical practice in the UV industry whereby the geometric mean of the surviving microbes from all three replicates at a given exposure time/water volume combination, irrespective of the cap used, was compared to the geometric mean of the untreated T1UV concentration. A second method was also reported, due to the large variability in reductions, where a "worst case" scenario was reported. Here the log reduction of the worst performing test at time/water volume combination was reported. This provided a log reduction for each test condition and is presented in Table 2. The UV transmittance of the test water was measured at 95.4% and the water temperature was 21.4°C.

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Figure 1. Larq test units including the UV LED caps and 1L and 680mL bottles.

Figure 2. Testing of the Larq device whereby the unit was turned on with a double press of the button, resulting in a dark blue pulse every 1 second. The unit was maintained in a upright position on the countertop and moved in a circular motion.

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Table 1. Test array.

Test Number	Bottle Size (mL)	Water Volume (mL)	Exposure Time (min)	Cap to use	Replicates
1	680	600	6	1219018 1219028	2 1
2	1000	900	6	1219018 1219028	2 1
3	680	600	9	1219018 1219028	2 1
4	1000	900	9	1219018 1219028	2 1

Table 2. Results from the bioassay of the Larq UV LED batch treatment units using T1UV bacteriophage – HER 468. See Table 1 above to relate the "Test Number" to a set of test conditions.

Test Number		Untreated	1	2	3	4
pfu/mL	Rep 1	$1.63x10^{7}$	$1x10^{-1}$	$1.83x10^{2}$	$6x10^{-1}$	$8x10^{-1}$
	Rep 2	$1.67 x10^{7}$	$2.1x10^{0}$	$<1x10^{-1}$	$4x10^{-1}$	$<1x10^{-1}$
	Rep 3	$1.48 x10^{7}$	$3x10^{-1}$	$1.27x10^{3}$	$2x10^{-1}$	$9.9x10^{0}$
Log (Rep 1)		7.21	-1.00	2.26	-0.22	-0.10
Log (Rep 2)		7.22	0.32	-1.00	-0.40	-1.00
Log (Rep 3)		7.17	-0.52	3.10	-0.70	1.00
Avg Log		7.20	-0.40	1.46	-0.44	-0.03
Average Log Reduction			7.60	5.75	7.64	7.24
Average % Reduction			>99.9999	99.9998	>99.9999	>99.9999
Worst Case Log Reduction			6.88	4.10	7.42	6.21
Worst Case % Reduction			>99.9999	99.9920	>99.9999	>99.9999

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If you have any questions regarding the analysis, please do not hesitate to call the lab anytime at (519) 681-0571.

Analyst:	Shawn Verhoeven	Manager Approval:	Jessica Patterson
Position:	Technical Manager	Position:	Lab Manager
Signature:	S. Verh	Signature:	J Patterson

These test results relate only to the samples submitted and the analyses requested.

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FINAL RESULTS FORM

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GAPLAB Environmental Microbiology

DATE SAMPLED:	06-Jun-24	**REPORT TO:**	LARQ
COLLECTED BY:	C. Odegaard	**ATTENTION:**	Josh Abell
DATE RECEIVED:	06-Jun-24	**ADDRESS:**	1900 South Norfolk St. Suite 350
RECEIVED BY:	S. Verhoeven		San Mateo, CA 94403
GAP JOB #:	A16839	**ANALYSIS START:**	06-Jun-24
CLIENT:	LARQ	**ANALYSIS FINISH:**	11-Jun-24
PROJECT:		**TEL:**	617-943-7239
PAGE #:	1 of 1	**EMAIL:**	josh.abell@livelarq.com
		FAX:	
		EMAIL:	

TEST RESULTS *-relate only to the samples as received and the analyses requested.*

Accredited Method Code: BACTPHAGE-0001

LAB #	SAMPLE DESCRIPTION	ASSAY DATE	T1UV - Replicate 1			T1UV - Replicate 2			T1UV - Replicate 3			Calculated Concentration (pfu/mL)		
			Dilution log	Aliquot (mL)	PFU	Dilution log	Aliquot (mL)	PFU	Dilution log	Aliqot (mL)	PFU	Replicate 1	Replicate 2	Replicate 3
5022	In Start	07-Jun-24	-5	1	156	-5	1	169				1.56E+07	1.69E+07	
5023	In Mid	07-Jun-24	-5	1	167							1.67E+07		
5024	In End	07-Jun-24	-5	1	148							1.48E+07		
5025	600mL, Cap A, 6 min, Rep 1	07-Jun-24	0	10	1							~1E-01		
5026	600mL, Cap B, 6 min, Rep 2	07-Jun-24	0	10	21							2.1E+00		
5027	600mL, Cap A, 6 min, Rep 3	07-Jun-24	0	10	3							~3E-01		
5028	600mL, Cap A, 9 min, Rep 1	07-Jun-24	0	10	6							~6E-01		
5029	600mL, Cap B, 9 min, Rep 2	07-Jun-24	0	10	4							~4E-01		
5030	600mL, Cap A, 9 min, Rep 3	07-Jun-24	0	10	2							~2E-01		
5031	900mL, Cap A, 6 min, Rep 1	10-Jun-24	0	1	183							1.83E+02		
5032	900mL, Cap B, 6 min, Rep 2	07-Jun-24	0	10	0							<1E-01		
5033	900mL, Cap A, 6 min, Rep 3	10-Jun-24	-1	1	127							1.27E+03		
5034	900mL, Cap A, 9 min, Rep 1	07-Jun-24	0	10	8							~8E-01		
5035	900mL, Cap B, 9 min, Rep 2	07-Jun-24	0	10	0							<1E-01		
5036	900mL, Cap A, 9 min, Rep 3	10-Jun-24	0	10	99							9.9E+00		

CALCULATED BY:	S. Verhoeven	**POSITION:**	Technical Manager
SIGNATURE:	S. Verhoeven	**MANAGER APPROVAL:**	J. Patterson (Lab Manager)
		SIGNATURE:	J. Patterson
		DATE:	21-Jun-24

< = Less Than; > = Greater Than; TNTC = Too Numerous To Count; OBSC = Obscured; NR = No Result; LA = Laboratory Accident
CFU = Colony Forming Unit; PFU = Plaque Forming Unit; MF = Membrane Filtration; MPN = Most Probable Number; SP = Spread Plate

ilac-MRA CALA Testing Accreditation No. A2914