



BioVir Laboratories, Inc.

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Aqua Ultraviolet
42371 Ave. Alvarado
Temecula, CA 92590

Please find enclosed the results of the bacteriological evaluation of your UV unit. The test was run according to the final protocol included in this document. One modification was the addition of a total plate count (not just for *K. terrigena*) procedure that was conducted on the samples 48 hrs., and 72 hrs (see below).

As you can see from the results (enclosed) there was a marked reduction in the *K. terrigena* over the 72 hour test. The removal rate of *K. terrigena* was observed to be > 99.99% within 48 hours of exposure.

During the course of the run, it was observed that a background non-coliform bacteria was growing with the *K. terrigena*. In the last 24 hour period (48 and 72 hrs) this (these) organisms were measured by TSA media. The *K. terrigena* fell below our detection limit, but the background organism remained. There was no evidence of lab contamination from the controls (agar, diluent, and air). Contributing factors to the apparent "re-growth" may include, the presence of a UV resistant organism from the pre-sanitized system; inadequate sanitization of plumbing and pump system; available nutrients from the UV degraded microorganisms; and/or, contamination of sampling port. If additional testing is desired, we can attempt to eliminate some of the variability by, i) increasing sanitization times; ii) additional washing the seed culture (to prevent introduction of media); and, iii) sampling directly from the reservoir. In addition, it may be beneficial in the future to attempt to run the system without spiked organisms and see if the same bacterial growth characteristics are repeated following sanitization. If the same re-growth occurs, the predominate species of bacteria could be identified.

If you have any questions with the following report, please contact me at (800) 442-7342.

Sincerely,

A handwritten signature in blue ink, appearing to read "Richard E. Danielson".

Richard E. Danielson, Ph.D.
Laboratory Director

Report for: Aqua Ultraviolet UV Disinfection Unit

Purpose:

To determine the efficacy of the Aqua Ultraviolet disinfection unit against *Klebsiella terrigena* in dechlorinated city tap water as a measure of bacteriocidal capabilities.

Materials:

Aqua Ultraviolet Disinfection Unit (AUV)
Recirculating Water Reservoir (100 gal minimum)
90.0 and 99.0 mL Sterile Dilution Bottles w/ Diluent
Trypticase Soy Agar (TSA) and Broth (TSB)
LES-Endo Agar
1.0, 5.0, 10.0 mL Pipettes
Absorbent Pads
Petri Dishes
Sterile 15 mL centrifuge tubes

Organism: *Klebsiella terrigena* ATCC 33257. Three progressive 24 hr cultures.

Procedure:

1. The AUV unit was set up with a recirculating reservoir with 100 gallons of dechlorinated City of Benicia tap water. The circulating pump was allowed to run unimpeded to achieve an estimated flow rate of about 50 g.p.m. (Dr. James, Aqua UltraViolet, personal communication). The entire system was pre-sanitized with bleach (150 mL household bleach/L) for one hour and neutralized with sodium thiosulfate in accordance with EPA 600/R-95/178.
2. In advance of the study, cultures of *K. terrigena* were prepared as per AOAC 965.13.
3. A final preparation of a 10 mL suspension of *K. terrigena* was made to obtain a concentration of ca. 10^{10} cfu total organisms.
4. The organism was added to the reservoir and allowed to run for 10 minutes, or five void volumes, with the AUV system turned off.
5. A 150 mL sample was withdrawn from the reservoir at time 0.
6. Dilutions were prepared on samples to obtain a countable range of 20-200 cfu/plate following Standard Methods 18th ed. 9222B.
7. At 24, 48 and 72 hours, 1.5 L samples were collected.
8. A combination of the following portions, 1.0 mL, 10 mL, 100 mL and 1 L, were

processed through membrane filters from each sample per SM 18th 9222B9. At the end of 48 ± 4 hrs, the final counts were recorded. At times 48 and 72 hours, duplicate non-selective plate counts (on TSA agar) were run.

Controls:

- A. Seed Stock
- B. Time 0, post-added organism.
- C. Diluent (1.0 mL of diluent into pour plate), Air, Broth and Agar

10. Results were recorded as colony forming units (CFU) per mL. The log removal from time 0 to 72 hours was determined. The results are presented in Table 1 and Table 2, below.

TABLE 1

BACTERIOLOGICAL RESULTS FOR *Klebsiella terrigena* REMOVAL BY THE AQUA ULTRAVIOLET UV DISINFECTION UNIT

	Result (CFU/mL)	$t_0 - t_{hr}$ Log Diff.	Percent Removal
STOCK SEED	2.25×10^9	NA ¹	NA
Time (Hrs) 0	5.6×10^4	NA	NA
24	0.22	4.09	99.99%
48	<0.01	>4.74	>99.995%
72	<0.01	>4.74	>99.995%

1. NA = Not Applicable.

CONTROLS	t_0 CFU/mL	t_{24} CFU/mL	t_{48} CFU/mL	t_{72} CFU/mL
Diluent	<1	<1	<1	<1
Broth	<1	<1	<1	<1
Agar	<1	<1	<1	<1
Air	<1	<1	<1	<1

TABLE 1
BACTERIOLOGICAL RESULTS ON M-ENDO

STOCK SEED	1 L			100 mL			10 mL			1 mL			Dilutions ¹	Result (CFU/mL)	t ₀ - t ₉ Log Diff.		
	1	2	3	1	2	3	1	2	3	1	2	3					
0													6,4,7	< 1	< 1	5.6 x 10 ⁻⁴	4.604
24	TNTC	22	2													0.22	8.695
48		< 1	< 1	< 1												< 0.01	> 9.332
72		< 1	< 1	< 1												< 0.01	> 9.332

1. Numbers represent replicate plate counts at that dilution. Dilutions for time = 0 at 10⁷ and 10⁸ (not shown) were < 1/mL.

CONTROLS	t ₀ CFU/mL	t ₂₄ CFU/mL	t ₄₈ CFU/mL	t ₇₂ CFU/mL
Diluent	< 1	< 1	< 1	< 1
Broth	< 1	< 1	< 1	< 1
Agar	< 1	< 1	< 1	< 1
Air	< 1	< 1	< 1	< 1

The results of the test performed by Biovir Laboratory on February 24, 1998 are impressive. This unit exceeded the requirements of a drinking water system. A reduction of more than 99.99% at the first sample collection shows that we could have challenged the unit to a more stringent test. Had we chosen a more stringent test protocol, we would have determined the maximum application parameters. However these results do show conclusively that this application is significantly below the units capability.

This unit had reduced the bacteria to below detection levels in less than 48 hours. The unit's capability over a time span of more than 48 hours is also greater than the test parameters.

Background checks of all solutions used and the laboratory air revealed no interferences that could be significant. They followed the test protocols and the experiment showed the unit to be more capable than the challenge to which it was subjected.