BioVir Laboratories

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TO: Petra Wadström, CEO
Solvatten AB

FROM: Richard Danielson, PhD.
Laboratory Director/Vice President

SUBJECT: Evaluation of the Solvatten Solar Safe Water System (SSSWS). Assessment of virus, bacteria and Cryptosporidium reduction in two challenge waters. BioVir #140264.

SUMMARY.

Solvatten, AB, requested that BioVir Laboratories, Inc. (BioVir) test the Solvatten Solar Safe Water System (SSSWS) for microbiological reduction. Two SSSWS units with no unique identification were received by BioVir and randomly assigned identifiers as 140264-A and 140264-B . Each unit consisted of two solar chambers with designated inlet and outlet ports. The SSSWS also has mechanical and digital indicators to signify when the effective temperature has been reached. The goal of this study was to demonstrate a 4-log reduction of enteric viruses, 6-log reduction of bacteria and 3-log reduction of oocysts at 53°, 55°, 57°, and 59°C .

A protocol was provided by BioVir working with Petra Wadström of Solvatten, AB. Briefly, the ability of the solar device to treat the US EPA Guide Standard for Water Purifiers defined test waters, General Test Water 1 and General Test Water 2, were tested for log reduction of enteric viruses, bacteria and oocysts. Although the SSSWS comes equipped with a pre-filter in the inlet port, this was removed to allow to test for the effect of temperature and solar exposure only. The protocol is attached.

The challenge organisms were added to the challenge fluid to achieve an approximate final concentration of 10^7 pfu per L for viruses, 10^7 cfu/100 mL of bacteria and 10^7 oocysts/L of *Cryptosporidium*. The challenge fluid was added to the Solvatten unit into each of two chambers. The Solvatten unit was positioned in direct sunlight (see Figure 1). A thermocouple was submersed into the chamber and the temperature was followed. When a target temperature was reached, a sufficient volume of challenge fluid was extracted and submitted for assay.

Viruses:
Stock concentration: The virus titer of the stock viruses was determined to be about 10^7 pfu/mL of each Poliovirus and Rotavirus. The stocks were added to attain at least 10^7 pfu/L of each virus in the final test waters.

Virus assays were conducted following the plaque-forming technique as described in BioVir SOP VIII.2. Portions of the samples were applied to cell culture undiluted and with dilution. After 10-days the incubation period was halted and the cell lines evaluated for the presence of plaque forming units (pfu).

Bacteria:

Stock concentration: *Escherichia coli* (ATTC 11229) was prepared following the procedure outlined in AOAC 991.47. The stock concentration was determined to be about 10^{10} cfu/mL. The stock was added to the challenge fluid to attain a concentration of at least 10^{8} cfu/L.

E. coli assays were conducted following the membrane filtration technique, Standard Methods 9222. Three 100 mL portions of the treated water was applied to a filter, placed onto mFC agar and incubated for 24 ± 2 hrs at 44.5°C. Typical colonies were counted per membrane.

Cryptosporidium parvum
Stock concentration: Stock *C. parvum* was acquired from Bunch Grass Farms, Deary, ID. The stock was added to attain at least 10^{6} oocysts/L in the final test waters.

Oocysts were concentrated from the challenge fluid and pre-treated to enhance infectivity on a HCT-8 cell line following the method of Slifko et al. (1999). Concentrations of oocysts were applied to four-well slides in order of 10^{5}, 10^{4}, 10^{3}, and 10^{2} oocysts per well per slide. The slides were stained and observed under epifluoresence microscopy for infective unit fluorescent foci (FF). The treated samples were compared to the untreated control. The untreated control sample yielded FF for all dilutions from 10^{2} - 10^{5} oocysts/well. In all cases, there were no FF observed for any of the treated samples.

The results of the assays are given below in Table 1. For bacteria, greater than 7-log reduction was observed at all sample points and in both water types ≥ 55°C. For viruses and *Cryptosporidium* oocysts, > 5-log and > 5-log reduction was observed at all temperatures, respectively.

In addition, the time-to-temperature is provided in Table 2.

Please contact me if you have any questions with this report.

Figure 1
Solvatten Solar Safe Water Systems. BV #140264

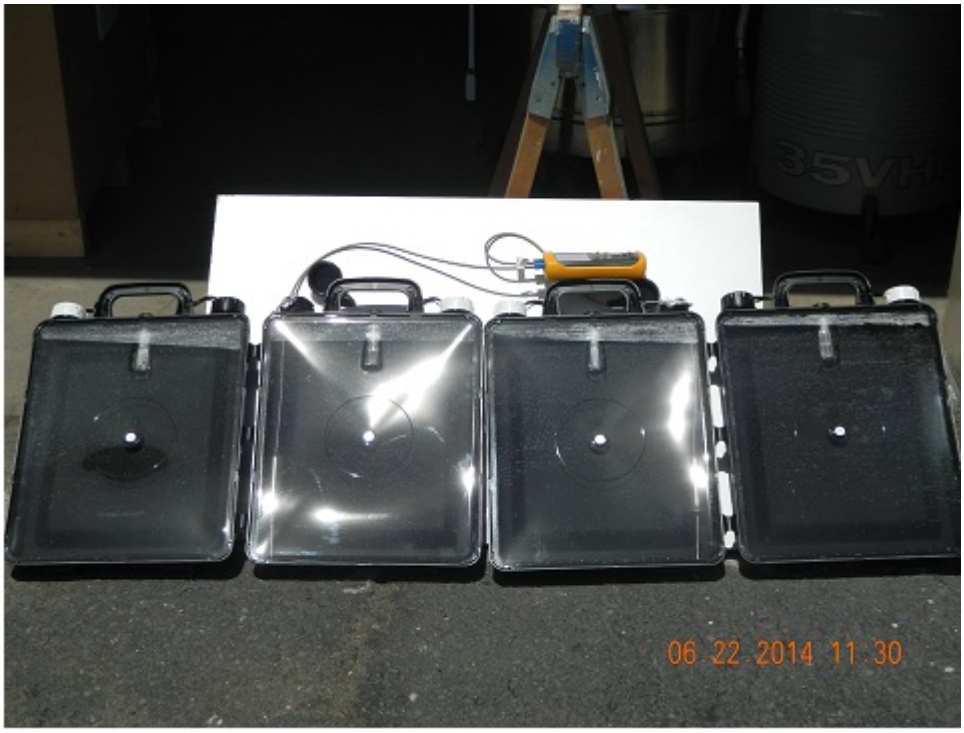

TABLE 1
Reduction of Microorganisms by the Solvatten Water Treatment Device in GTW 1 and 2
Project #140264

E.coli 11229

Temperature (°C)	GTW 1 (cfu/100 mL)	GTW 1 Log Reduction	GTW 2 (cfu/100 mL)	GTW 2 Log Reduction
25	2.80E+07		2.50E+07	
53	1.10E+02	5.41	7.70E+01	5.51
55	<1	>7.45	<1	>7.40
57	<1	>7.45	<1	>7.40
59	<1	>7.45	<1	>7.40

Poliovirus & Rotavirus

Temperature (°C)	GTW 1 (pfu/ mL)	GTW 1 Log Reduction	GTW 2 (pfu/ mL)	GTW 2 Log Reduction
25	2.10E+04		3.30E+04	
53	<0.1	>5.32	<0.1	>5.52
55	<0.1	>5.32	<0.1	>5.52
57	<0.1	>5.32	<0.1	>5.52
59	<0.1	>5.32	<0.1	>5.52

Cryptosporidium Oocysts

Temperature (°C)	GTW 1 # Infectious Oocysts	GTW 1 Log Reduction	GTW 2 # Infectious Oocysts	GTW 2 Log Reduction
25	5.00+00		5.00+00	
53	<1	>5.0	<1	>5.0
55	<1	>5.0	<1	>5.0
57	<1	>5.0	<1	>5.0
59	<1	>5.0	<1	>5.0

Table 2
Time (in hours) to Temperature for the Solvatten Units

Temp °C	GTW 1	GTW2
53	2:15	2:18
55	2:27	2:37
57	3:09	3:37
59	3:35	3:48

Note: ambient air temperature maximum at 35.5°C