



A & L Laboratory Inc.

Environmental Consulting ~ Drinking Water Analysis ~ Radon Testing

**Testing of SteriPEN™, a Portable Ultraviolet Light Water Purifier, Using
MS-2 Coliphage in Challenge Test Waters According to the U.S.E.P.A.
Protocol's Recommended Materials.**

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Research Conducted For:

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MAINE CERT #ME021

NH CERT #2501

Introduction

SteriPEN™ is a portable, handheld device designed to disinfect water by using a short wave germicidal ultraviolet (UV) light. The device, unlike traditional flow through UV water purifiers, treats batches of water up to 1 liter. Though the method of treatment is slightly different the concept is the same. The SteriPEN™ produces ultraviolet energy that is used to destroy microorganisms, without the use of chemicals. The SteriPEN™ is submerged in the water, where microorganisms are exposed to a dose of ultraviolet light in the 254-nanometer range. Ultraviolet light in this wavelength inactivates a wide range of microorganisms including bacteria, viruses and protozoan cysts. This inactivation occurs as the ultraviolet light disrupts the organism's DNA structure, making reproduction impossible. The intensity of the ultraviolet light and the microorganism's exposure time to the ultraviolet light are factors that influence which microorganisms are inactivated [6].

Prior studies conducted on the SteriPEN™ by The University of Arizona and The University of Maine have shown successful reduction of bacteria, viruses and protozoan cysts in visually clear water [2,3,4]. This study will examine the effects of the SteriPEN™ in a more stressful environment. Increased total dissolved solids, total organic carbon and turbidity along with decreased temperatures will reduce the transmittance of the UV light and provide a “worst case” scenario for using the SteriPEN™.

Test Organism

MS2 Coliphage was chosen as a test subject for this study for several reasons. MS2 offers a high linear response over a wide range of UV dose levels, UV inactivation results are highly reproducible, it's easily propagated to high titers, and it is non-pathogenic to humans [9].

Studies on MS2 Coliphage have shown that a 99.5% inactivation (2.3 log reduction) of MS2 coliphage after UV treatment is equivalent or greater than a 99.9999% inactivation or a 6-log reduction of bacterial pathogens and a 99.99% percent inactivation or a 4-log reduction of viral pathogens [9]. The UV inactivation rate of MS2 coliphage has been compared to common microbial contaminants and pathogens (B. subtilis, Hepatitis A, Rotavirus SA-11, and Poliovirus type 1) [9]. Of all these organisms, MS2 coliphage was found to be the most resistant to UV radiation.

Test Procedure

The testing procedure was based on the United States Environmental Protection Agency's Guide Standard and Protocol for Testing Microbiological Water Purifiers [7]. The method was slightly modified in order to accommodate for batch treatment rather than a flow through system. All other components of the USEPA protocol remain the same. .

Samples of challenge test water (EPA Test Water #4) were used to examine the effects of the SteriPEN on water of known contaminant levels. The challenge water was created from laboratory reagent water. The required physical and chemical characteristics of the water are listed in **Table #1**. The water did not contain chlorine or any other disinfectant residuals. PH in was measured by a Denver Instruments pH-ISE Meter model # 225. The pH was adjusted using a 1N solution of sodium hydroxide (NaOH) and/or hydrochloric acid (HCL). Total organic carbon (TOC) analyzed on a Shimadzu TOC-V Combustion Analyzer was adjusted in the challenge water using tannic acid. The turbidity in the challenge water was achieved through the addition of A.C. Fine Test Dust. Measurements of turbidity were taken on a Hach 2100A Turbidimeter. Total dissolved solids, measured by a YSI Conductivity Meter, were increased to the appropriate concentrations by the use of sea salts. The UV absorption was measured with a Shimadzu UV-2501PC Spectrophotometer and then the percent transmittance was calculated. Proper water

temperatures were monitored (Sper Scientific Infrared Thermometer 800048) and maintained throughout the entire experiment. Please refer to **Table #2** for the actual readings of each parameter used in the test.

Table #1. Required chemical and physical characteristics of test water per U.S.E.P.A . Guide Standard[7]

Parameter	Challenge Test Water
Chlorine Residual	None
pH	6.5 - 8.5
Total Organic Carbon (TOC)	>10.0 mg/L
Turbidity	>30 NTU
Temperature	4° +/- 1°C
Total Dissolved Solids (TDS)	1500 mg/L +/- 150 mg/L
Color U.V. Absorption	≥ 0.1550/cm
Color U.V. Transmittance	≤ 70 %

Table #2. Actual chemical and physical characteristics of test water.

Parameter	Challenge Test Water
Chlorine Residual	<0.10 mg/L
pH	7.5
Total Organic Carbon (TOC)	12 mg/L
Turbidity	33 NTU
Temperature	4°C
Total Dissolved Solids (TDS)	1495 mg/L
Color U.V. Absorption	0.640/cm
Color U.V. Transmittance	22.9%

Three SteriPENs™ were tested simultaneously. Each of the three SteriPENs™ was tested on 1 liter samples of the Challenge Test Water. In addition each SteriPEN™ was also tested using two treatments (180 seconds) of exposure.

The challenge waters were spiked with test organism MS-2 coliphage (Escherichia coli bacteriophage ATCC® 15597-B1™). A control sample was removed from each liter of water. A single (90 Second) or double (180 Second) UV dose for one liter of water was applied to each sample. The UV dose was administered according to the manufacturers instructions for treating between 0.5 -1.0 liter of water [5]. The on/off button was pushed once to begin the treatment of a one-liter sample. The green LED flashed indicating the SteriPEN™ was ready for use. The UV lamp end was then submerged into the sample contained in a 1000 ml Pyrex® beaker. The light illuminated, as the sensors came into contact with the water, indicating the 90-second dose had begun. The water was agitated by stirring the SteriPEN™ in various patterns at a moderate speed for the entire duration of the dose. Upon completion of the treatment, an aliquot of water was removed from each beaker and several dilutions were plated according to the agar layer method described by Adams using E. coli host (Escherichia coli ATCC® 15597™) [1].

Results

Table #3. General Test Water MS2 coliphage Titer -logarithmic reductions and percent kill

	Control	General Test Water	Log Reduction	% Kill
Single Treatment (90 Seconds)	2.98E+05	2.57E+03	2.0646	99.1382550%
Double Treatment (180 Seconds)	2.76E+05	9.60E+02	2.4586	99.6521739%

Conclusion

The use of SteriPEN™ on the challenge test water {Table #3} resulted in a 2.06-log reduction (99.1383%) of MS-2 coliphage after a single dose (90 second). Testing of the SteriPEN™ was also conducted after two treatment intervals on the challenge test water {Table #3} to examine the effects of longer exposures (increased contact time) to UV light. Increasing exposure time to two doses (180 seconds) resulted in a 2.46-log reduction (99.6522%).

The testing conducted on the challenge test waters indicates that SteriPEN™ meets the requirements set forth by the U.S.E.P.A. and is adequate for the inactivation of bacterial and viral contaminants in drinking water if two doses are administered. Due to the low transmittance of UV light (22.9%) in the challenge water a single treatment does not meet the 2.3 log reduction required by the U.S.E.P.A. The U.S. Environmental Protection Agency (EPA) Guide Standard and Protocol for Testing Microbiological Water Purifiers requires a minimum 6-log reduction/inactivation of bacteria, 4-log reduction/inactivation of viruses, and 3-log reduction/inactivation of protozoan cysts. A 2.3 log reduction (99.5%) of MS-2 coliphage is considered to be equivalent to a 6-log reduction (99.9999%) of bacterial contaminants and a 4-log reduction (99.99%) of viral contaminants [9]. Generally, UV light is most effective at inactivating *Cryptosporidium* and *Giardia*, followed by bacteria and then viruses [8]. Given that protozoan cysts inactivation requires significantly lower UV doses, it can be expected that an adequate reduction of bacterial and viral contaminants would automatically imply a satisfactory reduction/inactivation of cysts.



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Figure #1.

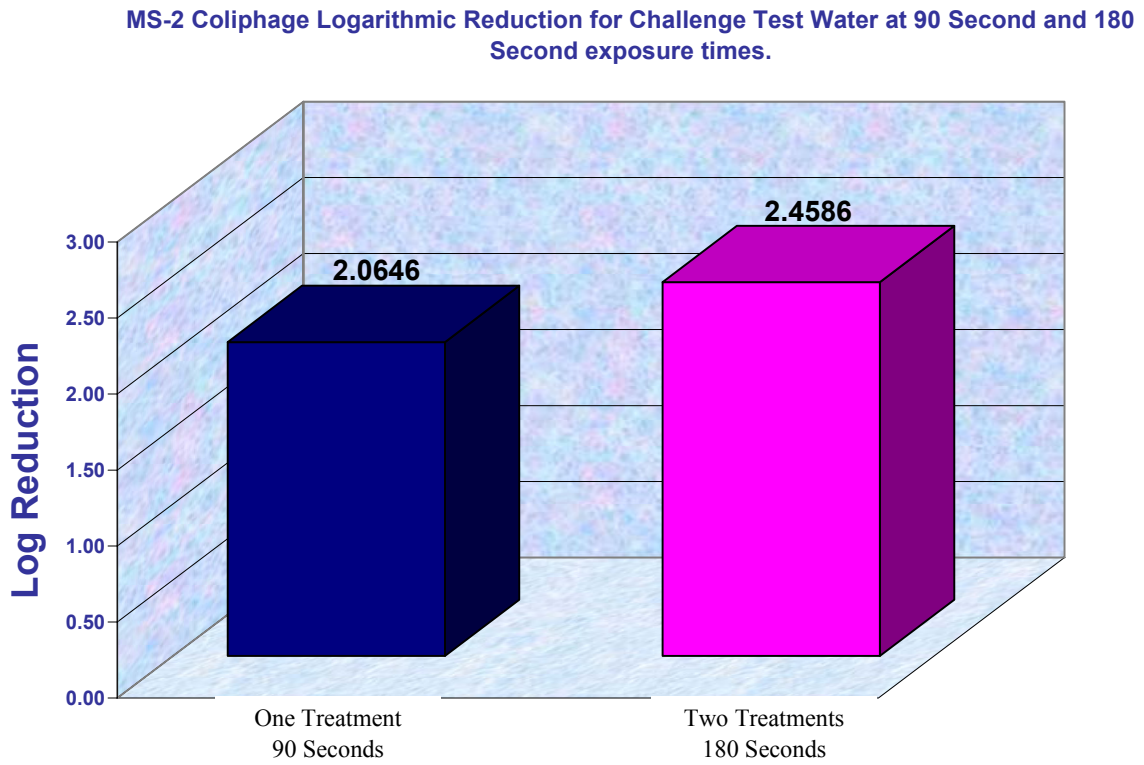
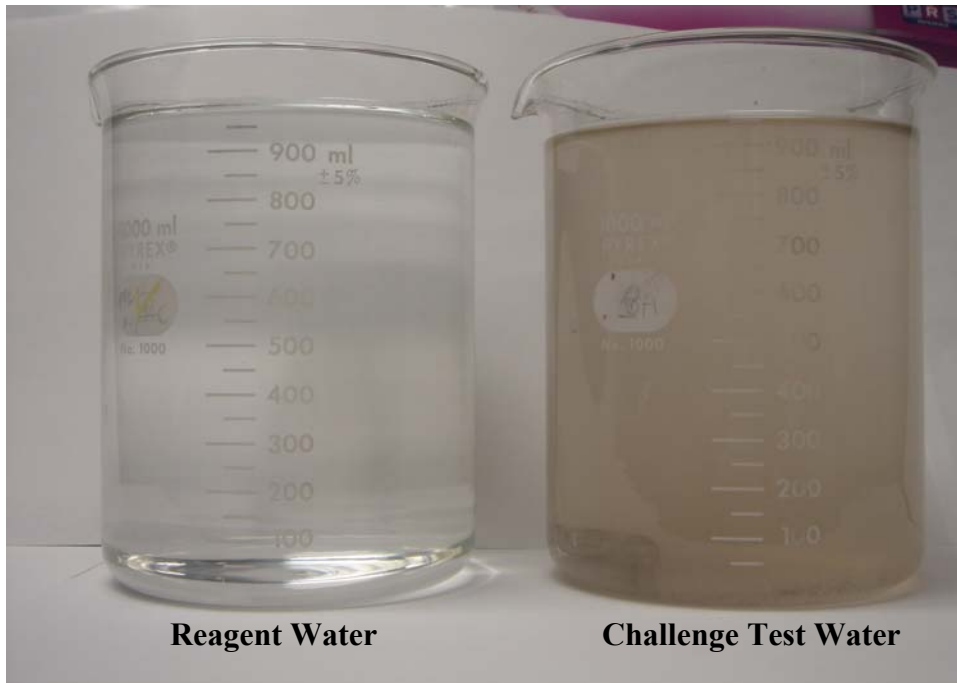


Figure #2.



References

1. Adams, M. H. 1959. *Bacteriophages*. Interscience Publishers, New York
2. Enriquez, C. and Gerba, c. 2001. *Evaluation of the Steri-Pen® Water Treatment System According to the US Environmental Protection Agency Guide Standard And Protocol For Testing of Microbiological Water Purifiers*.
3. Hanson, Anne 2000. *Testing of Steri-Pen, a Hand-held Ultraviolet Water Treatment Device using MS2 Coliphage*.
4. Hanson, Anne 2001. *Testing of Steri-Pen, a Hand-held Ultraviolet Water Treatment Device using MS2 Coliphage on Visually Turbid Natural Water*.
5. Hydro-Photon, Inc., 2005 *SteriPEN™ Users Guide*, Blue Hill, Maine <http://www.hydro-photon.com/PDF/SteriPENEnglish.pdf>
6. *Ultraviolet Light Disinfection Technology In Drinking Water Application - An Overview*. United States Environmental Protection Agency, Office of Water. EPA 811-R-96-002. September, 1996
7. U.S.E.P.A. - Task Force Report, 1987. *Guide Standard and Protocol for Testing Microbiological Water Purifiers*. United States Environmental Protection Agency, Registration Division, Office of Pesticide Programs and Criteria and Standards Division, Office of Drinking Water, Washington, DC
8. U.S. Army Center for Health Promotion and Preventive Medicine, 2005. *Technical Information Paper; Ultraviolet Light Disinfection in the Use of Individual Water Purification Devices*, Aberdeen Proving Ground, MD.
9. Wilson, B.R.P.F. Roessler, E. Van Dellen, M. Abbaszadegan and C.P. Gerba. *Coliphage MS2 as a UV Water Disinfection Efficacy Test Surrogate for Bacterial and Viral Pathogens*. University of Arizona, Tucson, AZ