



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 1 ½ Liter and 2 Liter Volumes of Water According to U.S.E.P.A. Protocol.

May 11, 2007

**Research Conducted For:
Miles Maiden
Hydro-Photon, Inc.
262 Ellsworth Road
Blue Hill, Maine 04614**

Jonathan T. Dyer
Laboratory Director
Rebecca L. Lebrun
Quality Control Officer

A & L Laboratory Inc. 3100 Hotel Road P.O. Box 1507 Auburn, Maine 04211-1507
Telephone: (207) 784-5354 Fax: (207) 782-5561 Email: allabs@adelphia.net



NELAP CERT #250103

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 [11].

Prior testing of SteriPEN™ has shown adequate disinfection/inactivation of MS-2 Coliphage in 0.5 Liter and 1.0 Liter volumes [2-9]. This study will examine the effects of the SteriPEN™ in increased volumes of water. Volumes of 1.5 Liters and 2.0 Liters of water will be tested in cook pot containers to determine the ability of SteriPEN™ to disinfect/inactivate MS-2 Coliphage in larger volumes of water.

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 [14].

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 [14]. 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) [14]. 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 [12]. The method was slightly modified in order to accommodate for batch treatment rather than a flow through system.

Samples of general test water (EPA Test Water # 1) were used to compare the effects of the SteriPEN™ in two different volumes of water. The general test 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. The pH of the water 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) was analyzed on a Shimadzu TOC-V Combustion Analyzer. 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. 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 [12].

Parameter	General Test Water
Chlorine Residual	None
pH	6.5 - 8.5
Total Organic Carbon (TOC)	0.1 mg/L - 5.0 mg/L
Turbidity	0.1 NTU - 5 NTU
Temperature	20°C +/- 5°C
Total Dissolved Solids (TDS)	50 mg/L - 500 mg/L

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

Parameter	General Test Water
Chlorine Residual	<0.10 mg/L
pH	7.4
Total Organic Carbon (TOC)	<1 mg/L
Turbidity	<1 NTU
Temperature	22°C
Total Dissolved Solids (TDS)	64 mg/L

Three SteriPENs™ were tested simultaneously. Each of the three SteriPENs™ was tested on 1.5 liter and 2.0 liter samples of the General Test Water contained in the two different size cook pots.. The general test water was 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 UV dose for one liter of water (90 seconds) 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 [10]. 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 container (Cook Pot). The light illuminated, as the sensors came into contact with the water, indicating the 90-second dose had begun. The water in the cook pot 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 container 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. MS2 coliphage Titer-logarithmic reductions and percent kill for 1.5 Liter and 2.0 Liter Volumes of General Test Water.

	Control	General Test Water	Log Reduction	% Kill
1.5 Liter Water Volume	7.59E+05	8.43E+02	2.9542	99.8888726%
2 Liter Water Volume	7.10E+05	1.47E+03	2.6849	99.7933918%

Conclusion

The use of SteriPEN™ on the general test water in 1.5 liter volumes resulted in a 2.95-log reduction (99.8889 %) of MS-2 coliphage after a single dose (90 seconds). The use of SteriPEN™ on the general test water in 2.0 liter volumes resulted in a 2.68-log reduction (99.7934%) of MS-2 coliphage after a single dose (90 seconds) **{Figure #1}**.

The testing conducted in both volumes of water 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 in both 1.5 liter and 2.0 liter volumes. 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 [12]. 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 [14]. Generally, UV light is most effective at inactivating *Cryptosporidium* and *Giardia*, followed by bacteria and then viruses [13]. 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.



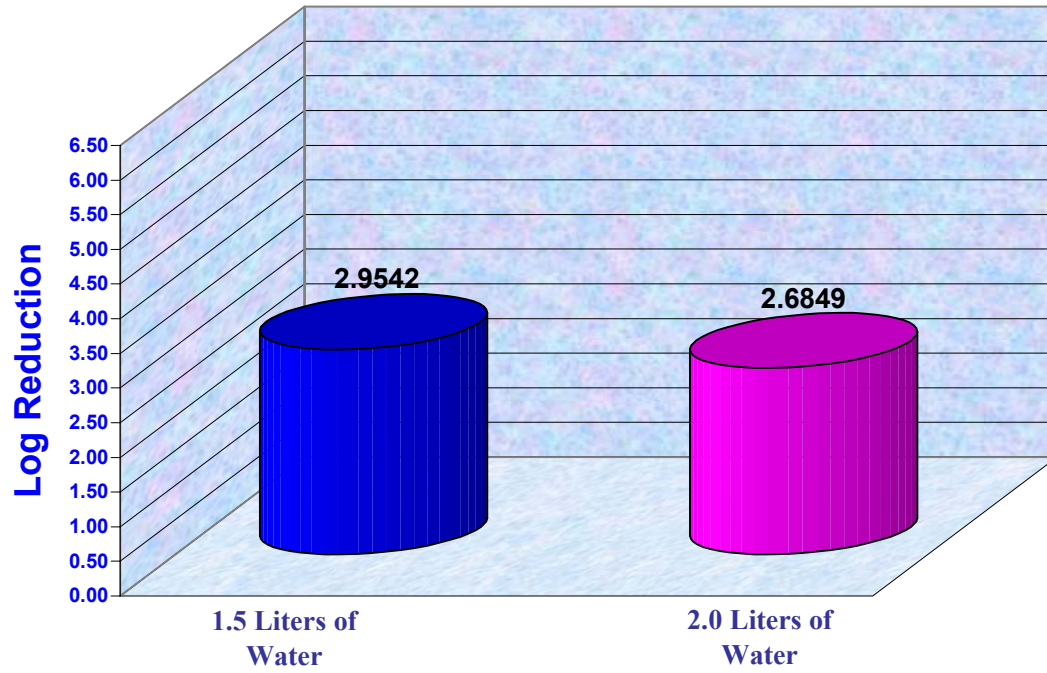
Jonathan T. Dyer / Laboratory Director



Rebecca L. Lebrun / Quality Assurance Officer

Figure #1

MS-2 Coliphage Logarithmic Reduction for General Test Water in Two Quantities of Water.



References

1. Adams, M. H. 1959. *Bacteriophages*. Interscience Publishers, New York
2. Dyer, J. and Lebrun, R. 2007. Testing of SteriPEN™, a Portable Ultraviolet Light Water Purifier, Using MS-2 Coliphage in Challenge Test Waters According to U.S.E.P.A. Protocol.
3. Dyer, J. and Lebrun, R. 2007. *Testing of SteriPEN™, a Portable Ultraviolet Light Water Purifier, Using MS-2 Coliphage in Different Shape Containers According to U.S.E.P.A. Protocol.*
4. Dyer, J. and Lebrun, R. 2007. *Testing of SteriPEN™, a Portable Ultraviolet Light Water Purifier, Using MS-2 Coliphage at Different Speeds and Diameters of Stirring According to U.S.E.P.A. Protocol.*
5. Dyer, J. and Lebrun, R. 2007. *Testing of SteriPEN™, a Portable Ultraviolet Light Water Purifier, Using MS-2 Coliphage in Various Baby Bottles According to U.S.E.P.A. Protocol.*
6. Dyer, J. and Lebrun, R. 2007. *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.*
7. 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.*
8. Hanson, Anne 2000. *Testing of Steri-Pen, a Hand-held Ultraviolet Water Treatment Device using MS2 Coliphage.*
9. Hanson, Anne 2001. *Testing of Steri-Pen, a Hand-held Ultraviolet Water Treatment Device using MS2 Coliphage on Visually Turbid Natural Water.*
10. Hydro-Photon, Inc., 2005 *SteriPEN™ Users Guide*, Blue Hill, Maine <http://www.hydro-photon.com/PDF/SteriPENEnglish.pdf>
11. *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
12. 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
13. 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.
14. 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