



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 U.S.E.P.A. Protocol.

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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. Reagents used for adjusting the total organic carbon and turbidity levels were altered from the U.S.E.P.A. protocol in order to obtain a transmittance of close to 70%. All other components of the USEPA protocol remain the same unless otherwise noted.

Samples of both general test water (EPA Test Water # 1) and challenge test water (EPA Test Water #4) were used to compare the effects of the SteriPEN on both visually clear water and water of known contaminant levels. The general test waters and the challenge waters were created from laboratory reagent water. The required physical and chemical characteristics of both waters are listed in **Table #1**. Neither water contained chlorine or any other disinfectant residuals. PH in both types of 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) analyzed on a Shimadzu TOC-V Combustion Analyzer was adjusted in the challenge water using potassium hydrogen phthalate. The turbidity in the challenge water was achieved through the addition of a formazin/A.C. Fine Test Dust mixture. Measurements of turbidity were taken on a Hach 2100A Turbidimeter. Total dissolved solids, measured by a YSI Conductivity Meter, were increased in both waters 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	General Test Water	Challenge Test Water
Chlorine Residual	None	None
pH	6.5 - 8.5	6.5 - 8.5
Total Organic Carbon (TOC)	0.1 mg/L - 5.0 mg/L	>10.0 mg/L
Turbidity	0.1 NTU - 5 NTU	>30 NTU
Temperature	20°C +/- 5°C	4° +/- 1°C
Total Dissolved Solids (TDS)	50 mg/L - 500 mg/L	1500 mg/L +/- 150 mg/L
Color U.V. Absorption	≥ 0.000/cm	≥ 0.1550/cm
Color U.V. Transmittance	≤ 100%	≤ 70 %

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

Parameter	General Test Water	Challenge Test Water
Chlorine Residual	<0.10 mg/L	<0.10 mg/L
pH	7.3	6.9
Total Organic Carbon (TOC)	<1 mg/L	12 mg/L
Turbidity	0.3 NTU	33 NTU
Temperature	19°C	4°C
Total Dissolved Solids (TDS)	55 mg/L	1430 mg/L
Color U.V. Absorption	0.0017/cm	0.1725/cm
Color U.V. Transmittance	99.61%	67.22%

Three SteriPENs™ were tested simultaneously. Each of the three SteriPENs™ were tested three times on 1 liter samples of the General Test Water and Challenge Test Water. In addition the SteriPENs™ were tested at increasing time intervals (90 Seconds (1 Treatment), 180 Seconds (2 Treatments), 270 Seconds (3 Treatments) on the challenge water in order to observe the changes in log reduction and percent kill based on exposure time.

The general test waters and 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 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 [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 (90 Seconds) MS2 coliphage Titer (PFU/mL)-logarithmic reductions and percent kill

	Control	General Test Water	Log Reduction	% Kill
Trial #1	6.47E+08	2.31E+05	3.4473	99.9642968%
Trial #2	6.18E+08	2.46E+05	3.4001	99.9601942%
Trial #3	6.75E+08	2.18E+05	3.4908	99.9677037%
Average			3.4461	99.9640649%

Table #4. Challenge Test Water (90 Seconds) MS2 coliphage Titer (PFU/mL)-logarithmic reductions and percent kill

	Control	Challenge Test Water (90 Second Treatment)	Log Reduction	% Kill
Trial #1	7.60E+08	8.47E+05	2.9529	99.8885526%
Trial #2	7.30E+08	7.60E+05	2.9825	99.8958904%
Trial #3	7.61E+08	8.17E+05	2.9692	99.8926413%
Average			2.9682	99.8923614%

Table #5. Challenge Test Water (180 Seconds) MS2 coliphage Titer (PFU/mL)-logarithmic reductions and percent kill

	Control	Challenge Test Water (180 Second Treatment)	Log Reduction	% Kill
Trial #1	7.60E+08	8.66E+02	5.9433	99.9998861%
Trial #2	7.30E+08	9.43E+02	5.8888	99.9998708%
Trial #3	7.61E+08	1.10E+03	5.8420	99.9998561%
Average			5.8914	99.9998710%

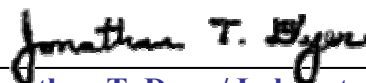
Table #6. Challenge Test Water (270 Seconds) MS2 coliphage Titer (PFU/mL)-logarithmic reductions and percent kill

	Control	Challenge Test Water (270 Second Treatment)	Log Reduction	% Kill
Trial #1	7.60E+08	4.22E+02	6.2555	99.9999445%
Trial #2	7.30E+08	3.56E+02	6.3119	99.9999512%
Trial #3	7.61E+08	4.41E+02	6.2369	99.9999420%
Average			6.2681	99.9999459%

Conclusion

The evaluation of SteriPEN™ on the general test water {Table #3} resulted in a 3.45-log reduction (99.964 %) of MS-2 coliphage after a single dose (90 seconds). The use of SteriPEN™ on the challenge test water {Table #4} resulted in a 2.97-log reduction (99.892%) of MS-2 coliphage after a single dose (90 second). The increased contaminants in the challenge test water slightly reduced the effectiveness of SteriPEN™ but not enough to decrease the log reduction to an inadequate level. Testing of the SteriPEN™ was also conducted at two additional time intervals on the challenge test water {Table #5 & #6} to examine the effects of longer exposures (increased contact time) to UV light. Increasing exposure time to two doses (180 seconds) resulted in a 5.89-log reduction (99.99987%). An additional increase to three doses (270 seconds) resulted in a slightly higher 6.27-log reduction (99.9999%). Please see Figure #1 & #2.

The testing conducted on both general test waters (3.45- log reduction) and challenge test waters (2.96- log reduction) 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. 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.



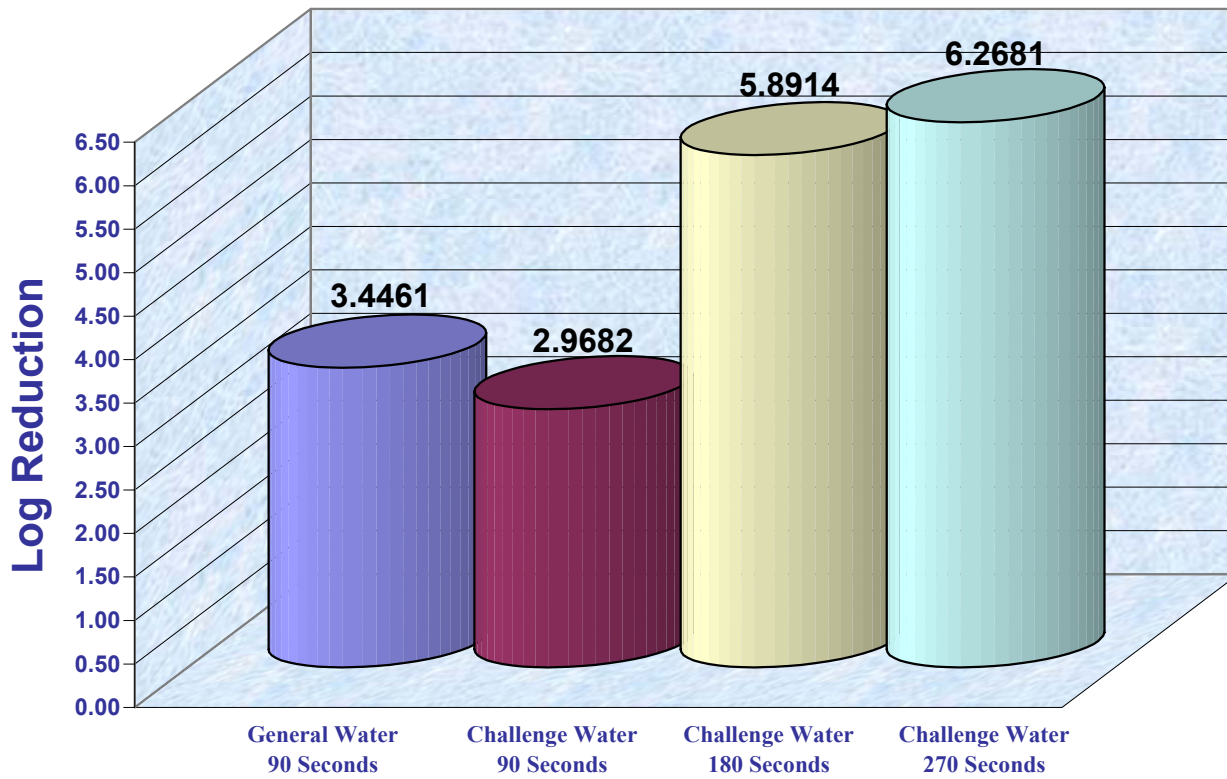
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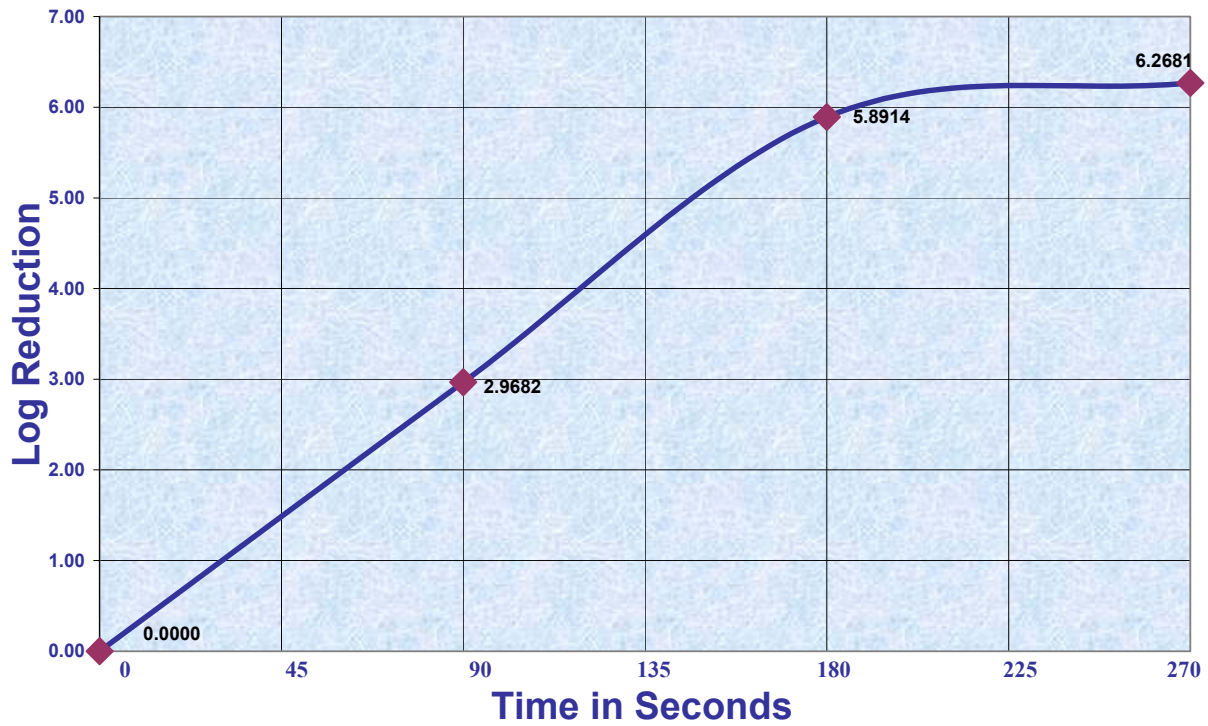
MS-2 Coliphage Logarithmic Reduction for General Test Water and Challenge Test Waters(Three Time Intervals).

Figure #1.

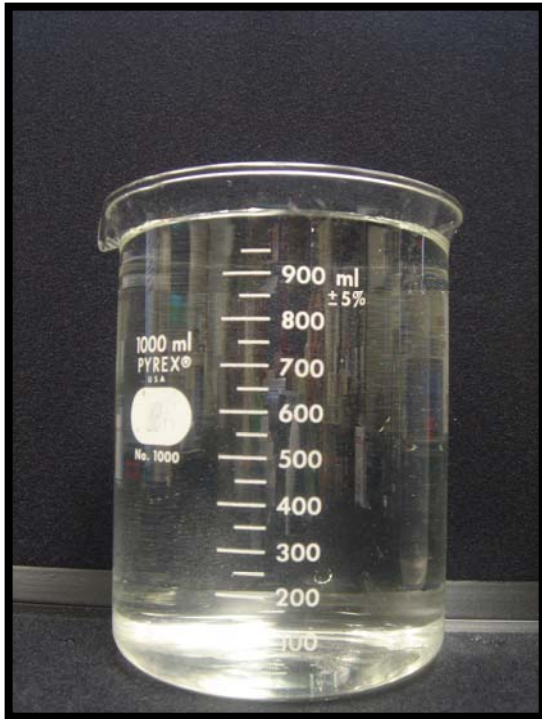


SteriPen™ Comparison of Logarithmic Reduction at Extended Time Intervals

Figure #2.



Figure#3



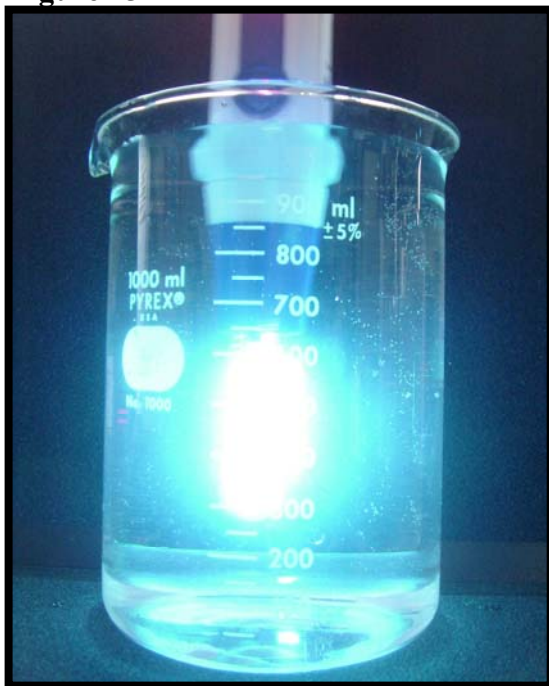
General Test Water

Figure #4



Challenge Test Water

Figure #5



**General Test Water
With SteriPEN™ In Use**

Figure #6



**Challenge Test Water
With SteriPEN™ In Use**

References

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