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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

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SUMMARY

The Steri-Pen® Water Treatment System was evaluated for its ability to eliminate *Klebsiella terrigena* (*K. terrigena*), poliovirus type 1, and simian rotavirus SA-11 according to the US Environmental Protection Agency (USEPA) Guide Standard and Protocol for Testing of Microbiological Water Purifiers. The Steri-Pen® Water Treatment System was used according to the manufacturer's instructions, and challenged with the test organisms in both average case (EPA test water # 1) and filtered worst case (EPA test water # 2) challenge test waters.

The removals (geometric average) by the water treatment in test water # 1 exceeded 6.37 $\log_{10}$ for *K. terrigena*, and 4.08 $\log_{10}$ for Poliovirus type 1 and rotavirus SA-11. In test water # 2, a similar level of removal was observed by the combination of filtration and UV treatment. The number of *K. terrigena*. was reduced by more than 7.23 $\log_{10}$, and the removal of the two viruses was of more than 4.75 $\log_{10}$.

According to these results, the Steri-Pen® Water Treatment System complies with the criteria guidelines suggested by the U.S. Environmental Protection Agency for the removal of bacteria and viruses.

MATERIALS AND METHODS

Experimental Design

The basic experimental design for evaluating the water purification units was based on the recommendations of the U.S. Environmental Protection Agency's Task Force Report on the *Guide Standard and Protocol for Testing Microbiological Water Purifiers* (Federal Register, May 26, 1986).

Bacterial Analysis

Klebsiella terrigena (ATCC-33257) was grown overnight in Trypticase Soy broth (Difco, Detroit, MI) at 37° C to obtain the organisms in the stationary growth phase. The bacterial cells were pelleted by centrifugation at 2,500 x g for 15 min, and resuspended in 0.025 M phosphate buffered saline (PBS). This procedure was repeated three times to remove organic matter present in the broth.

Bacterial assays were conducted by the membrane filtration method on m-Endo Agar LES (Becton and Dickinson, Cockesville, MD). Appropriate dilutions of untreated water samples were made in sterile PBS at pH 7.0. Volumes of 100-mL of undiluted treated water were also assayed. All assays were carried out in triplicate according to the *Standard Methods for the Examination of Water and Wastewater* (APHA, 1998).

Virus Analysis

Poliovirus type 1 (strain LSc2ab) ATCC-VR-59 and the simian rotavirus SA-11 (ATCC-VR-899) were obtained from the American Type Culture Collection (Rockville, MD), Viruses were propagated and assayed in the MA-104 continuous cell line. After extensive cytopathic effect, polioviruses and rotaviruses from infected MA-104, cells were harvested by three cycles of freeze-thawing, cell debris removed by centrifugation at 21,000 x g for 30 min, and stored at -70° until used (Smith and Gerba, 1982).

Titers of both poliovirus and rotavirus stocks, and untreated water samples, were determined in the MA-104 cell line by the plaque overlay method described by Smith *et al.* (1979), in tissue culture 6-well trays. Viral assays of effluent samples were conducted also by the plaque forming method but in 25-cm^2 tissue culture flasks. Inoculated flasks were incubated at 37° C for one hour to allow virus absorption, followed by the addition of maintenance media containing 1.0% agarose. Plaques were stained and counted after 3 days of incubation (Smith and Gerba, 1982).

Test Waters and Test Conditions

For the microbial challenge, the desired turbidity of the worst-case water was adjusted by the addition of approximately 100 mg/L of AC fine dust (GM, Flint, MI) to obtain a turbidity of 30 NTU. Total organic carbon (10 mg/L) (TOC) was obtained by the addition of approximately 23 mg/L of humic acid (Aldrich Chemical Company, WI), and Total Dissolved Solids (TDS) (1500 mg/L), by the addition of approximately 1.5 g/L of sea salts (Sigma Chemical Company, MO).

Both general test water (water # 1), and worst case water (water # 2) challenges were conducted in triplicate using 500-ml glass beakers (reaction vessels). Typical physicochemical characteristics of the challenge waters are shown in Table 1. Approximately $3.0x10^7$ plaque forming units (PFU) of a mixture of poliovirus 1 and rotavirus SA-11, and $2.0x10^8$ colony forming units (CFU) of *K. terrigena* were added to each one of the reaction vessels. A volume of 25 ml of spiked untreated water samples was collected in duplicate into sterile 50-ml polypropylene test tubes.

Water treatment

Approximately 480-ml of waters # 1 (general-test water) or # 2 (worst-case water), spiked with the test organisms, were treated with the Steri-Pen® Water Treatment System as follows. Upon activation of the unit (green light blinking), the lamp end of the Steri-Pen® was immersed in the test water, until the sensors turned the UV-light on. In the water # 1, the Steri-Pen® was agitated in a circular fashion throughout one entire dose period. In the case of water # 2, the spiked water was filtered before Steri-Pen® treatment, using a First Need ® Deluxe water purifier (General Ecology, Inc. Exton, Pennsylvania). This filtered water was then treated as described for water # 1.

RESULTS

Tables 2 and 3 show viral assay results in general test water, and worst case water, respectively, Table 4 shows bacterial assay results in both types of water, and Table 5 summarizes both viral and bacterial results, indicating microbial reduction levels.

The removals (geometric average) by the water treatment in test water # 1 exceeded 6.37 $\log_{10}$ for *K. terrigena*, and 4.08 $\log_{10}$ for poliovirus type 1 and rotavirus SA-11. In test water # 2, a similar level of removal was observed by the combination of filtration and UV treatment.. The number of *K. terrigena.* was reduced by more than 7.40 $\log_{10}$, and the removal of the two viruses was of more than 4.75 $\log_{10}$.

According to these results, the Steri-Pen® Water Treatment System complies with the criteria for virus and bacteria reduction guidelines suggested by the U.S. Environmental Protection Agency for the removal of bacteria and viruses, if these treatment procedures are followed.

The viral and bacterial reductions caused by Steri-Pen in these tests are indicative of a UV doses of 38 mJ/sq.cm. or greater. It is reasonable to conclude therefore that Steri-Pen would reduce the protozoans *Cryptosporidium parvum* and *Giardia lamblia, both of which have a very low UV tolerance,* by at least three logs.

This conclusion is further supported by Ster-Pen/Cryptosporidium testing done by Dr. E.A. Meyer of Oregon Health Sciences University. Dr. Meyer states:

"The results of the T1 experiment (the first UV exposure of 40 seconds) indicates that this treatment was highly effective against these oocysts. The most concentrated specimen employed here- 115,000 oocysts per mouse, failed to infect any of the mice. It is reasonable to conservately conclude that this initial UV exposure resulted in at least a 2-log reduction in oocyst viability. The oocyst viability reduction may well have been 3 logs or more, but we were not able not concentrate the oocysts sufficiently to test this."

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Table 1. Required characteristics of non-microbiological parameters of test waters[1]

Parameter	General test water	Worst case water
Chlorine residual	None	None
pH	6.5 - 8.5	6.5-8.5
Total organic carbon (TOC)	0.1 - 5.0 mg/L	≥ 10 mg/L
Turbidity	0.1 - 5.0 NTU	≥ 30 NTU
Temperature	20 ± 5° C	4 ± 1° C
Total dissolved solids (TDS)	50 - 500 mg/L	1,500 ± 150 mg/L

[1]Guidance manual for compliance with the filtration and disinfection requirements for public water systems using surface water sources. American Water Works Association. Denver, CO.

Table 2. Summary of test results of Steri-Pen® Water Treatment System Microbiological Water Treatment System

General test water # 1				
Organism	Influent titer/L	Effluent titer/L	Log_{10} Reduction	% Reduction
Polio/Rota	$1.2x10^7$ PFU	$<1.0x10^3$ PFU	>4.08	>99.992
K.terrigena	$5.8x10^6$ CFU	<2.5 CFU[1]	>6.37	>99.99996
Worst case water # 2				
Polio/Rota	$2.9x10^7$ PFU/L	$<1x10^3$ PFU/L	>4.46	>99.996
K.terrigena	$1.7x10^8$ CFU/L	<10 CFU/L	>7.23	>99.999994

[1]higher sensitivity was obtained by processing 400 ml of each effluent water sample