EVALUATION OF MICROBIAL REMOVAL/INACTIVATION BY THE INNOWAVE UVF ULTRAVIOLET SYSTEM FROM GENERAL CASE TEST WATER

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SUMMARY

The UVF Ultraviolet system was evaluated for removal/inactivation of waterborne pathogenic microorganisms from water. The evaluation was divided into three parts. The first part consisted of evaluating reduction/inactivation of the waterborne bacteria *Escherichia coli*, *Salmonella thyphimurium*, *Shigella dysentariae*, *Vibrio cholera*, and *Klebsiella terrigena*. Next the system was challenged with poliovirus type 1 and rotavirus SA-11. Finally physical removal of Cryptosporidium was assessed. UVF units were operated in accordance with the manufacturer's instructions.

The units exceeded the removal/inactivation parameter of 99.9999% for waterborne bacteria, 99.99% for viruses and 99.9% for parasites.

1.0 INTRODUCTION

Development of in-line water treatment devices has evolved from consumer interest in improving and ensuring the quality of drinking water. The need also extends to the water quality of families or communities having individual home and small system water sources.

One major concern in water treatment is the requirement for removing disease-causing microorganisms from water for consumption since it is recognized that infectious disease transmission by water is a significant public health concern. The majority of documented waterborne diseases in the United States are caused by infectious microorganisms (Craun, 1986). It is important that water treatment units or devices designed for the protection of human health be effective against pathogenic microorganisms and be capable of providing this capability over the designed operational life of the equipment.

To ensure the efficacy of microbiological water purifiers, a multi-disciplinary task force was formed by the U.S. Environmental Protection Agency to develop a guide standard and protocol for testing such units. This guide standard and protocol appeared in the Federal Register of May 26, 1986, and has been accepted on a provisional basis by the U.S. Environmental Protection Agency's Office of Drinking Water and Office of Pesticide Programs. This document recommends test and performance requirements for microbiological purifiers. While the document specifically deals with testing criteria for certain types of water treatment devices such as halogen disinfectants, ultraviolet light, ceramic filters, etc., its purpose was to serve as a guide for all types of water treatment devices. The guide establishes that any microbiological water purifier be capable of removing or killing enteric bacteria, viruses and protozoan parasites. Such units should be capable of reducing challenge levels of suggested microbial contaminates in each class of microorganism.

The units must demonstrate at least a 99.9999% removal of *Klebsiella terrigena*, a 99.99% for poliovirus and rotavirus, and a 99.9% for *Giardia* cysts. The devices must also be capable of achieving these results under a realistic "worst case" water quality situation. In 1993, FIFRA Scientific Advisory Panel Antimicrobial subpanel, Office of Pesticides Programs, recommended the substitution of *Giardia* cysts by *Cryptosporidium* oocysts. *Cryptosporidium* oocysts (4-6μm) are smaller than *Giardia* (8-12μm) and more likely to pass through units which depend upon filtration for parasite removal. *Cryptosporidium* is extremely resistant to common water disinfectants (Korich et al, 1990) and has caused several large waterborne outbreaks in the United States and Europe in recent years (Smith and Rose, 1990). Thus any device capable of removing/inactivating *Cryptosporidium* should be able to eliminate *Giardia* cysts.

It is important that water treatment devices designed for the protection of human health be effective against pathogenic microorganisms and be capable of providing this capability over the designed operational life of the equipment in waters likely to be encountered in the United States. This is a necessary consideration for protection of the public's health by both the water industry and the government.

2.0 MATERIALS AND METHODS

2.1 Experimental Design

The experimental design for evaluating the UVF system was based on the recommendation 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 ultraviolet light systems for "average case" challenge water).

UVF systems were provided by INNOWAVE, 10250 Regency Circle, Suite 110, Omaha, NE and operated according to the manufacturer's instructions. The systems were challenged with the test microorganisms after different test parts (Tables 3 to 5). Between challenges, dechlorinated (by passage of the tapwater through a column of activated carbon) University of Arizona tapwater was processed through the systems. The physical/chemical characteristics of this water are shown in Table 1.

2.2 PRODUCTION OF CHALLENGE ORGANISMS

The test organisms used for assessment of removal/inactivation of bacteria were *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysentariae*, and *Vibrio cholera*, kindly provided by Emily Pejovich from the bacterial collection of the Department of Microbiology, University of Arizona. Also used for the evaluation were *Klebsiella terrigena* (ATCC-33257) obtained from American Type Culture Collection (Rockville, MD).

All bacteria were prepared by overnight growth in Tryptic Soy Broth (Difco, Detroit, MI) to obtain the organism in the stationary growth phase (Asburg, 1983). The organisms were collected by centrifugation (in a Beckman Floor centrifuge, Model J2-21, Palo Alto, CA) and resuspended three times in the test water to be tested.

The test viruses were poliovirus type 1 (LSC) and rotavirus strain SA-11. All stocks were grown by the method described in Smith and Gerba (1982) and purified by the procedure of Berman and Hoff (1984) to produce largely monodispersed virion particles.

Cryptosporidium parvum oocysts were obtained from the feces of infected calves (Pleasant Hill Farms, Troy, ID) and then purified by discontinuous sucrose gradient.

2.3 WATER QUALITY PARAMETERS

2.3.1 General Test Water

This water was used for “general case” water quality challenges and to condition the systems between microbial challenges. Tap water provided at the University of Arizona was used for “general case” water. This water is obtained from deep wells located on the University of Arizona campus. It meets all of the requirements of “general case” water as defined in the USEPA Guide Standard. Its physical/chemical are listed in Table 1.

Table 1.
PHYSICAL/CHEMICAL PROPERTIES OF TAPWATER AT THE UNIVERSITY OF ARIZONA

pH	7.5-7.8
Total Organic Carbon (TOC)	<1.0 mg/L
Turbidity	<1.0 NTU
Temperature	23-25°C
Total Dissolved Solids (TDS)	200-300 mg/L

Before use the water was passed through an activated charcoal filter (Amway, Ada, MI) to remove any chlorine present. This water was passed through the systems between challenges.

2.4 CHALLENGE PROCEDURE

The INNOWAVE UVF system produces up to one gallon of water per minute. Untreated water is fed directly to the system and in turn is filtered and then exposed to ultraviolet light (UV). No storing of water before and after treatment is necessary because of the immediate production of treated water. Systems were aged by connecting them to the tapwater supply at the University of Arizona and running two liters through them before the beginning of the test.

For each microbial challenge, 10 liters of challenge water containing the test microorganisms was passed through the system and then samples were collected for microbial assays. Samples of 100 ml of challenge water were collected for influent and one liter sample effluent.

2.4.1 Bacterial Challenges

One INNOWAVE UVF system was used to evaluate the removal of the most important waterborne bacteria. The system was aged for one week and each bacteria was tested individually using specific selective differential media shown in Table 2.

Table 2.
BACTERIA AND MEDIA USED FOR BACTERIAL ANALYSIS

BACTERIA	MEDIA
Escherichia coli	m-endo Agar LES (BBL, Cockeysville, MD)
Shigella dysentariae	SS Agar (DIFCO, Detroit, MI)
Salmonella typhimurium	Hektoen Enteric Agar (DIFCO, Detroit, MI)
Vibrio cholera	Thiosulphate-citrate-bile salts-sucrose Agar (BBL, Cockeysville, MD)

2.4.2 EPA Guide Standard Protocol and Tests

Three UVF systems were tested for the removal/inactivation of Poliovirus Type 1, Rotavirus SA-11 and *Klebsiella terrigena* according the USEPA's Guide Standard and Protocol.

Three UVF systems were evaluated for the physical removal of *Cryptosporidium* oocysts.

2.5 ANALYTICAL METHODS

2.5.1 Bacterial Assays

Assays were conducted by the membrane filtration method on the specific media for each bacteria (Table 3). *Klebsiella terrigena* was tested using m-endo Agar LES (Becton Dickinson and Co., Cockeysville, MD. Cat #4311203). Appropriate dilutions of influent samples were made in sterile Tris-buffered saline (Trisma Base, Sigma Chemical, St. Louis, MO). A 10-mL sample of undiluted effluent was also assayed. All assays were in triplicate according to Standard Methods (APHA, 1992).

2.5.2 Viral Assay

Poliovirus type 1 and rotavirus SA-11 were assayed simultaneously by the plaque assay method on the MA-104 cell line using procedures described by Smith and Gerba (1982). Dilutions, if necessary, were conducted using Tris-buffered saline. All assays were done in triplicate.

2.5.3 Cryptosporidium Assay

Samples for parasite assays were examined using the hemocytometer method (Guidance Manual, 1990).

2.5.3.1 INFLUENT AND EFFLUENT

Influent samples of 10 mL and effluent samples of 100 mL were centrifuged in an IEC Clinical Centrifuge (Nedham Hts., MA) at 400 xg for 15 minutes to pellet oocysts. For both influent and effluent, samples were processed as follows; The supernatant was aspirated to 1 mL above the pellet. After resuspension of the pellet in PBS buffer, the oocysts were counted using SPolite Hemocytometer

(Baxter Healthcare Corp. McGraw Park, IL) using a phase contrast microscope (BH-2 Olympus, Japan) at 400x magnification. At least 12 chamber aliquots were counted for each sample according to the procedure outlined in the Guidance Manual (USEPA, 1990).

An average of all readings was done and multiplied with the conversion factor of $1.0x10^4$. Total number of oocysts were divided by 10 for influent and 100 for effluent to determine the number of oocysts per mL of sample.

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3.0 RESULTS

The results of the microbial removal studies are shown in Table 6 through 9. These results show that the units can achieve percent removal of 99.9999% for waterborne bacteria, 99.99% for enteric viruses, and 99.9% for *Cryptosporidium parvum*.

In summary, the UVF system can meet the microbial removals as required by the U.S. Environmental Protection Agency's Guide Standard and Protocol for Testing Microbiological Water Purifiers for "General case" test water.

REFERENCES

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Table 3.
BACTERIAL REMOVAL/INACTIVATION RESULTS
(CFU/mL)

BACTERIA	CHALLENGE	PRODUCT WATER	PERCENT REDUCTION
Escherichia coli	2.55×10^{9}	<50	>99.999998
Salmonella thyphimurium	2.34×10^{8}	<50	>99.99998
Klebsiella terrigena	4.00×10^{8}	<50	>99.99998
Shigella dysentariae	9.70×10^{7}	<50	>99.99995
Vibrio cholera	4.00×10^{8}	<50	>99.99998

CFU= colony forming units

TABLE 4.
REMOVAL/INACTIVATION OF POLIOVIRUS AND ROTAVIRUS
(PFU/LITER)

TEST NUMBER	INFLUENT	EFFLUENT	PERCENT REDUCTION
1	$2.00x10^{7}$	<166	>99.9992
2	$2.00x10^{7}$	<166	>99.9992

PFU= plaque forming unit

TABLE 5.
REMOVAL/INACTIVATION OF *CRYPTOSPORIDIUM*
(OOCYST/LITER)

ORGANISM	UNIT	INFLUENT	EFFLUENT	PERCENT REDUCTION
GIARDIA	1	$2.00x10^{6}$	<926	>99.95
CRYPTOSPORIDIUM	1	$5.90x10^{6}$	<926	>99.98
GIARDIA	2	$3.40x10^{6}$	<926	>99.97
CRYPTOSPORIDIUM	2	$1.36x10^{7}$	<926	>99.993
GIARDIA	3	$3.40x10^{6}$	<926	>99.97
CRYPTOSPORIDIUM	3	$1.36x10^{7}$	<926	>99.993

Sample Calculations

Calculator: Casio Fx-300s

Geometric Mean

$$\log X_g = \frac{\Sigma (\log X_i)}{n}$$

$$X_g = \text{antilog} (\log X_g)$$

Example: Table 8, test number 8 for viral challenges

$$\log X_g = \frac{\Sigma[(\log 111)+(\log 111)+(\log 111)]}{3}$$

$$= \frac{2.045323+2.045323+2.045323}{3}$$

$$X_g = \text{antilog} (2.045323)=111$$

Percent Reduction

Geometric average x 100= A
A is then divided by Challenge concentration = B
B then is subtracted from 100= Percent Reduction

Example: Table 8, test number 8 for viral challenge

111x100= 11,110

$11{,}100 \div 1.00 \times 10^6 = 0.0111$

0.0111-100= 99.989