Microbiological Testing of the PeaceFilter

Produced for

Kung Charities

Prepared by

Thomas Soerens[1], Andrew Nevin[2], Laura Ritenour[2], Holly Ross[2], Shaun Egolf[2], Megan Bleacher[2], Kiera Jeschke[2], Daniel Lemen[3], Alyssa Sargent[2], Chelsea Toburen[4]

[1]Department of Engineering, [2]Department of Biological Sciences, [3]Department of Chemistry and Biochemistry, [4]Department of Nursing

MESSIAH COLLEGE

One College Ave Suite 3034
Mechanicsburg, PA 17055
USA

April 21st, 2016

the Collaboratory for strategic partnerships and applied research

Summary

The PeaceFilter was tested for its ability to remove three microorganisms – *Escherichia coli*, *Serratia marcescens*, and *Micrococcus luteus* – using USEPA approved procedures. These organisms were added to test water to reach a $10^8 – 10^9$ cells/L initial concentration. The test water conditions met the criteria for "test water #3" as set by USEPA 1987. Each of the three tested PeaceFilters surpassed the target reduction of 6 log units, or 99.9999%, for each test organism. The PeaceFilter meets the USEPA standards for bacteria and protozoans.

Table 1. Mean log removal values (LRV) for three replicate filter tests. Water was collected and microbiologically analyzed after 100, 300 and 600 milliliters passed through the filter; standard error for these three samples is displayed.

	Filter Tested		
Organism	1	2	3
E. coli	8.46 (±0.00)	8.46 (±0.00)	8.46 (±0.00)
S. marcescens	8.88 (±0.00)	8.88 (±0.00)	8.88 (±0.00)
M. luteus	7.73 (±0.00)	7.73 (±0.00)	7.73 (±0.00)

Introduction

Filtration is "a pressure- or vacuum-driven separation process in which particulate matter larger than 1 µm is rejected by an engineered barrier primarily through a size exclusion mechanism and which has a measureable removal efficiency of a target organism that can be verified through the application of a direct integrity test" (40 CFR 141.2). The PeaceFilter underwent challenge testing with specific microorganisms to determine if it performed as an effective barrier to those organisms. Standard United States Environmental Protection Agency (USEPA) approved procedures were followed.

Three PeaceFilters provided by Kung Charities were tested. Each filter was conditioned with a 5% chlorine solution and sterile test water. The challenge microorganism (Table 2) was mixed with test water to obtain a $10^8 – 10^9$ cells/L concentration and was forced through the filter. 100 ml of filtrate were collected in a sterile Whirl-Pak® Bag after 100, 300 and 600 milliliters passed through the filter and analyzed for microbial growth using the membrane filtration technique following Standard Methods 9222 (APHA et al., 2012).

Surrogate organisms of similar size, approved by the USEPA, were used in place of the pathogenic target organisms to avoid unnecessary safety hazards.

Table 2. Challenge test organisms and USEPA approved surrogates. (USEPA, 2005 and NSF, 2005)

Target Organism	**Surrogate**	**Size range (µm)**
Pathogenic Coliforms	*Escherichia coli*	1-1.5
Cryptosporidium	*Serratia marcescens*	0.5
Giardia	*Micrococcus luteus*	7-12

The USEPA Guide Standard and Protocol for Testing Microbiological Water Purifiers (1987) requires a minimum reduction for protozoan parasites of 3 log units and a minimum of 6 log units for bacteria. For this test, *all* targeted log reductions for surrogates were set at 6 log units, or 99.9999% reduction. Surrogate organisms were chosen according to guidelines in the EPA Membrane Filtration Guidance Manual, published June 2003.

Methods

Test Water and Solutions

Test Water: The water used for testing was obtained from the Yellow Breeches Creek, which is the source for municipal drinking water in Cumberland and York Counties in Pennsylvania. Water was collected in a 20L carboy and autoclaved at 121°C (15 lb pressure) for 35 minutes to obtain sterile test water.

Standard methods (APHA et al., 2012) were followed to ensure test water conditions.

Trypticase Soy Broth (TSB) (BD Diagnostic Systems)

30 g dehydrated TSB was dissolved into 1 L of reagent grade distilled water. The agar was then dispensed in culture tubes and 250 ml flasks, covered with caps/foil and autoclaved at 121°C (15 lb pressure) for 15 minutes.

Trypticase Soy Agar (TSA) (BD Diagnostic Systems)

40 g dehydrated TSA was dissolved in a flask containing 1 L of reagent grade distilled water and heated to boiling with stirring until the ingredients dissolved. The agar was then autoclaved at 121°C (15 lb pressure) for 15 minutes. It was aseptically poured into 50x9 mm petri dishes to 4-5 mm depth (7 ml) and allowed to solidify. The plates were stored for up to two weeks in the refrigerator.

Bacterial Growth and Challenge Water Preparation

Stock cultures were quadrant streaked onto TSA plates and incubated at 30°C (*M. luteus*, *S. marcescens*) or 37°C (*E. coli*) for 24 hours. A pure culture was selected from the plate and used to inoculate a culture tube containing 10 ml of TSB. The tube was incubated with spinning at the temperatures given above overnight to grow the cells to stationary phase. The following day, the cultures with back – diluted into 10 ml of TSB at a concentration of 1:100. After approximately 2-4 hours of growth at 37°C on a shaker plate, the culture tubes were observed with a spectrophotometer in order to determine optical density (OD) and the phase of growth. When the cultures had achieved an OD of .55-.6 absorbance, to ensure the cells were still in exponential growth phase, the cultures were then diluted in 2.5 L test water to obtain a final concentration of 10^7-10^8 cells/100 ml (10^8-10^9 cells/L) after mixing. Test water was then distributed into 2 L vacuum bottles for challenge testing. Initial seed counts were confirmed by serial dilution using 99 ml sterile deionized distilled water blanks. The final dilutions for plated by membrane filtration (see below) were 10^{-5}, 10^{-6}, and 10^{-7}.

Sample Collection

All tubing, bottles, caps, and glassware were washed and autoclaved prior to each trial. Initial conditioning of the water filter was attained by passing 200 ml of 5% bleach solution followed by 1 L of sterile test water (without organisms) through the filter (Fig. 1). Negative controls were collected from the final 100, 300 and 600 milliliters of test water in sterile Whirl-Pak® Bags. Challenge test water with organisms was forced through the filter. Collection of filtrate was performed at 100, 300, and 600 milliliters in sterile Whirl-Pak® Bags.

Figure 1. Pressurizing device for forcing challenge water through the water filter. A hand pump was used to generate pressure inside the vacuum bottle, which then forced test water through the filter at 5-10 psi.

Microbiological Analysis

Standard Methods 9222 (APHA et al. 2012) were followed; the following description is abbreviated. The 100 ml sample was shaken vigorously and poured into the funnel. Vacuum was applied to filter the sample through. Vacuum was turned off and funnel top lifted. Using sterile forceps, the filter was transferred to the prepared petri dish by placing the filter grid right side up on the agar surface with a slight rolling motion. Plates were incubated at 30°C (*M. luteus*, *S. marcescens*) or 37°C (*E. coli*) for 24 hours, and the number of colonies was counted.

Calculations

Colony forming units (cfu)

Cfu/100 ml = 100 x (number of colonies) / volume of sample filtered in mL

Log removal value (LRV)

Target is 6 log unit reduction.

LRV= log (C_f) – log (C_p)
C_f = feed concentration (cfu/100 ml)
C_p = filtrate concentration (cfu/100 ml)

Results

All trials had outcomes of zero cfu/100 ml in each test organism and control filtrate (Table 3). All trials attained 6 log unit reduction or higher. (Table 4)

Table 3. Challenge filtration test trials for three water filters. Filtrate collected at 100, 500, and 900 milliliters were plated using the membrane filtration technique. Values are expressed as colony forming units per 100 milliliters (cfu/100 ml).

Trial	Organism	Initial seed	100	300	600
1	*E. coli*	2.95×10^8	0	0	0
	S. marcescens	7.61×10^8	0	0	0
	M. luteus	5.47×10^7	0	0	0
	Negative control		0	0	0
2	*E. coli*	2.95×10^8	0	0	0
	S. marcescens	7.61×10^8	0	0	0
	M. luteus	5.47×10^7	0	0	0
	Negative control		0	0	0
3	*E. coli*	2.95×10^8	0	0	0
	S. marcescens	7.61×10^8	0	0	0
	M. luteus	5.47×10^7	0	0	0
	Negative control		0	0	0

Table 4. Log removal values (LRV) for each trial. The target reduction was 6 log units or greater.

Trial	Organism	100	300	600
1	*E. coli*	8.46	8.46	8.46
	S. marcescens	8.88	8.88	8.88
	M. luteus	7.73	7.73	7.73
2	*E. coli*	8.46	8.46	8.46
	S. marcescens	8.88	8.88	8.88
	M. luteus	7.73	7.73	7.73
3	*E. coli*	8.46	8.46	8.46
	S. marcescens	8.88	8.88	8.88
	M. luteus	7.73	7.73	7.73

Discussion

All three PeaceFilters showed a 6 fold or greater reduction of all test organisms, indicating that the PeaceFilters successfully remove the organisms from the challenge water. The surrogate organism used for *Cryptosporidia* and *Giardia*, *Serratia marcesens*, achieved greater than a 6-log reduction in effluent. The EPA only mandates a 3-log reduction for these organisms so the PeaceFilters tested far exceeded the EPA minimum standards. Because 6 log reduction of all organisms was observed, these tests show that the PeaceFilter meets the USEPA standard for both bacteria and protozoan removal.

References

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF) 2012. Standard Methods for the Examination of Water and Wastewater. 22nd ed. American Water Works Association.

Federal Register 2012. National Primary Drinking Water Standards. 40 CFR 141.2

NSF International. 2005. EPA/NSF ETV Equipment Verification Testing Plan for the Removal of Microbiological and Particulate Contaminants by Membrane filtration. Ann Arbor, MI.

USEPA 1987. Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources Appendix O: Guide Standards and Protocol for Testing Microbiological Water Purifiers. Contract No. 68-01-6989 U. S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC.

USEPA 2005. Membrane Filtration Guidance Manual. EPA 815-R-06-009 U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, DC.