Microbiological and Chemical Testing of Microshield Safe Water System and the Bota of Boulder Outback Water Filtration System

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Summary

The ability of the Microshield Safe Water System and the Bota of Boulder Outback Water Filtration System to remove the human pathogens *Giardia lamblia, Cryptosporidium parvum, Legionella pneumophila, Salmonella typhimurium,* and *Escherichia coli* from stream water was conducted using the USEPA Guide Standard (1986) and Protocol for Testing Microbiological Water Purifiers. The Standard requires a minimum reduction for protozoan parasites (*Giardia* and *Cryptosporidium*) of 3 log units, or 99.9% removal, and a minimum of 6 log units, or 99.9999%, reduction for bacterial species (*Salmonella*, *Escherichia*, and *Legionella*).

The Microshield system removed greater than 99.99% of *Giardia* and *Cryptosporidium* and greater than 99.9999 % of the bacteria *Legionella*, *Salmonella*, and *Escherichia* (Table 1). As such, the purification system meets the EPA standard for both protozoans and bacteria. The Outback system removed greater than 99.99% of *Giardia* and *Cryptosporidium.* The Outback system is not designed to remove bacteria and was found to remove less than the minimum standard of 99.9999% of *Legionella*, *Salmonella*, and *Escherichia* (Table 2).

The ability of the same water purifiers to remove arsenic, copper, lead, zinc and chlorine was also determined. The Microshield system reduced the concentrations of arsenic, copper, lead, and chlorine by 90%, >99%, >99%, and 83%, respectively (Table 1). The Outback system reduced the concentrations of arsenic, copper, and lead by 57%, 98%, and 99%, respectively (Table 2). The Outback system did not measurably reduce chlorine concentrations. An increase rather than a decrease in zinc concentration was noted with both purification systems. This finding suggests that a component within the filter system contains zinc. The increase in zinc, however, was very slight (0.137mg/L, bringing final concentrations to approximately a tenth of EPA maximum acceptable levels).

Table 1. Results of Microshield Safe Water System microbiological and chemical tests

	Test Water Initial	**Test Water** After Purification	**Units**	**Reduction (%)**	**Reduction (log units)**
Protozoan parasites					
Giardia	1.3E+07	0 ± 0	(cysts/100mL)	**>99.99**	>4
Cryptosporidium	1.8E+06	0 ± 0	(oocysts/100mL)	**>99.99**	>4
Bacteria					
Legionella	4.7E+07	0 ± 0	(cells/100mL)	**>99.9999**	>6
Salmonella	4.7E+07	1.33 ± 1.3	(cells/100mL)	**99.999997**	>6
Escherichia	6.3E+07	57 ± 11	(cells/100mL)	**99.999909**	6
Chemistry					
Chlorine	2.20	0.38	(mg/L)	**82.7**	
Arsenic	534.8	54.4 ± 8.2	(µg/L)	**89.8**	
Copper	411.3	b.d.*	(µg/L)	**>99.8**	
Lead	316.9	b.d.*	(µg/L)	**>99.7**	
Zinc	185.1	291.4 ± 33.8	(µg/L)	**(57.4)****	

*b.d. indicates concentration was below the detection limit.
** (values) indicate an increase.

Table 2. Results of Bota of Boulder Outback Water Filtration System microbiological and chemical tests

	Test Water Initial	**Test Water** After Purification	**Units**	**Reduction (%)**	**Reduction (log units)**
Protozoan parasites					
Giardia	1.3E+07	0 ± 0	(cysts/100mL)	**>99.99**	>4
Cryptosporidium	1.8E+06	0 ± 0	(oocysts/100mL)	**>99.99**	>4
Bacteria					
Legionella	4.7E+07	>100	(cells/100mL)	**<99.9999**	<6
Salmonella	4.7E+07	>100	(cells/100mL)	**<99.9999**	<6
Escherichia	6.3E+07	>100	(cells/100mL)	**<99.9999**	<6
Chemistry					
Chlorine	2.20	2.20 ± 0	(mg/L)	**0**	
Arsenic	534.8	231.9 ± 33.4	(µg/L)	**56.6**	
Copper	411.3	10.2 ± 5.7	(µg/L)	**97.5**	
Lead	316.9	6.4 ± 5.4	(µg/L)	**98.0**	
Zinc	185.1	322.7 ± 67.7	(µg/L)	**(74.3)****	

*b.d. indicates concentration was below the detection limit.
** (values) indicate an increase.

Introduction

This introduction is intended to provide an explanation of the test without the use of unnecessary technical terminology. The sections following the introduction are intended to explain the methods, findings, and quality control procedures to a professional audience.

A suspension of each organism (*Giardia lamblia, Cryptosporidium parvum, Legionella pneumophilia, Salmonella typhimurium,* and *Escherichia coli)* was prepared in stream water collected from an active recreational area in Montana. The suspensions were passed through the water purification systems in a manner similar to way they would be

used by the consumer. All of the test water that passed through the purification system was collected and passed through an additional laboratory filter apparatus that captures all cell types. Since protozoan parasites (*Giardia* and *Cryptosporidium*) are difficult to culture, any protozoans that might have passed through the purifier and were therefore trapped on the laboratory filter were stained with a fluorescent DNA-binding dye and then observed for glowing cells under a fluorescent microscope. Since bacteria (*Legionella*, *Salmonella*, and *Escherichia*) grow relatively easily on plates containing a solidified nutrient source (nutrient plates), any cells collected on the laboratory filter were transferred directly to a nutrient plate. These plates were incubated and the number of viable colonies counted.

To test the ability of the purifiers to remove chemicals, two spiked solutions were prepared, one containing metal salts, and a second containing bleach as a chlorine source. For metal analysis, the water samples were sent to The University of Montana Environmental Biogeochemistry Laboratory and analyzed according to a standard EPA protocol. We calculated the chlorine concentration in the water samples before and after purification by adding a chlorine binding dye to the solution and then measured the amount of light absorbed by the dye.

Three identical purifiers were tested for each organism or chemical. Positive and negative controls were used to evaluate any possible laboratory error.

Methods

Test organisms: The test organisms and their sources are shown below.

Giardia lamblia cysts (Water borne, Inc., New Orleans, LA)
Cryptosporidium parvum oocysts (Water borne, Inc., New Orleans, LA)
Legionella pneumophila (ATCC 33152)
Salmonella typhimurium (ATCC 29058)
Escherichia coli (ATCC 23725)

Test water and Solutions: Test Water**:** Autoclaved, room temperature, water from Rattlesnake Creek (Missoula, MT), was used for the test water. Rattlesnake Creek flows out of the Rattlesnake Wilderness Area north of Missoula to its confluence with the Clark Fork River in the city of Missoula. Water was collected near the confluence. Samples of this water were archived and are available upon request. The water is considered representative of a normal water used by recreationalists.

Phosphate buffered saline solution (PBS): 0.05M PBS solution buffered at pH 7.0 was used for transfers and dilutions of cells.

Conditioning/Rinse water: Sterile, deionized water was used for conditioning water and rinse water.

Metal reduction test water: Deionized water was used as the base water for the metal reduction test. Metal salts were added to bring the solution metal concentration to 100 times the detection limit for the inductively coupled plasma emission spectrophotometer (ICP). Solution concentrations of salts were as follows: sodium arsenate (4.16 mg/L), cupric sulfate (2.36 mg/L), lead nitrate (3.80 mg/L) zinc sulfate (0.440 mg/L). Concentrations were calculated according to Table 3.

Table 3. Calculation of metal salt additions for use in metals reduction testing

Metal salt	g salt /mol	g metal/g salt	detection mg/L	metal mg/L	metal g/L	salt g/L	100x*
Sodium Arsenate	311.9	0.240	0.00500	1	0.001	0.0042	0.42
Cupric Sulfate	249.7	0.254	0.00300	0.6	0.0006	0.0024	0.236
Lead Nitrate	393.2	0.527	0.01000	2	0.002	0.0038	0.380
Zinc Sulfate	287.6	0.227	0.00050	0.1	0.0001	0.0004	0.044

*add 100x of salt to 100 mL, transfer 1mL to 1L tap water

Chlorine reduction test water: Tap water was used for the chlorine reduction test. 6mL household bleach (Chlorox, 6% sodium hypochlorite) was added to a liter of tap water to bring the total chlorine level to 2.20 mg/L.

Test organism preparation: Protozoans: Live giardia cysts were purchased in solution from WaterBorne, Inc (New Orleans, LA). The initial cyst concentration (1.52×10^7/ml) was verified using a Petroff-Hausser counting chamber. 8.5mL of the solution was added to 1L of test water to bring the final test concentration to 1.30×10^8 cysts/L. *Cryptosporidium* oocysts were prepared the same as giardia cysts. The oocyst concentration in the solution purchased from Waterborne Inc. was 2.30×10^6 oocysts/mL. 8mL of the solution was added to 1L of test water to bring the final concentration to 1.83×10^7 oocysts/L.

Bacteria: *Legionella pneumophila* (ATCC 33152) from a stock culture was streaked on a BCYE Agar (Difco Laboratories) plate and incubated at 35°C in a 5% CO_2 incubation chamber. A single colony was streaked onto a BCYE plate 48 hours before the test. On the day of the test, 5mL of PBS was pipetted onto the plate and the cells were massaged into solution. The 5mL of PBS containing *Legionella* were transferred to 50mL PBS. The cells were counted in a Petroff-Hausser counting chamber and then 1L of test water was inoculated to a density of approximately 5.0×10^8 cells/L to attain a final density of 5.0×10^7 cells/100mL. The final solution was rechecked using the counting chamber and determined to contain 4.7×10^8 cells/L (Tables 1 and 2). *Escherichia* and *Salmonella* from stock cultures were streaked onto TSA (Tripticase Soy Agar) plates and incubated at 35°C to check for purity. A 200mL flask of Trypticase Soy Broth (TSB) was inoculated with cells the night before the test and the cells grown to stationary phase. The cells were counted in a Petroff-Hausser counting chamber and then 1L of test water was inoculated to a density of approximately 5.0×10^8 cells/L to attain a final density of 5.0×10^7 cells/100mL. The *Salmonella* test water was rechecked using the counting chamber and contained 4.7×10^8 cells/L. The *Escherichia* test water contained 6.3×10^8 cells/L.

Microbial reduction water purifier test: Test Protocol: 100mL of a test solution containing a given microorganism was drawn through each of three identical units of both types of water purifiers. Between tests each purifier was rinsed with 1L of rinse water. A straw (included as part of the Microshield System) was affixed to a vacuum to draw test waters through the system. Air was drawn through the units after each test to insure that most of the 100mL of test water had passed through. The Outback System was tested by squeezing water through the unit using the included Bota of Bolder water bottle. A small amount of water was squeezed into a waste container before each test to insure that 100mL of test water could pass through the system. The test method is summarized in Figure 1.

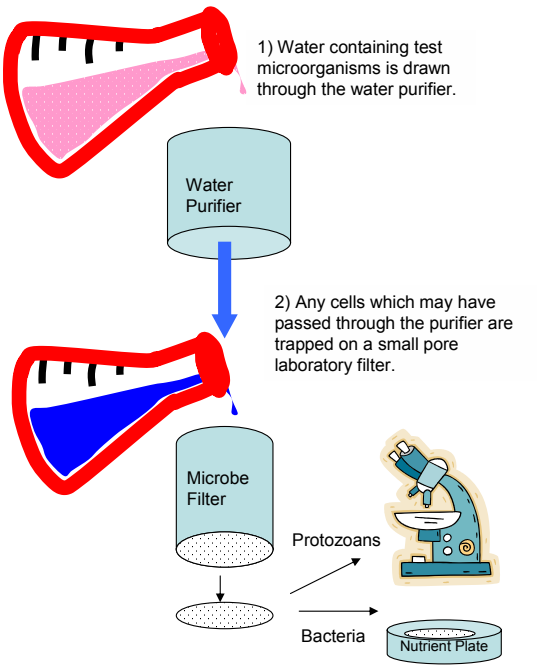

Figure 1

Determination of efficacy: Protozoans were collected on 0.2 µm black filters (Osmonics 11021) and stained with 4',6'-diamidino-2-phenylindole hydrochloride (DAPI) for microscopic counting using a Zeiss epifluorescent microscope. For positive controls the average number of cells in twenty fields of view was used to calculate cell numbers. To detect cysts and oocysts in test samples, the microscope was focused on the filter paper and the entire area of the filter paper was examined. All detected cysts and oocysts were recorded and photographed.

The bacteria in 100mL of effluent water were concentrated by filtration onto a 0.2µm polycarbonate filter and incubated as described above. *Legionella* filters were placed onto BCYE agar plates and incubated for five days in a 35°C in a 5% CO_2 chamber before counting. The *Salmonella* and *Escherichia* filters were placed on TSA plates and incubated for three days before counting. The order of testing proceeded from the largest organism to the smallest (*Giardia, Cryptosporidium, Legionella, Salmonella,* then *Escherichia*). Microbiological tests were followed by the metal reduction and then the chlorine reduction test. Three filters were evaluated for each test organism and the mean +/- one standard error reported.

Metal reduction filter test: 50mL of metal reduction test water were drawn through each purifier unit and sent to The University of Montana Environmental Biogeochemistry Laboratory. A 50mL sample of the test water was used to calculate the starting concentrations of the metals (high concentration control). Tap water was used as a low concentration control. 1 L of conditioning water was used to flush each filter before the

next test. Metal content was quantified on an ICP-MS (IRIS model, Thermoelemental, Franklin, MA) by U.S. EPA method 200.7.

Chlorine reduction test: 50mL of chlorine reduction test water containing 0.06% bleach as a chlorine source was drawn through each purifier unit and collected for analysis. The Hach Total Chlorine Assay (Hach method 8167), a spectrophotometric method, was used to determine total chlorine concentration. The test water was used as a high concentration control and for determination of reduction efficiency. Tap water was used as the low concentration control.

Quality control: One unit was tested in triplicate. Triplicate testing allowed variation in purifier performance to be differentiated from variation in laboratory replicates. Unfiltered test water was used to show that organisms were detectable. Test water without added cells was drawn through each unit as a negative control.

Safety: All organisms used in this testing were potentially pathogenic. Analysts wore gloves and transferred infectious materials under aseptic conditions in a laminar flow hood. Surfaces were wiped with disinfectant before and after use. At each break in the procedure ultraviolet lights were used to disinfect the room.

Results and Conclusions

Microbiological test results: Table 4 displays the numbers of each type of organism that were detected after passing the test solution through the indicated water purifier. The approximate size of the organism tested is also given as a reference. The efficacy results are summarized in Tables 1 and 2 (above). The Microshield System reduced *Giardia*, *Cryptosporidium*, and *Legionella* to below detection levels. Four *Salmonella* cells were passed through one (of 3) of the Microshield filter systems, representing a 99.99999% reduction in cell numbers. An average of 57 cells (standard error = 11) of *Escherichia* passed through the Microshield system, representing a 99.99991% reduction in cell numbers. *Escherichia* cells were the smallest cells tested (2-3μm). Overall the Microshield System passed the minimum standard for protozoans and bacteria as set by the EPA standard.

The Outback System reduced *Giardia* and *Cryptosporidium* to below detection levels. Two of the purifiers (unit 1 and 2) greatly reduced *Legionella* and *Salmonella*. None of the three filters measurably reduced *Escherichia* (i.e. the filter papers contained too many cells to count). The Outback System unit 3 did not measurably reduce cell numbers of any of the bacteria. Outback System unit 3 was selected as the filter that would be analyzed in triplicate before testing began. Results from the triplicate analysis of this filter are shown in Table 4 (Quality Check 1-3). The results were consistent for all tests except for the *Cryptosporidium* test. A total of three oocysts were detected in the two repeated tests of unit 3. The oocysts detected in the two replicate samples would not have caused the Outback System to fail the *Cryptosporidium* test had they been detected

in the replicate considered the "test" sample. Overall, the Outback System passed the minimum standard for protozoan removal as set by the EPA standard.

Table 4. Results of microbiological tests

	Giardia (cells/100mL)	*Cryptosporidium* (cells/100mL)	*Legionella* (cells/100mL)	*Salmonella* (cells/100mL)	*Escherichia* (cells/100mL)
Microshield System Unit 1	0	0	0	0	79
Microshield System Unit 2	0	0	0	0	41
Microshield System Unit 3	0	0	0	4	52
Outback System Unit 1	0	0	0	8	>100
Outback System Unit 2	0	0	9	10	>100
Outback System Unit 3	0	0	>100	>100	>100
Quality Check 1	0	0	>100	>100	>100
Quality Check 2	0	2	>100	>100	>100
Quality Check 3	0	1	>100	>100	>100
Plate/Filter Count	5.5E+05	2.2E+05	2.7E+06	4.7E+06	2.5E+06
Organism Size (μm)	10-12	5-7	2-5	2-4	2-3

Chemical Test Results: Table 5 displays the results of the chemical tests applied to the purifier units. The results are summarized in Tables 1 and 2. The Microshield System reduced the concentrations of arsenic, copper, lead, and chlorine by 90%, >99%, >99%, and 83%, respectively. Chlorine reduction ranged from 95% to 58%, indicating some variability in the performance of the filter units with regard to chlorine. The Outback system reduced the concentrations of arsenic, copper, and lead by 57%, 98%, and 99%, respectively. The Outback system did not measurably reduce chlorine concentrations. An increase rather than a decrease in zinc concentration was noted with both purifier types. This finding may indicate that a compound within the purifier contains zinc. The increase in zinc concentrations due to the purifier composition was very slight (0.137mg/L, bringing final concentrations to approximately a tenth of EPA maximum acceptable levels). The most likely explanation for the increase in zinc is that a material in the filter contains zinc that leaches into the filtered water in small quantities.

Table 5. Results of chemical tests

	Arsenic (μg/L) 0.5	Chlorine (mg/L) <0.1	Copper (μg/L) 1	Lead (μg/L) 1	Zinc (μg/L) 1
Microshield System Unit 1	69.5	0.10	b.d.*	b.d.*	355.2
Microshield System Unit 2	52.3	0.92	b.d.*	b.d.*	278.5
Microshield System Unit 3	41.4	0.12	b.d.*	b.d.*	240.4
Outback System Unit 1	265.1	2.20	20.5	17.3	453.7
Outback System Unit 2	165.0	2.20	b.d.*	b.d.*	286.8
Outback System Unit 3	265.5	2.20	9.15	b.d.*	227.7
High Control	534.8	2.20	411.3	316.9	185.1
Low Control	5.7	0.16	310.5	14.5	140.6

*b.d. indicates concentration was below the detection limit.