Attached is our independent lab report performed by Hydreion Labs. This lab reports covers the following filters:

PointOne™ Filter
Bio Filter
7/6B Filter
Squeeze Filter
Sawyer 3 in 1 Filter
Sawyer 4 way Filter
Sawyer Bag to Bag filters
Sawyer Water Bottle Filters
Sawyer Bucket Filter Kits

There have been no changes to the filter since the lab tests were performed.

Please note on the last page under Results and Conclusions that on each of the test filters there was zero detection of any pathogens. This far **exceeds** the EPA Standards.

Microbiological Testing of the Sawyer 7/6B Filter

Report No. S05-03
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Summary

The ability of the Sawyer 7/6B filter to remove the human pathogens *Giardia lamblia, Cryptosporidium parvum,* and *Klebsiella terrigena* from surface 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 bacteria (*Klebsiella).*

The Sawyer 7/6B filter removed greater than 99.999% of protozoan parasites and greater than 99.9999 % of bacteria (Table 1). As such, the filter system meets the EPA standard for both protozoa and bacteria. Further, no pathogenic organisms were detected to have passed through the filter.

Table 1. Results of Sawyer 7/6B filter microbiological tests

	# detected Initial	**# detected** After filtration	**Units**	**Reduction (%)**	**Reduction (log units)**
Protozoan parasites					
Giardia	1.0E+06	0 ± 0	(cysts/100mL)	**>99.999**	>5
Cryptosporidium	1.0E+06	0 ± 0	(oocysts/100mL)	**>99.999**	>5
Bacteria					
Klebsiella	1.8E+07	0 ± 0	(cells/100mL)	**>99.9999**	>6

Introduction

This introduction provides an explanation of the test conducted 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,* and *Klebsiella terrigena)* was prepared in stream water. These suspensions will be referred to as test waters. The test waters were passed through the water filter in a manner similar to how they would be used by the consumer (20 psi). Test water that passed through the Sawyer 7/6B filter was collected and passed through an additional laboratory filter apparatus that captures all cell types onto a small round filter film. Since protozoan parasites (*Giardia* and *Cryptosporidium*) are difficult to culture, the laboratory films were stained with a fluorescent DNA-binding dye and then observed for glowing cells under a fluorescent microscope. The bacteria (*Klebsiella terrigena*) grows easily on plates containing a solidified nutrient source (nutrient plates). The laboratory filter papers were transferred to a nutrient plate where cells could grow into visible colonies for counting.

Three identical filter systems were tested for each organism. 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 (Hyperion Research, LTD, Alberta, Canada)
Cryptosporidium parvum oocysts (Hyperion Research, LTD, Alberta, Canada)
Klebsiella terrigena (ATCC 33628)

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 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.

Test organism preparation: Protozoans: Live *Giardia* cysts were purchased in solution from Hyperion Research, LTD. The initial cyst concentration (1.0×10^7/ml) was verified using a Petroff-Hausser counting chamber. 10 mL of the solution was added to 1L of test water to bring the final test concentration to 1.0×10^7 cysts/L. *Cryptosporidium* oocysts were prepared the same as *Giardia* cysts. The oocyst concentration in the solution purchased from Hyperion Research, LTD was 1.0×10^7. 10 mL of the solution was added to 1L of test water to bring the final concentration to 1.0×10^7 oocysts/L.

Bacteria: *Klebsiella terrigena* from stock cultures were streaked onto TSA (Trypticase 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 2.0×10^8 cells/L to attain a final density of 2.0×10^7 cells/100mL.

Microbial reduction test: Test Protocol: 100mL of a test solution containing a given microorganism was drawn through each of three identical units of the Sawyer 7/6B filter. Between tests, each filter system was rinsed with 1L of rinse water. The filters were purged with air after each test to insure that most of the 100mL of test water had passed through. The test method is summarized in Figure 1.

Pressurizing device: Air pressure was regulated with a NorLab air regulator (model HPS270-125-590-4f, Norco Inc. Boise, ID). A 2 L Nalgene heavy-duty vacuum bottle (model DS2126, Nalge Nunc, Rochester, NY) was used as a pressure chamber. Conditioning water was forced through the filters at 40 psi. Test waters were forced through the filters at 20 psi. The gas regulator was used to maintain pressure in the chamber for tests. Water added to the chamber was forced through the filters by opening a clamp at the terminal end of the apparatus.

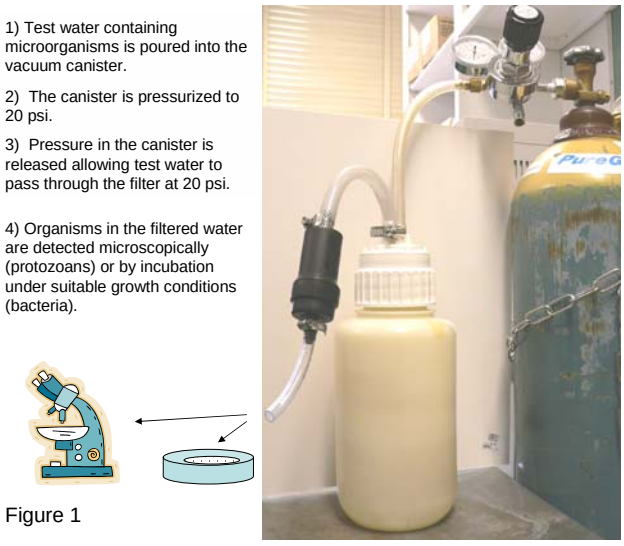

Figure 1

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. Filters were placed on TSA plates and incubated for 48 hours before counting. Three filters were evaluated for each test organism and the mean +/- one standard error reported.

Quality control: One unit was tested in triplicate. Triplicate testing allowed variation in filter system 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

Test results: Table 4 displays the numbers of each type of organism that were detected after passing the test solution through the indicated water filter system. The approximate size of the organism tested is also given as a reference. The efficacy results are summarized in Table 1. The Sawyer 7/6B filter reduced *Giardia*, *Cryptosporidium*, and *Klebsiella* to below detection levels.

Table 3. Results of microbiological tests

	Giardia (cells/100mL)	*Cryptosporidium* (cells/100mL)	*Klebsiella* (cells/100mL)
Sawyer 7/6B unit 1	0	0	0
Sawyer 7/6B unit 2	0	0	0
Sawyer 7/6B unit 3	0	0	0
Negative control	0	0	0
Plate/Filter Count	1.0E+06	1.0E+06	1.8E+00
Organism Size (µm)	10-12	5-7	2-4