Effective Removal Of *E. coli* And Coliform Bacteria By The TivaWater Biosand Filter

Introduction

The developers of the TivaWater Biosand filter (BSF) are providing a low cost filter for the removal of *E. coli* and coliform bacteria for household use in developing countries. This BSF was first tested for use for areas around Kampala, Uganda and was designed in Knoxville, Tennessee. In the Kampala, Uganda area, TivaWater provides sustainable employment for area residents as they sell, distribute and service these BSFs.

Historically, BSFs have provided an excellent point-of-use (POU) treatment technology which is efficient in removal of pathogens that cause significant intestinal disease in these developing areas, both rural and urban. BSFs provide an affordable and economic, low maintenance filter for tap, well/bore holes, and surface water sources.

The following is documentation of the methods and testing results that TivaWater has produced since the development of this filter. There are currently three completed experiments within the document regarding the efficiency and effectiveness of the filtration of the TivaWater filters. The first experimental review contains the long-term analysis of coliform and *E. coli* removal by the Colilert IDEXX reagent method. Designed to test the lifespan of the filters, this method proves the capability of TivaWater filters in removing coliforms and *E. coli* by both presence/absence and quantification of the targeted organisms. The second experiment is an attempt to analyze the interaction of copper metal within the TivaWater filters. By understanding the mobility of copper(II) sulfate through the filter, it is possible to discern the capabilities of the filter in removing copper, arsenic, lead, and/or other possible contaminant metals similar in ion size which often pollute source waters. The last review concerns an experiment which implements detection methods of microspheres passed through four different types of TivaWater filters. The experiment attempts to prove which sand types and masses of sand are more effective in removing particles, as well as gaging what sizes of particles those filters are capable of removing from source waters.




TivaWater Development Time-line:

2008

- Introduced to concrete Bisosand Filters (BSF) during field visit to Uganda.
- Extensive technical and field research confirmed that BSF is the most practical filter method for potential business model.

2009

- In collaboration with Neal Caldwell, designed modified BSF plastic version that included pre-filter, water storage and tap.
- Purchased IDEXX Colilert system and performed extensive testing for *E. coli* and coliform with functional model.
- Conducted multiple studies comparing flow rates and sand configuration.
- Flow rates and water quality compares favorably to Hydraid BSF product.
- Field market research focused on product implementation in Uganda.

2010

- Patent Application dated June 14, 2010 and plastic mold ordered.
- First filters manufactured at Dalen Products plant.
- Testing on 6 Prototypes at Dalen Lab with Colilert System initiated.
- Service team in Kampala, Uganda established and field sand production initiated.
- Delivered 50 filters to Uganda for home installation and additional water quality testing.

2011

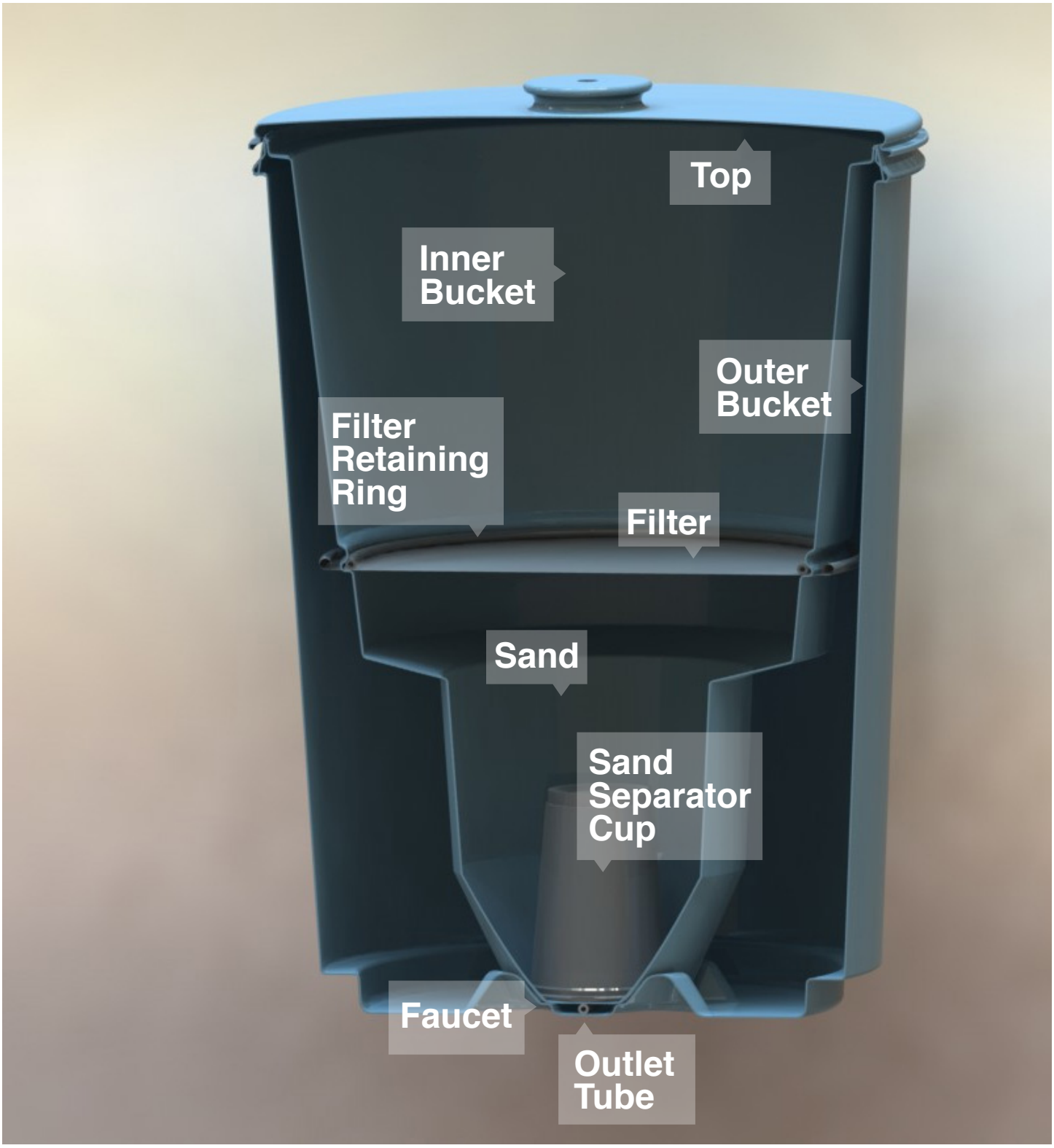
- 3,200 filters delivered by container to Kampala, Uganda.
- Installed filters at 5 test sites in Uganda, including source water from surface, tap and wells.
- Awarded water quality certification from Uganda National Bureau of Standards.
- Testing for copper reduction conducted at University of Tennessee (UT) Center for Environmental Biotechnology (see attached data reports).

2012

- 4,200 filters delivered by container to Kampala, Uganda.
- Field testing expanded to include many sites in Uganda.
- Experiments with various types and masses of sand conducted at TivaWater lab.
- Phase I of UT conducts tests for efficiency of biolayer using fluorescent dye, see attached data reports

2013

- Phase II of UT tests simulating particle sizes of Cryptosporidium, Vibrio cholera and Rotavirus for reduction in competing filter masses. (see attached data reports)


Top
Inner Bucket
Outer Bucket
Filter Retaining Ring
Filter
Sand
Sand Separator Cup
Faucet
Outlet Tube

History of Coliform and E. coli Removal

Method

Testing was performed by the Colilert IDEXX reagent method, using a patented Defined Substrate Technology (DST). This method of testing uses two nutrient indicators ONPG and MUG, which can be metabolized by the coliform enzymes. Coliform, using β-galactosidase to move from a colorless to a yellow product, and *E. coli*, using β-glucoronidase to metabolize MUG to create fluorescence. Non-coliform bacteria are suppressed by the Colilert's specifically formulated matrix. Three levels of control were utilized per the manufacturer's directions for new lots of reagent material. Documentation of QC frequency and performance can be submitted upon request. During the testing process all QC were found to be in-range and passed. Quantification of coliform and *E. coli* levels are interpreted by the Most Probable Number (MPN) technique which provides the density of target organisms per 100ml sample.

Data

Table 1. Percent and Log Removal of Coliform and *E. coli* through Filters 1-5 and Comparison to Hydraid Product

			Average % Removal		Average Log Removal	
Filter #	No. Dates	Flow Rate (ml/min)	Coliform	*E. coli*	Coliform	*E. coli*
1 (sand >145)	65	22	99.82	99.55	2.962	2.844
2 (sand >100)	14	35	99.93	99.55	3.272	2.809
3 (un-amended)	65	9	99.94	99.38	3.381	2.862
4 (sand >145)	14	41	99.62	99.45	2.732	2.539
5 (sand >145)	14	41	99.92	99.55	3.228	2.809
Hydraid	13	192	89.63	98.26	1.358	2.143

Discussion

Table 1 shows efficiency levels for removal of total coliforms and *E. coli* bacteria by both percent and logarithmic removal of a range of TivaWater filters. Logarithmic removal is the standard system for examining filtration performance in water testing. Filters 1 and 3 were designated long-term filters in order to determine their lifespan under constant conditions. The Hydraid filter used its own designated media mixture of rocks and sand and not the same sand as the TivaWater filters. All data points reading less than 1 MPN were converted to 0.5 since zeros do not compute in the logarithmic scale. The final averages are limited by the maximum *E. coli* measurement by Colilert in undiluted source water of a MPN greater than 2419.6/100ml. Thus, if *E. coli* concentration in the source water was greater than 2419.6, than the percent removal would be underestimated.

Copper(II) Sulfate Influence and Removal

Method

The copper experiment is a simulation of metal contamination by charging TivaWater filters with copper(II) sulfate ($CuSO_4$)from CuVer 5ml Copper Reagent Powder Pillows. The simulation provides insight on the mobility of heavy metals such as arsenic and lead because of their similarities with the Copper ion. A stock solution of $CuSO_4$ was first formulated at a concentration of 3.9g/100ml. Four standard concentrations of 4, 1, 0.4, and 0.1mg/L were equivalent to 3.32, 0.86, 0.33, and 0.08mg/L respectively. Each run consisted of adding known concentrations of the stock solution to each of the filters. Filter 1 received 15ml, 2 - 5ml, 3 - 3ml, and 4 a control receiving no amount of solution. Each input of stock solution was added to 5 L of water and the concentration of the mix before filtration recorded. After the filtration time period of 24 hours, the volume of output was recorded as well as the concentration of $CuSO_4$ remaining.

Data

Table 2. Percent Removal of Copper(II) Sulfate in triplicate experiments.

Filter	Average Concentration $CuSO_4$ Added (mg/L)	Average Concentration $CuSO_4$ Finished (mg/L)	Average % Removal	Average $CuSO_4$ Remaining (mg) in Sand
1	137.5	0.413	99.69	137.1
2	52.87	0.327	97.42	52.5
3	28.9	0.521	98.34	28.4
4	4.68	0.602	88.48	4.1

Discussion

The amount of copper(II) sulfate removed by the sand filters was determined in triplicate experiments. Copper(II) sulfate was added to tap water because the use of creek water led to interference in the copper reagent powder pillow measurements. Input water consisted of 5 Liters of water with varying amounts of $CuSO_4$. The average output of finished water was 4.78 L.. Water retention which remains in the sand layer of the filter accounts for the difference between input water and finished water. Filter 4 received no $CuSO_4$ indicating trace amounts of copper was present in the tap water. The amount of $CuSO_4$ remaining in the sand is shown in Table 2. It is unknown as to how much $CuSO_4$ can be retained in the sand before breakthrough.

Fluorescent Microsphere Removal

Method

Mixes of Constellation Microspheres from Life Technologies of assorted colors and sizes were added to various TivaWater filters in order to determine the range of removal of sized particles by filtration. The sizes of the microspheres ranged from 0.02 micrometers to 4 micrometers and the colors ranged from blue across the spectrum to red. The Accuri C6 Flow Cytometer was used to identify populations of beads within input and output samples as well as the sizes of the beads present in before and after filtration samples. A template of known bead size populations from a previous experiment was overlaid across a standard of the beads to predict the sizes of the beads. All filters involved with the experiment were unique in that two sets of sand types and sand masses were tested. The efficiency of coliform and *E. coli* removal by the colilert test on five different runs was used to verify the functionality of the biolayer in TivaWater filters.

Data

Table 3. Percent Removal of Fluorescent Microspheres among Varying Filter Sands and Masses

		% Colilert Removal	
Filter #	% Bead Removal	Coliform	*E. coli*
Silica 10 lb.	80.14	72.22	85.98
Silica 20 lb.	99.16	87.74	96.18
USAS 10 lb.	96.19	93.19	98.80
USAS 20 lb.	98.86	98.48	95.49

Discussion

In all experiments, three populations of microspheres of two distinct sizes contained 95% of the measured beads. Populations 1 and 2 were1 micrometer but differed in color with 1 and 2 being green. Population 3 was 2 micrometers and labeled a red color. The sensitivity of the cytometer was limited to quantifying particles equal or greater than 1 micrometer. The percent bead removal was 80% to 99% with a higher percentage removal in the 20 lb filters than the 10 lb sand filters which was consistent with the higher percentage removal of *E. coli* and coliforms.

Conclusion

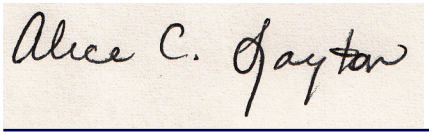
Coliform and *E. coli* removal experiments indicate that filters with 20 lbs of sand consistently removed over 99.62% of coliforms and 99.37% of *E. coli* for more than 2 years. The minimum log removal of coliforms was 2.73 and the maximum log removal of coliforms 3.38. Similarly, the minimum *E. coli* log removal was 2.53 and the maximum *E. coli* log removal was 2.86. These minimum log removal values exceeded the average Hydraid log removal values by log 1.17 and 0.72 for coliforms and *E. coli*, respectively.

Copper(II) sulfate removal experiments indicate that 20 lb sand filters removed between 97.42 and 99.69% of the added copper(II) sulfate. Copper was used as a metal surrogate for arsenic and lead because use of arsenic was prohibited due to its toxicity and disposal issues. It is currently unknown whether pollution of heavy metals is a problem in the city of Kampala, Uganda.

The fluorescent microsphere bead experiments indicate that beads in the size range of 1 to 2 micrometers are removed more efficiently in the 20 lb. sand filters compared to the 10 lb. sand filters and ranged from 80% in the 10 1b. silica sand filter to greater than 99% in the 20 lb silica sand filters. Particles that are 1 micrometer in size are smaller than most bacterial and protozoan pathogens such as *Vibrio cholerae* (2-3 microns) and *Cryptosporidium* (5-6 microns).

In summary, the combined colilert testing and microsphere testing suggests that the filters will effectively remove bacterial and protozoan pathogens. While the lifespan of the TivaWater filter is undetermined, the Colilert experiment concludes that efficacy is not diminished after two years of constant filtration. More testing will be needed to verify removal of metals.

The TivaWater Filter Experiments were performed under the supervision of Dr. Alice C. Layton, Research Associate Professor of the University of Tennessee's Center for Environmental Biotechnology Lab. Mr. Ben Curry, Graduate Research Assistance and Ph.D. student of the Univeristy of Tennessee, also provided assistance in analyzing experimental data. The experiments were carried out by Mr. Samuel Blount, Undergraduate Research Assistant at the University of Tennessee and TivaWater Researcher. Dr. Layton has reviewed the final report.



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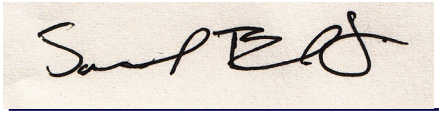

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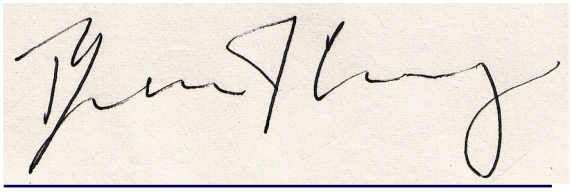

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