



March 9, 2010

The following contaminant filtration efficacy study results were compiled by the WaterGeeks Laboratories Inc. in Gainesville, Florida. The testing was done according to USEPA Guide Standards (1986) and Protocol for Testing Microbiological Water Purifiers. The method is based on the use of Standard Methods (APHA, 2005) and analytical procedures.

Challenge Bacterial Culture Preparation and Enumeration.

E. coli (ATCC 11775), *Salmonella typhimurium* (ATCC 19585) and Methicillin Resistant *Staphylococcus aureus* (MRSA; BAA-44) stock cultures were obtained from American Type Culture Collection and were maintained at -80°C. For Challenge experiments, overnight cultures from colony purified frozen stocks were grown in 10 ml of Tryptic Soy Broth (TSB, Beckton Dickinson, MD) at 36°C prior to the date of the experiments. At the day of challenge, the broth cultures were centrifuged at 3K x G for 5 minutes and suspended in 10 ml of phosphate buffered saline (PBS, Fisher scientific, PA). This was repeated and the pellet was suspended in 10 ml PBS. A 1/10 dilution of each of the suspended bacteria was then performed in PBS supplemented with 2% fetal bovine serum (FBS, Atlanta Biologicals, GA). The cultures were mixed together.

The number of viable bacterial species was enumerated as colony forming units (cfu) using spread plating onto Tryptic Soy Agar (TSA, Beckton Dickinson, MD) supplemented with ferric ammonium citrate and ferric thiosulfate. Plates were incubated at 36.5°C for 48 hours. Colonies of MRSA, *Salmonella*, and *E. coli* were visually identified and counted. The colony forming units (cfu) per milliliter were determined for each plated sample.

Challenge Viral Culture Preparation and Enumeration.

Poliovirus Lsc1 Chat strain (ATCC VR-1562) was propagated and enumerated as plaque forming units (pfu) using EPA ICR Methodology (EPA 600/R-95/178, 1998). For enumeration, aliquots containing poliovirus were inoculated on freshly prepared monolayers of Buffalo Green Monkey (BGM) kidney cells. Plaque assays were performed using 2X dMEM (MediaTech, USA) and 2X Bacto Agar containing 0.0001 % Neutral Red as per methodology outlined in EPA 600/R9-95/178. Cell flasks were incubated at 36.5°C and in 5% CO₂ for 72-96 hours.

Plaques on the respective flasks were counted following additional Neutral Red staining.

Stock cultures of Poliovirus were obtained from ATCC and propagated to a high titer on BGM cells. Viral stocks were maintained at -80°C. For Challenge experiments, virus stocks (approximate titer 1×10^8 pfu/ml) were thawed on the day of experiment. They were then used to inoculate the water for the filter challenge.

Challenge *Cryptosporidium* and *Giardia* Preparation and Enumeration.

Cryptosporidium parvum and *Giardia lamblia* were obtained from Waterborne Inc. (New Orleans, LA). The parasites were maintained in PBS buffer. The cysts and oocysts were stained and enumerated by the Easystain® (BTF Bio. Ltd. Australia) *Cryptosporidium* and *Giardia* direct immunofluorescent (IMF) assay (US EPA 1623). Water samples containing low numbers of parasites were concentrated by centrifuging for 15 minutes at 5K RPM and the visualized by the IMF assay.

Supplied disinfectant:

On December 23, 2009, six sport water bottles and replacement filters were received from The WaterGeeks Laboratories Inc. A picture of the bottle and filter as shown in Figure 1.



Figure 1

Microbial Contaminant Challenge Study:

Aliquots of the above mentioned and purified microorganisms were seeded separately into 2 liters of dechlorinated City of Gainesville tap water. The pH of the tap water was 8.1 and temperature was 19.4°C. The seeded water was stirred for 5 minutes and approximately 700 ml was placed into a sport water bottle; this volume is the water bottle capacity. A new supplied red filter was attached to the bottle top and the bottle was sealed. The outlet side of the water bottle was then connected via flexible tubing to a peristaltic pump (Cole- Palmer, USA). At the start of the experiment the pump was turned on and water was

evacuated from the water bottle via the tubing and collected in a sterile 1 Liter beaker. Following the passage of the liquid, the pump was turned off. The bottle was then refilled with the seeded water and the experiment was repeated and filter water was collected in a new sterile beaker. Next, aliquots of the influent (seeded) water and filter effluents were collected and assayed for the respective microorganisms as described above. Ten fold dilutions of the recovered microbial suspensions were performed in PBS.

All analysis for each sample was conducted in duplicates. The average of all values obtained from the first and second filter effluent was averaged and compared to the filter influent in order to determine filter removal efficacy. Table 1 below presents the results of the above-mentioned test.

Chemical Contaminant Challenge Study:

City of Gainesville tap water was passed through the filters to determine the chlorine removal efficacy of the supplied water bottle filters. A total of 20 liters of tap water was collected in plastic carboy jar. The pH of the tap water was 8.1, the average total chlorine concentration was 2.2 ppm, and the temperature was 19.4°C. A new supplied red filter was attached to the bottle top and the bottle was filled with 700 ml of the tap water (this volume is the water bottle capacity) and then sealed. The outlet side of the water bottle was then connected via flexible tubing to a peristaltic pump (Cole-Palmer, USA). The pump was turned on, water was evacuated from the water bottle via the tubing, and the filtered 700 ml filtered fraction was collected in a sterile 1 Liter beaker. This was labeled as fraction 1. Following the passage of the liquid, the pump was turned off. The bottle was then refilled with another 700 ml tap water and the experiment was repeated and the effluent was collected. This was labeled as fraction 2. This was repeated for a total of 15 fractions which equals to a total filtered volume of 10.5 liters. Following the collection of each fraction, the total chlorine was determined in fraction 1, 2, 3, 4, 5, 10, and 15 using the DPD test. Total chlorine concentration was measured by a calibrated hand held colorimeter (Series 942 Mini-Analyst, Orbeco-Hellige Inc., USA). All measurements were taken in duplicates and average data for the 10th fraction is reported in Table 1.

Additionally, one liter of City of Gainesville tap water was seeded with toluene, mercuric chloride and lead (II) acetate trihydrate and the pesticide simazine. The water was stirred for 30 minutes and was then 700 ml (water bottle capacity) was passed as described above through a sport water bottle with a brand new filter. The 700 ml effluent fraction was collected in a clean beaker and 100 ml of the filter influent and effluent water was collected in clean certified glass sampling bottles. The results are summarized in Table 1.

Table 1. The efficacy of microorganism filtration, tap water chlorine filtration, and chemical contaminant filtration of the supplied WaterGeeks sport bottle filters at a flow rate of 7 ml/sec

Microorganism Contaminant	% Reduction
MRSA ¹	>99%
Salmonella ¹	>99%
E. coli ¹	>99%
Cryptosporidium ²	>99%
Giardia ²	>99%
Polio Type 1 ³	46.4%
Chemical Contaminant	% Reduction
Chlorine ⁴	86.5%
Toluene (VOC) ⁵	84.7%
Mercury ⁵	59.2%
Lead ⁵	85.8%

¹Methycillin Resistant *Staphylococcus Aureus*, *E. coli*, and *Salmonella typhimurium* were enumerated by spread plating onto Tryptic Soy Agar (TSA, Beckton Dickinson, MD) supplanted with ferric ammonium citrate and sodium thiosulfate. Plates were incubated at 36.5°C for 48 hours.

²*Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts were detected by the direct immunofluorescent (IMF) assay (US EPA 1623).

³Poliovirus was enumerated by the plaque assay procedure using Buffalo Green Monkey (BGM) kidney cells as per EPA 600/R9-95/178. Cell flasks were incubated at 36.5°C and 5% CO₂ for 72-96 hours.

⁴City of Gainesville (FL) tap water was collected in a 20 liter container and was determined to contain a total chlorine concentration of 2.2 ppm. The sport bottle was filled with 700 ml (water bottle capacity) of the collected tap water. The water was flowed through the WaterGeeks sport bottle water filter and the effluent was collected in a clean beaker. This was repeated 15 times (10.5 liter total volume) and each effluent fraction was collected separately and labeled consecutively. The above-indicated 10th fraction (700 ml) were analyzed for total chlorine and was measured by a calibrated hand held colorimeter (Series 942 Mini-Analyst, Orbeco-Hellige Inc., USA).

⁵City of Gainesville (FL) tap water was seeded with the indicated chemical species and passed through the WaterGeeks sport bottle water filter.