



Biological Consulting Services
of North Florida, Inc.

February 11, 2011

Dennis B. Brown
Chief Operating Officer
Aquamira Technologies, Inc.
917 West 600 North
Logan, UT 84321
ph (360)-306-5586
dbrown@aquamira.com

RE: *Cryptosporidium* filtration efficacy testing of provided drinking water filters.

Dear Mr. Brown,

We have conducted the filtration efficacy testing of the provided filters. The testing was conducted according to a protocol developed to demonstrate the ability of water filters to remove *Cryptosporidium* oocysts from water. Testing was done using viable infectious *Cryptosporidium parvum* and also fluorescent 3 µm latex beads. Based on the observed results, the filters demonstrated >99.9% filtration efficacy when tested as described.

In the following pages, you will find a summary of the methodology used and the results of our analysis. Should you have any concerns please do not hesitate to contact me.

Best Regards,

George Lukasik, Ph.D.
Laboratory Director

- Page 1 of 7-

BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Building A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

Microbial and Latex Bead Challenge

Cryptosporidium parvum Oocysts (Iowa isolate (Harley Moon) were obtained from Waterborne Inc. (LA, USA). Oocysts were enumerated by immuno-fluorescence using the EasyStain kit (BTF Pty, Ltd., Australia) as per EPA method 1623. Oocysts were viewed and counted using UV fluorescence microscopy.

Three micron green fluorescent latex beads (Fluoresbrite® YG Microspheres 3.00µm, PolySciences Inc. PA, USA) were used as surrogates for *Cryptosporidium* oocysts. The beads were enumerated by fixing onto SingleSpott Slides (IDEXX, USA) and viewing by UV fluorescence microscopy.

Supplied Filters:

On February 04, 2011 four water filters were received from Aquamira Technologies Inc.; three filters were labeled 10114C and one labeled #21. The filter samples were assigned BCS numbers 1102020, 1102021, 1102021, and 1102022.

- Page 2 of 7-

BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Building A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

Filtration efficacy of provided water filters using Fluorescent 3.0 µm latex beads and *Cryptosporidium* oocysts.

Each of the provided water bottle filters were filled with 500 ml of Class I reagent water. Each filter was then connected to an air Pressure Control Station (Stratagene, USA) via Tygon tubing. The system was pressurized to 1.5 PSI and water was pushed through the filters. This was repeated one additional time in order to ensure the adequate rinsing and wetting of the filter material. Florescent latex beads (Fluoresbrite® YG Microspheres 3.00µm) were added to 2 L of Class I water and stirred thoroughly. Sufficient beads were added to obtain an initial minimum concentration of 10^5 particles/ml. Five hundred ml of the bead solution was then passed through each of filters 10114C and #21. The filter effluent was collected in a sterile bottle. The flow rate was measured to be approximately 5-6 ml / second.

For *cryptosporidium* challenge, oocysts were added to 2 liters of Class I water to achieve a minimum concentration of 10^3 oocysts/ml. The solution was stirred thoroughly. Five hundred milliliters of the solution was passed through each of two 10114C filters. The filter effluent was collected in a sterile bottle. The flow rate through the filter was measured at 6 ml / second.

- Page 3 of 7-

BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Building A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

Filter influent and effluents from the above studies were enumerated for the respective challenge species as indicated. The resulting data of the described study is summarized in the following Tables. The results presented pertain only to the samples analyzed and batch number indicated. They are not representative nor are they indicative of a process.

Positive and negative controls were performed as outlined in the Method and as per Good Laboratory Practices. All analysis was performed in accordance to NELAC accreditation standards that are equivalent to ISO 17025.

- Page 4 of 7-

BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Building A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

Table 1. Filtration efficacy of the Aquamira Technologies Inc. drinking water bottle filter ID 10114C.

Microorganism/Challenge	Average Filter Influent Concentration / milliliter	Average Filter Effluent Concentration / milliliter	Filtration Efficiency³
<i>Cryptosporidium parvum</i> Oocysts¹	2.5 x 10³	<1.0	>99.96%
		<1.0	
Fluoresbrite® YG Microspheres 3.00µm²	3.1 x 10⁴	5.0	99.98%

¹ Oocysts were enumerated by immuno-fluorescence using the EasyStain kit (BTF Pty, Ltd., Australia) as per EPA method 1623. Oocysts were viewed and counted using UV fluorescence microscopy.

² Three micron green fluorescent latex beads (Fluoresbrite® YG Microspheres 3.00µm, PolySciences Inc. PA, USA) were used as surrogates for cryptosporidium oocysts. The beads were enumerated by UV fluorescence microscopy.

³ Five hundred milliliters of the solution containing the indicated challenge was added to the provided water bottle. The filter was then placed into the bottle and the bottle was sealed. The solution was passed through filter by 1.5 PSI air pressure. The filter effluent was collected in a sterile bottle. Filter influent and effluent were enumerated for the respective challenge species and percent removals were determined

BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Building A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630

www.microbioservices.com

FL DOH Laboratory #E82924, EPA# FL01147

Table 2. Filtration efficacy of the Aquamira Technologies Inc. drinking water bottle filter ID #21.

Microorganism/Challenge	Average Filter Influent Concentration / milliliter	Average Filter Effluent Concentration / milliliter	Filtration Efficiency ²
Fluoresbrite® YG Microspheres 3.00µm ¹	3.1 x 10 ⁴	<5.0	>99.98%

¹ Three micron green fluorescent latex beads (Fluoresbrite® YG Microspheres 3.00µm, PolySciences Inc. PA, USA) were used as surrogates for cryptosporidium oocysts. The beads were enumerated by UV fluorescence microscopy.

² Five hundred milliliters of the solution containing the indicated challenge was added to the provided water bottle. The filter was then placed into the bottle and the bottle was sealed. The solution was passed through filter by 1.5 PSI air pressure. The filter effluent was collected in a sterile bottle. Filter influent and effluent were enumerated for the respective challenge species and percent removals were determined

Figure 1. Image of filtration set up used in the described study



- Page 7 of 7-

BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Building A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630

www.microbioservices.com

FL DOH Laboratory #E82924, EPA# FL01147