January 5, 2016

Icon Lifesaver Ltd.
Hall Chase, London Road
Marks Tey, Colchester
Co6 1EH, UK
+44(0)1206 580999

Re: Biological filtration efficacy testing of the LIFESAVER® bottles, model 6000UF; BCS ID 1510394, 1510395, and 1510396.

To whom it may concern,

We have conducted the requested biological filtration efficacy study on the LIFESAVER® bottles, model 6000UF. The experimental set up and challenge of the water filters was designed to evaluate the filters' microbiological contaminant removal efficacy. The contaminant species and water parameters selected were based on client's request and adaptation of NSF/ANSI P231 water purifier test protocol.

Following, you will find our report on the results of the challenge study. Should you have any questions, please do not hesitate to contact me.

Sincerely,

George Lukasik, Ph.D.
Laboratory Director

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Test Article(s):

On October 29th, 2015 four personal water filtration units: LIFESAVER® bottles, model 6000UF were received at BCS labs. The units were assigned BCS ID's 1510394, 1510395, 1510396, and 1510397, respectively. BCS ID 1510394, 1510395, and 1510396 were used for testing and the remaining bottle was kept in reserve.

Challenge Water:

The study was conducted with both General Test Water (GTW) Type 1 and GTW Type 3 water. The GTW1 water was prepared from dechlorinated municipal water and had a pH of 7.20. The Total Dissolved Solids (TDS) was measured at 420 ppm, turbidity at 2.0 NTU, alkalinity at 40 mg/L, and water hardness at 140 ppm.

The GTW 3 was also prepared from dechlorinated municipal water. The pH was 8.98, TDS was measured at 1610 ppm, turbidity at 52.7 NTU, temperature at 4.6°C, alkalinity at 62 mg/L, water hardness at 142 ppm, and the TOC at 16.65.

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Table 1. Critical equipment and supplies used in study for measurement and analysis

Equipment and Measurement Parameter	Manufacturer	BCS Lab ID
Balance	Sartorius Laboratory Instruments	BL-5
Tubidity meter	Hach Turbidity Meter	TM-01
Total hardness test kit	LaMotte	4911208
Alkalinity test kit	LaMotte	511220
Incubator	Sanyo MIR-253	I-2
pH	Denver Instrument Traceable UB-5	PH-1
Conductivity/TDS	Omega Traceable Conductivity Meter CDH-27	CM-1
Timer	VWR Traceable 61161-346	T-10
Centrifuge	Eppendorf C-5702	C-12
Temperature	VWR traceable IR Thermometer	IR-4
4 Liter standardized graduated cylinder	Nalgene	GC-4L-A
500 milliliter standardized graduated cylinder	Nalgene	GC-500ML-C
"PTFE" Printed 3-well slides	Electron Microscopy Sciences	526284
Epi-fluorescence microscope	Olympus BH-2	MIC-3
Total organic Carbon	Shimadzu TOC-5000 analyzer	Engineering Performance Solutions, Jacksonville Florida

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Study Date:

Study was initiated on October 30th, 2015 and completed on November 2nd, 2015.

Performed by: George Lukasik, Ph.D. and David Sekora
October 30th, 2015

Analyzed by: David Sekora
October 30th, 2015

Study Supervisor: George Lukasik, Ph.D.
October 30th, 2015 – November 2nd, 2015

Test System / Challenge Species:

Bacteria: *Raoultella terrigena* ATCC ® 33257 reference stock culture was obtained from Microbiologics® (MN, USA) and maintained as per supplier's recommendations. The lyophilized culture was hydrated and propagated on Tryptic Soy Agar (TSA, Neogen Inc., MI). Prior to the date of the study, a broth culture (Tryptic Soy Broth (TSB), Neogen Inc., MI) was started from a single colony. The culture was incubated at 36.5 ± 0.5 ºC for 16-18 hrs. On the day of the study, the culture was centrifuged once at 4,000 rpm for 10 minutes and suspended in laboratory grade reagent water. Throughout the study, bacteria were enumerated by spread plating onto TSA. Duplicate 0.1 and 1.0 ml aliquots of each of the collected filters' effluent and influent (1/1000 dilution) samples were plated and incubated at 36.5°C for 18-20 hours as per Standard Method 9215C

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(APHA, 2012). Colonies were enumerated and corresponding concentrations were determined.

Virus: Bacteriophage MS2 (ATCC 15597-B1; 30 nm RNA virus specific for *Escherichia coli* C3000 ATCC 15597) was used in this study as a surrogate for viral pathogens. The stock culture was obtained from the American Type Culture Collection (ATCC). The virus was cultivated to $>10^{11}$ plaque forming units (pfu)/mL in the laboratory prior to the challenge study. Bacteriophage stock was pre-filtered through a 0.1 µm membrane filter (Millipore, USA). Stock titer was determined by performing one-hundred fold dilutions of bacteriophage stock prepared in sterile Phosphate Buffered Water (3M Corporation, USA) and enumeration by the agar overlay plaque assay procedure using the respective host, as per EPA 1601. For the study, duplicate 0.1 and 1.0 ml aliquots of each of the collected filters' effluent and influent (1/1000 dilution) samples were plated and incubated at 36.5°C for 18-20 hours prior to enumeration of the plaques. Bacteriophage stock was maintained at 4°C until the initiation of the challenge study.

Parasite/Cysts surrogate: 3.0 micrometer Fluoro-Max Green Fluorescent Polymer Microspheres (Lot 43393) were obtained from Thermo Scientific (USA) and validated to the correct size using scanning electron microscopy (SEM, University of Florida, US).

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Two well slides (S100-2 SuperStick® – 15 mm, Waterborne Inc., US) were used for sample mounting and enumeration under fluorescent UV microscopy (FITC Filter). Briefly, enumeration was conducted using duplicate 0.5 ml aliquots of each of the collected samples and influent (1/1000 dilution). The samples were applied to the well slides and dried at 36.5º C. The number of microspheres on each slide were counted and concentrations were determined.

Challenge study Description / Methodology:

The provided water bottles were first primed and conditioned per manufacturer's instructions. Each bottle was flushed with an additional 5 bottle holding capacity volume of laboratory reagent water prior to challenge test. The activated carbon filters were not installed in the bottles. The silicone teat was removed from each bottle and an 18 cm silicone tube was attached to aid in sample collection and reduce the risk of potential contamination. For challenge water preparation, aliquots of the indicated species were added to 25 liters of carbon dechlorinated municipal water (GTW1). The water was homogenized and a sample was removed for enumeration. Following, each bottle was filled to capacity and sealed.

The bottle was pumped as per instructions to achieve flow through the filter. The effluent from the challenge was collected in three fractions of the filtration process into

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sterile 50 mL tubes (Corning®). Upon completion of the GTW1 study, the challenge was repeated using GTW3 in an identical manner. The filters' influent and effluent samples were assayed as per Standard Methods and Lab Standard Operating Procedures (SOP F-1). All analysis was conducted, at minimum, in duplicates for each sample volume and dilution analyzed. The respective percent reductions were determined based on the concentration obtained in the filter influent and effluent at each specific challenge point.

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Study data are summarized in the provided table(s). The results presented pertain only to the study conducted on the test articles/samples provided by the client (or client representative). The study was authorized and commissioned by the client. The results presented pertain only to the samples analyzed and identifier number(s) indicated. The data provided is strictly representative of the study conducted using the material/samples/articles provided by the client (or client's representative) and it's (their) condition at the time of test. The study and data obtained under the laboratory conditions may not be representative or indicative of a real-life process and/or application. Positive, negative, and neutralization controls were performed as outlined in the method and as per Good Laboratory Practices. All analyses were performed in accordance with laboratory practices and procedures set-forth by our NELAP/TNI accreditation standards (ISO 17025) unless otherwise noted. BCS makes no express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or product.

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Project: **LIFESAVER® 6000 UF Efficacy Test**
Sample(s): **BCS 1510394, 1510395, and 1510396 received October 29th, 2015**
Test: **Filtration Efficacy – General Test Water (GTW) Type 1 Water**
Test Parameter: ***Raoultella terrigena* (Bacteria), MS-2 Bacteriophage (virus), and 3.0 µM Fluorescent Microspheres as *Cryptosporidium parvum* oocyst surrogate**
Performed and Analyzed by: **David Sekora; October 30th, 2015**

Challenge Species	Filter influent average concentration	Average concentration of the challenge species in the filters' effluent		
		Filter A BCS 1510394	Filter B BCS 1510395	Filter C BCS 1510396
Bacteria: *Raoultella terrigena*[1]	3.3 x 10^5 cfu/mL	< 0.4 cfu/mL*	< 0.4 cfu/mL*	< 0.4 cfu/mL*
Virus: MS-2 Bacteriophage[2]	3.2 x 10^5 pfu/mL	0.9 pfu/mL	8.2 pfu/mL	10.5 pfu/mL
3.0 µM Fluorescent microspheres [3]	4.0 x 10^4 particle/mL	< 1.0 particle/mL*	< 1.0 particle/mL*	< 1.0 particle/mL*

[1] *Raoultella terrigena* (ATCC 33257) was obtained from ATCC and propagated on Tryptic Soy Agar (TSA, Becton Dickinson, USA). It is used to evaluate filters' bacterial removal efficacy. Bacteria was enumerated as colony forming units (cfu) following incubation at 36.5°C for 24 hours as per Standard method 9215C (APHA, 2012).
[2]Bacteriophage MS-2 (ATCC 15597-B1) was used as a model for human viruses. It is of similar shape and size to human enteroviruses and thus is used to determine filter's viral capture efficacy. It was enumerated using *E. coli* C3000 (ATCC 15597) as a host using the single layer plaque assay agar procedure as per EPA 1601.
[3]Three micron green fluorescent latex microspheres (Fluoro-Max™ Green Fluorescent Microspheres 3.00µm, Thermo Scientific CA, USA) were used as surrogates for *Cryptosporidium* oocysts. It is used to determine filter's parasitic removal efficacy. The microspheres were enumerated by fixing onto 3-Well PTFE Slides (Electron Microscopy Sciences, USA) and viewing by UV fluorescence microscopy.
* No species were detected in the filter effluent for the total volume analyzed. Filter effluent samples were analyzed in duplicates at the minimum following collection.

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FL DOH #E82924, ISO/IEC 17025:2005 L2422 (L-A-B), EPA# FL01147

Project: **LIFESAVER® 6000 UF Efficacy Test**
Sample(s): **BCS 1510394, 1510395, and 1510396 received October 29th, 2015**
Test: **Filtration Efficacy – General Test Water (GTW) Type 1 Water**
Test Parameter: **Test Parameter: *Raoultella terrigena* (Bacteria), MS-2 Bacteriophage (virus), and 3.0 µM Fluorescent Microspheres as *Cryptosporidium parvum* oocyst surrogate**
Performed and Analyzed by: **David Sekora; October 30th, 2015**

Challenge Species	Filter influent average concentration	Average percent removal** of the challenge species by:		
		Filter A BCS 1510394	Filter B BCS 1510395	Filter C BCS 1510396
Bacteria: *Raoultella terrigena*	3.3 x 10^5 cfu/mL	> 99.9999%*	> 99.9999%*	> 99.9999%*
Virus: MS-2 Bacteriophage	3.2 x 10^5 pfu/mL	99.9997%	99.997%	99.997%
3.0 µM Fluorescent microspheres	4.0 x 10^4 particle/mL	> 99.998%*	> 99.998%*	> 99.998%*

* No species were detected in the filter effluent for the total volume analyzed. Filter effluent samples were analyzed in duplicates at the minimum following collection.
** Purifier NSF/ANSI standard microbial removal claims are 99.9999% or greater for bacteria, 99.99% or greater for virus, and 99.9% or greater for parasite cysts.

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Project: **LIFESAVER® 6000 UF Efficacy Test**
Sample(s): **BCS 1510394, 1510395, and 1510396 received October 29th, 2015**
Test: **Filtration Efficacy – General Test Water (GTW) Type 3 Water**
Test Parameter: ***Raoultella terrigena* (Bacteria), MS-2 Bacteriophage (virus), and 3.0 µM Fluorescent Microspheres as *Cryptosporidium parvum* oocyst surrogate**
Performed and Analyzed by: **David Sekora; October 30th, 2015**

Challenge Species	Filter influent average concentration	Average concentration of the challenge species in the filters' effluent		
		Filter A BCS 1510394	Filter B BCS 1510395	Filter C BCS 1510396
Bacteria: *Raoultella terrigena*[1]	2.4 x 10^5 cfu/mL	< 0.4 cfu/mL*	< 0.4 cfu/mL*	< 0.4 cfu/mL*
Virus: MS-2 Bacteriophage[2]	7.1 x 10^4 pfu/mL	5.0 pfu/mL	7.3 pfu/mL	3.6 pfu/mL
3.0 µM Fluorescent microspheres [3]	4.0 x 10^4 particle/mL	< 1.0 particle/mL*	< 1.0 particle/mL*	< 1.0 particle/mL*

[1] *Raoultella terrigena* (ATCC 33257) was obtained from ATCC and propagated on Tryptic Soy Agar (TSA, Becton Dickinson, USA). It is used to evaluate filters' bacterial removal efficacy. Bacteria was enumerated as colony forming units (cfu) following incubation at 36.5°C for 24 hours as per Standard method 9215C (APHA, 2012).
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Challenge Species	Filter influent average concentration	Average percent removal** of the challenge species by		
		Filter A BCS 1510394	Filter B BCS 1510395	Filter C BCS 1510396
Bacteria: *Raoultella terrigena*	2.4 x 10^5 cfu/mL	> 99.9999%*	> 99.9999%*	> 99.9999%*
Virus: MS-2 Bacteriophage	7.1 x 10^4 pfu/mL	99.993%	99.99%	99.995%
3.0 µM Fluorescent microspheres	4.0 x 10^4 particle/mL	> 99.998%*	> 99.998%*	> 99.998%*

* No species were detected in the filter effluent for the total volume analyzed. Filter effluent samples were analyzed in duplicates at the minimum following collection.
** Purifier NSF/ANSI standard microbial removal claims are 99.9999% or greater for bacteria, 99.99% or greater for virus, and 99.9% or greater for parasite cysts.

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