



inc. BIOLOGICAL CONSULTING SERVICES
OF NORTH FLORIDA, INC.

April 28, 2016

Icon Lifesaver Ltd.
Hall Chase, London Road
Marks Tey, Colchester
CO6 1EH, UK
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RE: Biological filtration efficacy test study of the provided Icon Lifesaver® C2 filter unit equipped with filter cartridges C2P_0001 and C2P_0002; BCS IDs 1604207 and 1604208 respectively.

To whom it may concern,

We have conducted the requested filtration efficacy study on the filter unit received on April 21st, 2016. 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 guidance from NSF/ANSI P231 water purifier test protocol. The units' challenge parameters were selected to simulate operation of the filter unit by personnel.

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

Best Regards,

George Lukasik, Ph.D.
Laboratory Director

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FL DOH #E82924, ISO/IEC 17025:2005 L2422 (L-A-B), EPA# FLO1147

FILE: ICON LIFESAVER C2 FILTER TESTING BCS 1604207-208 04.28.2016

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Test Article(s):

On April 21st, 2016, a Lifesaver ICON C2 filter unit was received at BCS Laboratories. Each of the unit's 2 filter cartridges, C2P_0001 and C2P_0002, were assigned a BCS identifier numbers: 1604207 and 1604208 respectfully.

Study Date:

The study was initiated on April 25th, 2016 and completed on April 26th, 2016.

Performed by: David Sekora, M.S.
Analyzed by: David Sekora, M.S.
Study Supervisor: George Lukasik, Ph.D.

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Physical parameter measuring devices and critical equipment utilized:

Equipment and Measurement Parameter	Manufacturer	BCS Lab ID
Balance	OHAUS Scout SPX Balance	BL-7
Epi-fluorescence microscope	Olympus BH-2	MIC-3
Digital Colorimeter; DPD-06	Hach DR 890	COL-03/DPD-06
Turbidity meter	Hach Turbidity Meter	TM-01
Alkalinity test kit	Hach Alkalinity Test Kit	ALK-1
Total hardness test kit	LaMotte	H-1
Incubator	Sanyo MIR-253	I-2
pH	Denver Instruments pH Meter	PH-1
Conductivity/TDS	VWR Traceable Conductivity Meter 89094-958	CM-05 NIST
Timer	VWR Traceable Lab Top Timer 62344-910	T-07 NIST
Centrifuge	Eppendorf C-5702	C-12
Temperature	Micro IR Thermometer NIST	IR-5 NIST
4-Liter standardized graduated cylinder	Nalgene	GC-4L-B
Pressure regulator	Bellofram Pressure regulator	PR-02
Pressure Transducer NIST 2 BAR	Sper Scientific PS100-2BAR	PM 28 NIST
NIST digital pressure meter 0-100 PSI	Omega DPG 1001B-100G	PD-03

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Test Matrix; General Test Water 1:

General Test Water 1 (GTW1) was made up of the dechlorinated municipal water. Municipal water was dechlorinated by addition of Sodium thiosulfate (GFS Chemicals, USA). Total dissolved solids, turbidity, and pH were measured and adjusted (if necessary) to NSF P231 guidelines. The pH of the water was 7.47, turbidity was 0.42 NTU, total dissolved solids were measured at 210 ppm, and Total Organic Carbon (TOC) was <1.0 ppm. Temperature was maintained between 22°C and 23°C. TOC analysis was conducted by TestAmerica Laboratories (Tampa, FL).

Test Matrix; Challenge Test Water 3:

Challenge Test Water 3 (CTW3) was prepared from dechlorinated municipal water and adjusted to NSF P231 for total dissolved solids, turbidity, and pH. The pH of the test water was 8.82, turbidity was 42.3 NTU, total dissolved solids were measured at 1550 ppm, and Total Organic Carbon (TOC) was >10 ppm. Temperature was maintained between 4.1°C and 4.3°C. TOC analysis was conducted by Test America Laboratories (Tampa, FL).

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,

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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 °C for 15-18 hrs. On the day of the study, the culture was washed by repeat centrifugation and suspended in Phosphate buffered saline (PBS). Bacteria concentration was determined by performing serial 100 fold dilutions of culture in sterile PBW and enumeration as per laboratory Standard Operating Procedure (SOP). Bacteria in the study were enumerated as per Standard Method 9215C (APHA, 2012). Briefly, duplicate 0.1 and 1.0 mL samples of the filters' effluent and influent (10^{-3} dilution) were analyzed. The plates were incubated at 36.5 ± 0.5 °C for 18-20 hours prior to colony enumeration.

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 virus was cultivated to $>10^{10}$ plaque forming units (pfu)/mL in the laboratory prior to the challenge study. Bacteriophage stock was pre-filtered through a 0.22 µm membrane filter (Millipore, USA). Titer was determined by performing serial 100 fold dilutions in sterile PBW and enumeration as per laboratory SOP. Bacteriophage stock was maintained at 4°C until the initiation of the challenge study. Coliphage assay (BCS SOP V-10) was used for the enumeration of MS2. Briefly; duplicate 0.1 and 1.0 mL samples of each of the filters' effluent and influent (1/1000 dilution) samples were analyzed by an agar overlay plaque assay using *E. coli* ATCC 15597 as the host. Plates were incubated

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at 36.5±5°C for 18-20 hours prior to plaque enumeration.

Parasite 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). Well slides ('PTFE' Printed slides – 14 mm, Electron Microscopy Sciences., US) were used for sample mounting and enumeration under fluorescent UV microscopy (FITC Filter) as per laboratory SOP. Enumeration was conducted as per EPA1623.1 methodology. All collected samples were analyzed in duplicates at the minimum.

Challenge study Description / Methodology:

The unit was thoroughly cleaned and the filters were installed. The filters were conditioned, and primed following the manufacture's guidelines before initiation of study. During conditioning, approximately 750 liters were passed through each C2 Filter. For the study, the unit was emptied and 500L of General Test Water type 1 (dechlorinated municipal water) were added to the tank. Aliquots of the indicated challenge species were added to the water and homogenized. A sample of the challenge water was removed and stabilized. Following, the unit was sealed and the tank was charged with compressed air to an internal pressure of 4.5 PSI. The pressure was continuously provided by a compressed air supply connected to the C2 Tank unit. The water dispensing valves were rapidly opened and the filtered water was allowed to flow. The

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initial water flow was measured at 15 Liters/min. Duplicate 50 mL samples from each the filters' effluents were collected following the passage of 50, 100, 200, and 400 liters through the unit. Pressure and time elapsed for the volumes collected were recorded using a validated measuring device. Upon completion of the GTW1 study, a second sample was taken from the remaining challenge water.

The unit was emptied of any remaining water and 500 liters of Challenge Test Water Type 3 were added to the tank. Challenge species were homogenized into the water, a sample was removed, and the tank was sealed and charged to 4.5 PSI. The study was repeated as described and samples of the filters' effluents were collected following the passage of 50, 100, 200, and 400 liters through the unit. The water flow through the filter systems was 13.6 Liters/min. Pressure and time elapsed for the volumes collected were recorded using a validated measuring device. Upon completion of the CTW3 study, a second sample was taken from the remaining challenge water.

All collected filters' influent and effluent samples were assayed as per Standard Methods and Lab Standard Operating Procedures. All collected samples were analyzed, at a minimum, in duplicate for each sample volume and dilution. The respective percent reductions were determined based on the average concentration obtained from the duplicate samples of filters' influent and effluent samples 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/units provided by the client (or client representative). The study was authorized and commissioned by the client. The analytical results pertain only to the samples analyzed relating to the respective 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 ISO 17025-2005 and NELAP/TNI accreditation standards 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: Icon LIFESAVER® C2 Efficacy Test
Sample(s): BCS 1604207 and 1604208 received April 21st, 2016
Test: Filtration Efficacy – General Test Water Type 1 (GTW1)
Test Parameter: *Raoultella terrigena* (Bacteria)
Test Date: April 25th, 2016

Challenge Species	Volume (Liters) passed through the unit	Filter influent average concentration	Average bacteria concentration (cfu/mL) in the filters' effluents at the indicated volume**	
			C2P_0001 BCS 1604207	C2P_0002 BCS 1604208
Bacteria: <i>Raoultella terrigena</i> ¹	50	4.3 x 10 ⁵ cfu/mL	< 0.25 cfu/mL* >99.99994%***	< 0.25 cfu/mL* >99.99994%***
	100		< 0.25 cfu/mL* >99.99994%***	< 0.25 cfu/mL* >99.99994%***
	200		< 0.25 cfu/mL* >99.99994%***	< 0.25 cfu/mL* >99.99994%***
	400		< 0.25 cfu/mL* >99.99994%***	< 0.25 cfu/mL* >99.99994%***

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).

* 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.

** Provided filters were subjected to the challenge study as described in the methods section. Collected samples of filter units' influent and effluent were assayed for the respective challenge species as per Standard Methods and Lab Standard Operating Procedures. The respective percent reductions were determined based on the species' concentration obtained in the filter influent and effluent samples.

*** 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: Icon LIFESAVER® C2 Efficacy Test
Sample(s): BCS 1604207 and 1604208 received April 21st, 2016
Test: Filtration Efficacy – General Test Water Type 1 (GTW1)
Test Parameter: MS-2 Bacteriophage (virus)
Test Date: April 25th, 2016

Challenge Species	Volume (Liters) passed through the unit	Filter influent average concentration	Average virus concentration (pfu/mL) in the filters' effluents at the indicated volume**	
			C2P_0001 BCS 1604207	C2P_0002 BCS 1604208
Virus: MS-2 Bacteriophage ²	50	3.3 x 10 ⁵ cfu/mL	3.4 pfu/mL 99.999%***	1.8 pfu/mL 99.9995%***
	100		2.5 pfu/mL 99.9992%***	3.2 pfu/mL 99.999%***
	200		3.2 pfu/mL 99.999%***	4.5 pfu/mL 99.999%***
	400		3.9 pfu/mL 99.999%***	4.5 pfu/mL 99.999%***

²Bacteriophage 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.

** Provided filters were subjected to the challenge study as described in the methods section. Collected samples of filter units' influent and effluent were assayed for the respective challenge species as per Standard Methods and Lab Standard Operating Procedures. The respective percent reductions were determined based on the species' concentration obtained in the filter influent and effluent samples.

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Project: Icon LIFESAVER® C2 Efficacy Test
Sample(s): BCS 1604207 and 1604208 received April 21st, 2016
Test: Filtration Efficacy – General Test Water Type 1 (GTW1)
Test Parameter: 3.0 µM Fluorescent Microspheres as *Cryptosporidium parvum* oocyst surrogate
Test Date: April 25th, 2016

Challenge Species	Volume (Liters) passed through the unit	Filter influent average concentration	Average concentration (Microspheres/mL) in the filters' effluents at the indicated volume**	
			C2P_0001 BCS 1604207	C2P_0002 BCS 1604208
3.0 µM Fluorescent microspheres ³	50	4.5 x 10 ⁴ particle/mL	< 1.0 particle/mL* >99.999%***	< 1.0 particle/mL* >99.999%***
	100		< 1.0 particle/mL* >99.999%***	< 1.0 particle/mL* >99.999%***
	200		< 1.0 particle/mL* >99.999%***	< 1.0 particle/mL* >99.999%***
	400		< 1.0 particle/mL* >99.999%***	< 1.0 particle/mL* >99.999%***

³Three 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.

** Provided filters were subjected to the challenge study as described in the methods section. Collected samples of filter units' influent and effluent were assayed for the respective challenge species as per Standard Methods and Lab Standard Operating Procedures. The respective percent reductions were determined based on the species' concentration obtained in the filter influent and effluent samples.

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Project: Icon LIFESAVER® C2 Efficacy Test
Sample(s): BCS 1604207 and 1604208 received April 21st, 2016
Test: Filtration Efficacy – Challenge Test Water Type 3 (CTW3)
Test Parameter: *Raoultella terrigena* (Bacteria)
Test Date: April 25th, 2016

Challenge Species	Volume (Liters) passed through the unit	Filter influent average concentration	Average bacteria concentration (cfu/mL) in the filters' effluents at the indicated volume**	
			C2P_0001 BCS 1604207	C2P_0002 BCS 1604208
Bacteria: <i>Raoultella terrigena</i> ¹	50	3.5 x 10 ⁵ cfu/mL	< 0.25 cfu/mL* >99.99993%***	< 0.25 cfu/mL* >99.99993%***
	100		< 0.25 cfu/mL* >99.99993%***	< 0.25 cfu/mL* >99.99993%***
	200		< 0.25 cfu/mL* >99.99993%***	< 0.25 cfu/mL* >99.99993%***
	400		< 0.25 cfu/mL* >99.99993%***	< 0.25 cfu/mL* >99.99993%***

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).

* 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.

** Provided filters were subjected to the challenge study as described in the methods section. Collected samples of filter units' influent and effluent were assayed for the respective challenge species as per Standard Methods and Lab Standard Operating Procedures. The respective percent reductions were determined based on the species' concentration obtained in the filter influent and effluent samples.

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Project: Icon LIFESAVER® C2 Efficacy Test
Sample(s): BCS 1604207 and 1604208 received April 21st, 2016
Test: Filtration Efficacy – Challenge Test Water Type 3 (CTW3)
Test Parameter: MS-2 Bacteriophage (virus)
Test Date: April 25th, 2016

Challenge Species	Volume (Liters) passed through the unit	Filter influent average concentration	Average virus concentration (pfu/mL) in the filters' effluents at the indicated volume**	
			C2P_0001 BCS 1604207	C2P_0002 BCS 1604208
Virus: MS-2 Bacteriophage ²	50	3.3 x 10 ⁵ cfu/mL	14.7 pfu/mL 99.996%***	15.7 pfu/mL 99.995%***
	100		14.7 pfu/mL 99.996%***	14.1 pfu/mL 99.996%***
	200		11.8 pfu/mL 99.996%***	16.4 pfu/mL 99.995%***
	400		15.9 pfu/mL 99.996%***	17.7 pfu/mL 99.994%***

²Bacteriophage 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.

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Project: Icon LIFESAVER® C2 Efficacy Test
Sample(s): BCS 1604207 and 1604208 received April 21st, 2016
Test: Filtration Efficacy – Challenge Test WaterType 3 (CTW3)
Test Parameter: 3.0 µM Fluorescent Microspheres as *Cryptosporidium parvum* oocyst surrogate
Test Date: April 25th, 2016

Challenge Species	Volume (Liters) passed through the unit	Filter influent average concentration	Average concentration (Microspheres/mL) in the filters' effluents at the indicated volume**	
			C2P_0001 BCS 1604207	C2P_0002 BCS 1604208
3.0 µM Fluorescent microspheres ³	50	4.5 x 10 ⁴ particle/mL	< 1.0 particle/mL* >99.999%***	< 1.0 particle/mL* >99.999%***
	100		< 1.0 particle/mL* >99.999%***	< 1.0 particle/mL* >99.999%***
	200		< 1.0 particle/mL* >99.999%***	< 1.0 particle/mL* >99.999%***
	400		< 1.0 particle/mL* >99.999%***	< 1.0 particle/mL* >99.999%***

³Three 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.

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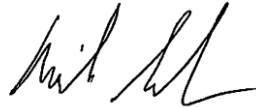
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I hereby certify to the accuracy, quality, and data integrity of the reported study. I also certify that the study was appropriately executed and is fully defensible. All physical measurements and their source have been documented. Measurements were obtained using approved protocols and NIST traceable and/or validated instruments. Analysis execution and results were fully documented. Analytical methods used to produce the study's raw data are within the laboratory's ISO 17025 accreditation. The results and conclusions of the study accurately reflect the real raw data obtained in the study.

Signature of Sr. Analyst



David Sekora, M.S.

Date: 04/28/2016



George Lukasik, Ph.D.

Date: 04/28/2016

I certify that I have personally examined and am familiar with the information submitted herein. Based on my inquiry of the individuals immediately responsible for obtaining the information, I certify the submitted information to be true, accurate, and complete. The data provided is solely representative of the analysis conducted on the material/samples/articles provided by the client (or client's representative) it's (their) condition at the time of study. They may not be representative of a process or product. The sample(s) were analyzed in accordance with the method described for each analyte. Due to the inherent limitation(s) of analytical method(s), BCS Laboratories offers no express or implied warranties concerning the quality, safety, and/or purity of any sample, batch, source, or the process they are derived from. The species analysis and corresponding presented results in this report meet the requirements of The NELAC Institute (TNI), ISO 17025, and The State of Florida Department of Public Health's Laboratory Certification Program, as applicable unless otherwise noted.

Signature of Study Director



George Lukasik, Ph.D.

Date: 02/28/2016

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