February 09, 2016

Icon Lifesaver Ltd.
Hall Chase, London Road
Marks Tey, Colchester
CO6 1EH, UK
+44(0)1206 580999

RE: Biological filtration efficacy test study of the provided Icon Lifesaver® jerrycan filter units JCP10k_5-7; BCS IDs 1601087, 1601088, and 11601089.

To whom it may concern,

We have conducted the requested filtration efficacy study on the filter units received on January 25th, 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 adaptation of NSF/ANSI P231 water purifier test protocol. The units' specific use conditions were selected to simulate actual operation of the units 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# FL01147

**Test Article(s):**

On January 25th, 2016, 4 jerrycan filter units were received from Icon Lifesaver. The four jerrycan filter units had the designation JCP10k_5-8, the units were issued BCS identifiers 1601087, 1601088, 11601089, and 1601090 respectively. BCS IDs 1601087, 1601088, and 11601089 were selected for the study and 1601090 was kept in reserve.

**Study Date:**

The study was initiated on January 28th, 2016 and completed on February 09, 2016.

**Performed by:** David Sekora, M.S. and George Lukasik Ph.D.

**Analyzed by:** David Sekora, M.S. and George Lukasik Ph.D.

**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 | Sartorius Laboratory Instruments | BL-4 |
| Epi-fluorescence microscope | Olympus BH-2 | MIC-3 |
| Digital Colorimeter; DPD-02 | Hach DR 890 | COL-03/DPD-02 |
| Tubidity meter | Hach Turbidity Meter | TM-01 |
| Alkalinity test kit | LaMotte | 511220 |
| Total hardness test kit | LaMotte | 4911208 |
| Incubator | Sanyo MIR-253 | I-2 |
| pH | Denver Instrument Traceable UB-5 | PH-4 |
| Conductivity/TDS | VWR Traceable Conductivity Meter 89094-958 | CM-05 |
| Timer | VWR Traceable 61161-346 | T-10 |
| Centrifuge | Eppendorf C-5702 | C-12 |
| Temperature | VWR NIST traceable IR Thermometer 33501-413 | IR-5 |
| 4-Liter standardized graduated cylinder | Nalgene | GC-4L-A |
| Pressure regulator | Ingersoll Rand PR4021-200 | (PR-1) |
| Pressure Vessel; 55 liter | Alloy Products 55L 67349008 | PV-06 |
| Pressure Transducer NIST 2 BAR | Sper Scientific PS100-5BAR | PM 29 |
| NIST digital pressure meter 0-100 PSI | Omega DPG 1001B-100G | PM 27 |





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**Test Matrix; General Test Water 1:**

Carbon block dechlorinated municipal water was used throughout the study; General Test Water 1 (GTW1) was made up of the dechlorinated municipal water. Total dissolved solids, turbidity, and pH were adjusted (if necessary) to NSF P231 guidelines. The pH of the water was 7.01-7.05, turbidity was 0.51 NTU, total dissolved solids were measured at 187 ppm, and Total Organic Carbon (TOC) was <1.0 ppm. Temperature was maintained between 20°C and 21°C. TOC analysis was conducted by XENCO 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 guidance for total dissolved solids, turbidity, and pH. The pH of the test water was 8.95, turbidity was 30.5 NTU, total dissolved solids were measured at 1370 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 XENCO Laboratories (Tampa, FL).

**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 15-18 hrs. On the day of the study, the culture was centrifuged at 3K x G for 10 minutes and suspended in laboratory grade reagent water. Bacteria were enumerated by spread plating onto TSA. Filter influent samples were diluted 1,000 fold in Phosphate Buffered Water (PBW, 3M, USA). Standard Method 9215C (APHA, 2012) was used for the enumeration of *Raoultella terrigena.* Briefly, duplicate 0.1 and 1.0 mL samples of the filters' effluent and influent ($10^{-3}$ dilution) were plated. 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





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challenge study. Bacteriophage stock was pre-filtered through a 0.22 µm membrane filter (Millipore, USA). Titer was determined by performing 1,000 fold dilutions of bacteriophage stock in sterile PBW and enumeration as per laboratory standard methodology (SOP V-10). Bacteriophage stock was maintained at 4°C until the initiation of the challenge study. For the enumeration of MS-2 in collected samples, duplicate 0.1 and 1.0 mL aliquots of each of the collected samples were analyzed by an agar overlay plaque assay using *E. coli* ATCC 15597 as the host. Plates were incubated 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). Three well slides (PTFE Slides, Electron Microscopy Sciences, USA) were used for sample mounting and enumeration under fluorescent UV microscopy (FITC Filter). Enumeration was conducted as per EPA1623.1 methodology. All collected samples were analyzed in duplicates at the minimum.





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**Challenge study Description / Methodology:**

The provided filters were fitted with appropriate connections to the source of GTW1. Each unit was first conditioned with GTW1 as per manufacturer's instructions. Approximately 80 liters were passed through each filter prior to challenge. The line pressure was adjusted to 4.5 PSI throughout the challenge study. The pressure was chosen as it was equivalent to pumping a jerrycan filter unit 20-22 times with the valve closed. The flow rate varied between the units and was measured at 1040-1080 mL/ min at 4.5 PSI. For challenge water preparation, aliquots of the challenge species were added to 70 liters of GTW1. The water was homogenized and an influent sample was removed and preserved for enumeration. Following the initial conditioning of the filters, each jerrycan unit was emptied and filled with challenge water. Each of the units were pressurized and 10 filter bed volumes were allowed to pass through each of the filters prior to collecting two consecutive 50 mL samples of the effluent. 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.





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The filter challenge was then repeated using CTW3 in an identical manner. 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® jerrycan Efficacy Test
**Sample(s):** BCS 1601087, 1601088, and 1601089 received January 25th, 2016
**Test:** Filtration Efficacy – General Test Water (GTW) Type 1
**Test Parameter:** *Raoultella terrigena* (Bacteria), MS-2 Bacteriophage (virus), and 3.0 µM Fluorescent Microspheres as *Cryptosporidium parvum o*ocyst surrogate
**Test Date:** January 28th, 2016

| Challenge Species | Filter influent average concentration | Average concentration of the challenge species in the filters' effluent | | |
|---|---|---|---|---|
| | | JCP10k_5 BCS 1601087 | JCP10k_6 BCS 1601088 | JCP10k_7 BCS 1601089 |
| Bacteria: *Raoultella terrigena*[1] | 8.3 x $10^5$ cfu/mL | < 0.45 cfu/mL* | < 0.45 cfu/mL* | < 0.45 cfu/mL* |
| Virus: MS-2 Bacteriophage[2] | 6.1 x $10^5$ pfu/mL | 2.3 pfu/mL | 4.3 pfu/mL | 0.93 pfu/mL |
| 3.0 µM Fluorescent microspheres [3] | 4.3 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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**Test:** Filtration Efficacy – General Test Water (GTW) Type 1
**Test Parameter:** Test Parameter: *Raoultella terrigena* (Bacteria), MS-2 Bacteriophage (virus), and 3.0 µM Fluorescent Microspheres as *Cryptosporidium parvum* oocyst surrogate
**Test Date:** January 28th, 2016

| Challenge Species | Filter influent average concentration | Average percent removal** of the challenge species by: | | |
|---|---|---|---|---|
| | | JCP10k_5 BCS 1601087 | JCP10k_6 BCS 1601088 | JCP10k_7 BCS 1601089 |
| Bacteria: *Raoultella terrigena* | 8.3 x $10^5$ cfu/mL | > 99.9999%* | > 99.9999%* | > 99.9999%* |
| Virus: MS-2 Bacteriophage | 6.1 x $10^5$ pfu/mL | 99.9996% | 99.9993% | 99.9998% |
| 3.0 µM Fluorescent microspheres | 4.3 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:** Icon LIFESAVER® jerrycan Efficacy Test
**Sample(s):** BCS 1601087, 1601088, and 1601089 received January 25th, 2016
**Test:** Filtration Efficacy – Challenge Test Water (CTW) Type 3
**Test Parameter:** *Raoultella terrigena* (Bacteria), MS-2 Bacteriophage (virus), and 3.0 µM Fluorescent Microspheres as *Cryptosporidium parvum o*ocyst surrogate
**Test Date:** January 28th, 2016

| Challenge Species | Filter influent average concentration | Average concentration of the challenge species in the filters' effluent | | |
|---|---|---|---|---|
| | | JCP10k_5 BCS 1601087 | JCP10k_6 BCS 1601088 | JCP10k_7 BCS 1601089 |
| Bacteria: *Raoultella terrigena*[1] | 8.7 x $10^5$ cfu/mL | < 0.45 cfu/mL* | < 0.45 cfu/mL* | < 0.45 cfu/mL* |
| Virus: MS-2 Bacteriophage[2] | 5.4 x $10^5$ pfu/mL | 10.2 pfu/mL | 20.2 pfu/mL | 13.9 pfu/mL |
| 3.0 µM Fluorescent microspheres [3] | 4.1 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: | | |
|---|---|---|---|---|
| | | JCP10k_5 BCS 1601087 | JCP10k_6 BCS 1601088 | JCP10k_7 BCS 1601089 |
| Bacteria: *Raoultella terrigena* | 8.7 x $10^5$ cfu/mL | > 99.9999%* | > 99.9999%* | > 99.9999%* |
| Virus: MS-2 Bacteriophage | 5.4 x $10^5$ pfu/mL | 99.998% | 99.996% | 99.997% |
| 3.0 µM Fluorescent microspheres | 4.1 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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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 _____ Date: 02/09/2016

David Sekora, M.S.

_____ Date: 02/09/2016

George Lukasik, Ph.D.

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 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 _____ Date: 02/09/2016

George Lukasik, Ph.D.





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