



BIOLOGICAL CONSULTING SERVICES  
OF NORTH FLORIDA, INC.

June 8, 2016

Sagan, LLC  
11035 Technology Place, Suite 100  
San Diego, CA 92127  
858-675-7017 ext. 2000

RE: Biological filtration efficacy test study of the provided Sagan® Journey Filters; BCS ID 1605405 and 1605406.

To whom it may concern,

We have conducted the requested filtration efficacy study on the filter units received on April 21<sup>st</sup>, 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 extended operation of the filter units

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,

A handwritten signature in black ink, appearing to read 'George Lukasik'.

George Lukasik, Ph.D.  
Laboratory Director

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

FILE: SAGAN JOURNEY FILTER RT, MS2 AND CYST REMOVAL STUDY BCS 1605405-406 06.07.2016  
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**Test Article(s):**

On May 31<sup>st</sup>, 2016, 3 Sagan Journey filter units were received from Sagan LLC. The three filter units were issued BCS identifiers 1605405, 1605406, and 1605407 respectively. BCS IDs 1605405 and 1605406 were selected for the study and 1605407 was kept in reserve.

**Study Date:**

The study was initiated on May 31<sup>st</sup>, 2016 and completed on June 6<sup>th</sup>, 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:**

<b>Equipment and Measurement Parameter</b>	<b>Manufacturer</b>	<b>BCS Lab ID</b>
Epi-fluorescence microscope	Olympus BH-2	MIC-3
Vacuum Pump	Schuco Inc.	Pump-03
Turbidity meter	Hach Turbidity Meter	TM-01
Total hardness test kit	LaMotte	H-1
Incubator	Sanyo MIR-253	I-2
pH	SevenCompact pH/Ion pH meter, Model S220	PH-4
Conductivity/TDS	VWR Traceable Conductivity Meter, 89094-958	CM-05 NIST
Timer	VWR Traceable Lab Top Timer 62344-910	T-10 NIST
Centrifuge	Eppendorf C-5702	C-12
1-Liter standardized graduated cylinder	Nalgene	GC-1L-A
Pressure meter	Weiss Solar Metrix Digital Pressure meter	PM 06
CP Masterflex Gear Pump Digital	Masterflex, 577903	Pump 27
CP Masterflex Gear Pump Digital	Masterflex, 986006	Pump 25

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## Test Water Matrix; General Test Water 1:

General Test Water 1 (GTW1, NSF P231) was made up of the dechlorinated municipal water. Municipal water was dechlorinated by filtration through carbon block filters.

Total dissolved solids, turbidity, and pH were measured and adjusted (if necessary) to NSF P231 guidelines. The pH of the water was  $7.42 \pm 0.1$ , turbidity was  $0.56 \pm 0.1$  NTU, total dissolved solids were measured at  $210 \pm 4$  ppm, and Hardness was  $160 \pm 4$  ppm.

## 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  $36.5 \pm 0.5$  °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 enumeration as per Standard Method 9215C (APHA, 2012).

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**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. Coliphage assay (BCS SOP V-10) was used for the enumeration of MS2. Briefly; enumeration was performed by an agar overlay plaque assay using *E. coli* ATCC 15597 as the host.

**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 and EPA1623.1 methodology.

### **Challenge study Description / Methodology:**

The provided filters were connected to individual digital gear drive pumps (Masterflex, USA) and submerged in a reservoir of General Test Water 1 (GTW1). The test water was drawn up through the filters at an approximate flow rate of 650mL/min. After the passage of 1 gallon of GTW1 the filters were subjected to the filtration efficacy challenge. Briefly, aliquots of the described biologicals were added to 3.5 liters of GTW1 and homogenized. A sample of the challenge water was removed and stabilized. The

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filters were submerged in the challenge water and connected to a vacuum pump to draw the challenge water up through the filters. One liter of the prepared challenge water was passed through each filter and collected for analysis. Following the challenge, the filters were returned to the GTW1 source and the challenge was repeated after the passage of 25, 50, 75, 100, 125, 150, 175, 200, and 225 gallons of GTW1.

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 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:** Sagan, LLC Journey Filter Efficacy Test  
**Sample(s):** BCS 1605405 and 1605406 received May 31<sup>st</sup>, 2016  
**Test:** Filtration Efficacy  
**Test Parameter:** *Raoultella terrigena*<sup>1</sup> (Bacteria)  
**Test Dates:** May 31<sup>st</sup>, 2016 – June 6<sup>th</sup>, 2016

Filter ID	Average concentration (cfu/100mL) in the filters' effluents following each challenge**										
	Average Influent Concentration	1 Gallon	25 Gallons	50 Gallons	75 Gallons	100 Gallons	125 Gallons	150 Gallons	175 Gallons	200 Gallons	250 Gallons
BCS 1605405	36,000,000-54,000,000 cfu/100 mL (3.6 x 10 <sup>7</sup> - 5.4 x 10 <sup>7</sup> cfu/100 mL)	<45* >99.9999% ***	<45* >99.9999% ***	<0.45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***
BCS 1605406		<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***	<45* >99.9999% ***

<sup>1</sup> *Raoultella terrigena* (ATCC 33257) was 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 99.95% or greater for parasite cysts.

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**Project:** Sagan, LLC Journey Filter Efficacy Test  
**Sample(s):** BCS 1605405 and 1605406 received May 31<sup>st</sup>, 2016  
**Test:** Filtration Efficacy  
**Test Parameter:** MS-2 Bacteriophage<sup>2</sup> (virus)  
**Test Dates:** May 31<sup>st</sup>, 2016 – June 6<sup>th</sup>, 2016

Filter ID	Average concentration (pfu/100mL) in the filters' effluents following each challenge**										
	Average Influent Concentration	1 Gallon	25 Gallons	50 Gallons	75 Gallons	100 Gallons	125 Gallons	150 Gallons	175 Gallons	200 Gallons	250 Gallons
BCS 1605405	26,000,000-34,000,000 pfu/100 mL (2.6 x 10 <sup>7</sup> - 3.4 x 10 <sup>7</sup> pfu/100 mL)	110 99.9996%***	110 99.9996%***	450 99.999%***	650 99.998%***	660 99.998%***	770 99.997%***	810 99.997%***	840 99.997%***	2050 99.992%***	4610 99.99%***
BCS 1605406		45 99.999%***	110 99.9996%***	570 99.998%***	810 99.998%***	1180 >99.996%** *	930 99.997%***	950 99.996%***	1000 99.997%***	2270 99.991%***	4520 99.99%***

<sup>2</sup>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.

\*\* 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:** Sagan, LLC Journey Filter Efficacy Test  
**Sample(s):** BCS 1605405 and 1605406 received May 31<sup>st</sup>, 2016  
**Test:** Filtration Efficacy  
**Test Parameter:** 3.0 µM Fluorescent Microspheres<sup>3</sup> as *Cryptosporidium parvum* oocyst surrogate (Parasite)  
**Test Dates:** May 31<sup>st</sup>, 2016 – June 6<sup>th</sup>, 2016

Filter ID	Average concentration (particle/100mL) in the filters' effluents following each challenge**										
	Average Influent Concentration	1 Gallon	25 Gallons	50 Gallons	75 Gallons	100 Gallons	125 Gallons	150 Gallons	175 Gallons	200 Gallons	250 Gallons
BCS 1605405	3,800,000-4,200,000 particle/100 mL (3.8 x 10 <sup>6</sup> – 4.2 x 10 <sup>6</sup> particle/100 mL)	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***
BCS 1605406		<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***	<100 99.9996% ***

<sup>3</sup>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.

\*\* 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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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: 06/06/2016

*George Lukasik*  
\_\_\_\_\_  
George Lukasik, Ph.D.

Date: 06/06/2016

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 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: 06/06/2016

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