May 01, 2015

Sagan LLC
11035 Technology Place, Suite 100
San Diego, CA 92127
858-675-3088 xt 200

Re: Biological filtration efficacy testing of the provided gravity fed bucket prototype filter; BCS ID 1504028

To whom it may concern,

We have conducted the extensive biological filtration efficacy study on the filter received. The provided filtration system was tested in a gravity fed configuration. The experimental set up and challenge of the water filters was designed to **evaluate the filters' lifetime** bacterial, viral, and protozoan cysts removal efficacy. The contaminant species and water parameters selected were based on client's request and NSF/ANSI water purifier testing protocols.

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

BCS LABORATORIES INC.-GAINESVILLE
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WWW.MICROBIOSERVICES.COM
FL DOH LABORATORY #E82924, EPA# FL01147

Project: **Sagan Gravity Fed Bucket Prototype Filter Test (Grade 5283 with 5289)**
Study Sponsor: **Sagan**
Sample(s): **BCS 1504028 received April 8th, 2015**
Test: **Filtration Efficacy**
Test Parameter: ***Raoultella terrigena* (Bacteria)**
Performed and Analyzed by: **George Lukasik, Ph.D. & Kintin Ng; 4/13/2015-4/28/2015**

Filter	Filter influent concentration throughout study	Average concentration (cfu/100ml) of the bacterial challenge in the filter's effluent following the passage of the indicated volumes (gallons) of Municipal dechlorinated water				
		10	**200**	**400**	**600**	**700**
BCS 1504028 Subjected to the specified **"challenge concentration" every 25 gallons**	*Raoultella terrigena*[1] 17,000,000-42,000,000 cfu/100ml ($1.7x10^7$-$4.2x10^7$/100ml)	< 25*	< 25*	< 25*	< 25*	< 25*

[1] *Raoultella terrigena* (ATCC 33257) was obtained from ATCC and propagated on Tryptic Soy Agar (TSA, Becton Dickinson, USA). It is used as a bacterial model to evaluate filters' bacterial removal efficacy. The 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's effluent for the volume analyzed (<0.25 cfu or pfu/ml). Effluent samples from challenge studies performed at the 25 gallon intervals yielded the same result; the table presents selected representative results of challenge points.

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Performed and Analyzed by: **George Lukasik, Ph.D. & Kintin Ng; 4/13/2015-4/28/2015**

Filter	Filter influent concentration throughout study	Average percent removal** of the challenge species by the filter following the passage of the indicated volumes (gallons) of Municipal dechlorinated water				
		10	**200**	**400**	**600**	**700**
BCS 1504028 Subjected to the specified **"challenge concentration" every 25 gallons**	*Raoultella terrigena*[1] 17,000,000-42,000,000 cfu/100ml ($1.7x10^7$-$4.2x10^7$/100ml)	> 99.9999%*	> 99.9999%*	> 99.9999%*	> 99.9999%*	> 99.9999%*

[1] *Raoultella terrigena* (ATCC 33257) was obtained from ATCC and propagated on Tryptic Soy Agar (TSA, Becton Dickinson, USA). It is used as a bacterial model to evaluate filters' bacterial removal efficacy. The 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's effluent for the volume analyzed (<0.25 cfu or pfu/ml). Effluent samples from challenge studies performed at the 25 gallon intervals yielded the same result; the table presents selected representative results of challenge points.

** Purifier NSF/ANSI, and US EPA standards for 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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Study Sponsor: **Sagan**
Sample(s): **BCS 1504028 received April 8th, 2015**
Test: **Filtration Efficacy**
Test Parameter: ***MS-2 Bacteriophage (virus)***
Performed and Analyzed by: **George Lukasik, Ph.D. & Kintin Ng; 4/13/2015-4/28/2015**

Filter	Filter influent concentration throughout study	Average concentration (pfu/100ml) of the challenge species in the filter's effluent following the passage of the indicated volumes (gallons) of Municipal dechlorinated water				
		10	**200**	**400**	**550**	**600**
BCS 1504028 Subjected to the specified **"challenge concentration" every 25 gallons**	*MS-2 Bacteriophage*[1] 21,000,000-290,000,000 pfu/100ml ($2.1x10^7$-$2.9x10^8$/100ml)	< 45	< 45	2,182	9,000	17,136

[1]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.

* No species were detected in the filter effluent for the total volume analyzed (<0.45 cfu or pfu/ml). Filter effluent samples were analyzed in duplicates at the minimum following collection.

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Performed and Analyzed by: **George Lukasik, Ph.D. & Kintin Ng; 4/13/2015-4/28/2015**

Filter	Filter influent concentration throughout study	Average percent removal** of the challenge species by the filter following the passage of the indicated volumes (gallons) of Municipal dechlorinated water				
		10	**200**	**400**	**550**	**600**
BCS 1504028 Subjected to the specified **"challenge concentration" every 25 gallons**	*MS-2 Bacteriophage*[1] 21,000,000-290,000,000 pfu/100ml ($2.1x10^7$-$2.9x10^8$/100ml)	> 99.9999%*	> 99.9999%*	99.998%	99.996%	99.94%

[1]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.
* No species were detected in the filter effluent for the total volume analyzed (<0.45 cfu or pfu/ml). Filter effluent samples were analyzed in duplicates at the minimum following collection.
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Project: **Sagan Gravity Fed Bucket Prototype Filter Test (Grade 5283 with 5289)**
Study Sponsor: **Sagan**
Sample(s): **BCS 1504028 received April 8th, 2015**
Test: **Filtration Efficacy**
Test Parameter: **3.0 µM Fluorescent Microspheres as *Cryptosporidium parvum* Oocyst Surrogate**
Performed and Analyzed by: **George Lukasik, Ph.D. & Kintin Ng; 4/13/2015-4/28/2015**

Filter	Filter influent concentration throughout study	Average concentration (pfu/100ml) of the challenge species in the filter's effluent following the passage of the indicated volumes (gallons) of Municipal dechlorinated water				
		10	**200**	**400**	**600**	**700**
BCS 1504028 Subjected to the specified **"challenge concentration" every 25 gallons**	3.0 µM Fluorescent microspheres[1] 2,400,000 – 14,000,000/100ml ($2.4x10^6$-$1.4x10^7$/100ml)	< 100*	< 100*	< 100*	< 100*	< 100*

[1]Three micron green fluorescent latex microspheres (Fluoro-Max Green Fluoroscent Polymer Microspheres 2.9µm, 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 SuperStick Slides (Waterborne, Inc, USA) and viewing by UV fluorescence microscopy.
* No species were detected in the filter's effluent for the volume analyzed. Effluent samples from challenge studies performed at the 25 gallon intervals yielded the same result; the table presents selected representative results of challenge points.

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Performed and Analyzed by: **George Lukasik, Ph.D. & Kintin Ng; 4/13/2015-4/28/2015**

Filter	Filter influent concentration throughout study	Average percent removal** of the challenge species by the filter following the passage of the indicated volumes (gallons) of Municipal dechlorinated water				
		10	**200**	**400**	**600**	**700**
BCS 1504028 Subjected to the specified **"challenge concentration" every 25 gallons**	3.0 µM Fluorescent microspheres[1] 2,400,000 – 14,000,000/100ml ($2.4x10^6$-$1.4x10^7$/100ml)	> 99.998%*	> 99.998%*	> 99.998%*	> 99.998%*	> 99.998%*

[1]Three micron green fluorescent latex microspheres (Fluoro-Max Green Fluoroscent Polymer Microspheres 2.9µm, 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 SuperStick Slides (Waterborne, Inc, USA) and viewing by UV fluorescence microscopy.
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***** Biological filtration challenge study description: Initially, ten gallons of dechlorinated City of Gainesville Municipal water was passed through the provided filter using gravity filtration. Dechlorination was achieved using a Pentek EP-BB, 0.5 micron carbon block filter cartridge. For challenge water preparation, the indicated species were added to seven liters of dechlorinated City of Gainesville Municipal water (pH 7.5±0.5). The challenge water was homogenized and transferred to the vessel that the filter is housed in. The challenge solution was allowed to pass through the filter by gravity filtration. A minimum of two liters of the challenge solution was passed through the filter prior to collecting an effluent sample. The filter effluent sample was collected in a sterile container in duplicates. The flow rate was validated using a NIST traceable timer. The flow rate of the filter was averaged at approximately 400-500 ml/min at the beginning of the study. The flow rate gradually decreased throughout the study. At the end of the study, flow rate was measured to be approximately 75-90 ml/min. for the challenge study, a sample of the influent was removed prior to the beginning of the study and at the end. The samples were assayed to determine the concentration of the filter's influent challenge. The influent and effluent samples were assayed for the respective species as per Standard Methods (APHA 2012), EPA 1623.1, and EPA 1601. Following the initial challenge, dechlorinated City of Gainesville Municipal water was added to the filtration system at a rate that ensured the filter was submerged at all times. The challenge study was repeated following the passage of 25 gallons and every 25 gallons thereafter up to 700 gallons. The respective percent reductions were determined based on the concentration obtained in the filter influent and effluent. The tables report the average reduction of the filter tested at the indicated test points.

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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 its (their) condition at the time of test. The study and data are obtained under laboratory conditions and 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 claims with regards to the express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or product.

Signature of Laboratory Director/Authorized Rep. _____ Date: May 1, 2015

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