

March 30, 2015

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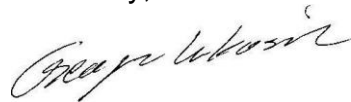
Re: Biological filtration efficacy testing of the provided prototype filters; BCS ID 1502057 and 1502058

To whom it may concern,

We have conducted the extensive biological filtration efficacy study on the filters received. The provided filters were retrofitted to be tested in an "in-line" 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

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FILE: SAGAN PROTOTYPE WATER FILTERS BACTERIA VIRUS AND CYSTS CHALLENGE BCS 1502057 AND 1502058 MARCH 9 2015.DOCX



**Project:** Sagan Prototype Filter Test  
**Study Sponsor:** Sagan  
**Sample(s):** BCS 1502057 and 1502058 received February 6<sup>th</sup>, 2015  
**Test:** Filtration Efficacy  
**Test Parameter:** *Raoultella terrigena* (Bacteria)  
**Performed and Analyzed by:** George Lukasik, Ph.D. & Kintin Ng; 2/9/2015-2/17/2015

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)				
		1	100	150	200	230
<b>Filter A BCS 1502057</b> Subjected to the specified "challenge concentration" every 10 gallons	<i>Raoultella terrigena</i> <sup>1</sup> 30,000,000-40,000,000 cfu/100ml (3.0x10 <sup>7</sup> -4.0x10 <sup>7</sup> /100ml)	< 45*	< 45*	< 45*	< 45*	< 45*
		1	100	150	200	300
< 45**		< 45**	< 45**	< 45**	< 45**	

<sup>1</sup> *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 (<0.45 cfu or pfu/ml). Effluent samples from challenge studies performed at the 10 gallon intervals yielded the same result; the table presents selected representative results of challenge points.

\*\* No species were detected in the filter's effluent for the volume analyzed (<0.45 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; 2/9/2015-2/17/2015

Filter	Filter influent concentration throughout study	Average percent removal*** of the challenge species by the filter following the passage of the indicated volumes (gallons)				
		1	100	150	200	230
<b>Filter A BCS 1502057</b> Subjected to the specified “challenge concentration” every 10 gallons	<i>Raoultella terrigena</i> <sup>1</sup> 30,000,000-40,000,000 cfu/100ml (3.0x10 <sup>7</sup> -4.0x10 <sup>7</sup> /100ml)	> 99.9999%*	> 99.9999%*	> 99.9998%*	> 99.9999%*	> 99.9999%*
		1	100	150	200	300
<b>Filter B BCS 1502058</b> Subjected to the specified “challenge concentration” every 25 gallons		> 99.9999%*	> 99.9998%*	> 99.9999%*	> 99.9999%*	> 99.9999%*

<sup>1</sup> *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 (<0.45 cfu or pfu/ml). Effluent samples from challenge studies performed at the 10 gallon intervals yielded the same result; the table presents selected representative results of challenge points.

\*\* No species were detected in the filter’s effluent for the volume analyzed (<0.45 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.



**Project:** Sagan Prototype Filter Test  
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**Sample(s):** BCS 1502057 and 1502058 received February 6<sup>th</sup>, 2015  
**Test:** Filtration Efficacy  
**Test Parameter:** MS-2 Bacteriophage (virus)  
**Performed and Analyzed by:** George Lukasik, Ph.D. & Kintin Ng; 2/9/2015-2/17/2015

Filter	Filter influent concentration throughout study	Average concentration (pfu/100ml) of the challenge species in the filter effluent following the passage of the indicated volumes (gallons)				
		1	100	150	200	230
<b>Filter A BCS 1502057</b> Subjected to the specified "challenge concentration" every 10 gallons	MS-2 Bacteriophage <sup>1</sup> 15,000,000-37,000,000 pfu/100ml (1.5x10 <sup>7</sup> -3.7x10 <sup>7</sup> /100ml)	< 45*	45	1.8	4.5	3.6
		<b>1</b>	<b>100</b>	<b>150</b>	<b>200</b>	<b>300</b>
<b>Filter B BCS 1502058</b> Subjected to the specified "challenge concentration" every 25 gallons		< 45*	45	< 45*	4.1	2.3

<sup>1</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. 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; 2/9/2015-2/17/2015

Filter	Filter influent concentration throughout study	Average percent removal** of the challenge species by the filter following the passage of the indicated volumes (gallons)				
		1	100	150	200	230
<b>Filter A BCS 1502057</b> Subjected to the specified "challenge concentration" every 10 gallons	MS-2 Bacteriophage <sup>1</sup> 15,000,000-37,000,000 pfu/100ml (1.5x10 <sup>7</sup> -3.7x10 <sup>7</sup> /100ml)	>99.9998%	99.9999%	99.9995%	99.998%	99.998%
		<b>1</b>	<b>100</b>	<b>150</b>	<b>200</b>	<b>300</b>
>99.9998%		99.9999%	>99.9999%	99.999%	99.998%	
<b>1</b>		<b>100</b>	<b>150</b>	<b>200</b>	<b>300</b>	

<sup>1</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. 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.

\*\* 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.



**Project:** Sagan Prototype Filter Test  
**Study Sponsor:** Sagan  
**Sample(s):** BCS 1502057 and 1502058 received February 6<sup>th</sup>, 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; 2/9/2015-2/17/2015

Filter	Filter influent concentration throughout study	Average concentration (microspheres/100ml) of the challenge species in the filter effluent following the passage of the indicated volumes (gallons)				
		1	100	150	200	230
<b>Filter A BCS 1502057</b> Subjected to the specified “challenge concentration” every 10 gallons	3.0 µM Fluorescent microspheres <sup>3</sup> 5,000,000 – 6,800,000/100ml (5.0x10 <sup>6</sup> -6.8x10 <sup>6</sup> /100ml)	< 100*	< 100*	< 100*	< 100*	< 100*
		<b>1</b>	<b>100</b>	<b>150</b>	<b>200</b>	<b>300</b>
< 100**		< 100**	< 100**	< 100**	< 100**	

<sup>3</sup>Three micron green fluorescent latex microspheres (Fluoro-Max Green Fluorescent 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 10 gallon intervals yielded the same result; the table presents selected representative results of challenge points.

\*\* No species were detected in the filter’s effluent for the volume analyze. 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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Filter	Filter influent concentration throughout study	Average percent removal** of the challenge species by the filter following the passage of the indicated volumes (gallons)				
		1	100	150	200	230
<b>Filter A BCS 1502057</b> Subjected to the specified “challenge concentration” every 10 gallons	3.0 µM Fluorescent microspheres <sup>3</sup> 5,000,000 – 6,800,000/100ml (5.0x10 <sup>6</sup> -6.8x10 <sup>6</sup> /100ml)	>99.997%*	>99.997%*	>99.997%*	>99.997%*	>99.997%*
		<b>1</b>	<b>100</b>	<b>150</b>	<b>200</b>	<b>230</b>
<b>Filter B BCS 1502058</b> Subjected to the specified “challenge concentration” every 25 gallons		>99.997%*	>99.997%*	>99.997%*	>99.997%*	>99.997%*
		<b>1</b>	<b>100</b>	<b>150</b>	<b>200</b>	<b>300</b>

<sup>3</sup>Three micron green fluorescent latex microspheres (Fluoro-Max Green Fluorescent 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 10 gallon intervals yielded the same result; the table presents selected representative results of challenge points.

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**Test Parameter:** *Raoultella terrigena* (Bacteria), MS-2 Bacteriophage (virus), 3.0 µM Fluorescent Microspheres as *Cryptosporidium parvum* Oocyst Surrogate  
**Performed and Analyzed by:** George Lukasik, Ph.D. & Kintin Ng; 2/9/2015-2/17/2015

\* Biological filtration challenge study description: Initially, two liters of dechlorinated City of Gainesville Municipal water was passed through each of the provided filters using 1.9-2.0 PSI pressure. 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 three liters of dechlorinated City of Gainesville Municipal water (pH 7.5±0.5). The challenge water was homogenized and transferred to a pressure vessel. The system was pressurized to 2 PSI and the solution was passed through each of the filters. Half a liter of the challenge solution was passed through each filter. The filter effluent was collected in a sterile container. The flow rate was validated using a NIST traceable timer. The flow rate of the filters was averaged at approximately 450-500 ml/min. A sample of the influent was removed prior to the beginning of the challenge study and at the end and assayed to determine the concentration of the filters 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, each of the provided filters was connected to pressure regulated (1.5-2.5 PSI) dechlorinated City of Gainesville Municipal water supply. Filter A BCS 1502057 was subjected to the described challenge study following the passage of the initial 10 gallons and every 10 gallons thereafter up to 230 gallons. Filter B BCS 1502058 was subjected to the challenge study following 25 gallons and again every 25 gallons thereafter up to 300 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 filters tested.





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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: March 30, 2015

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