



Biological Consulting Services
of North Florida, Inc.

September 09, 2013

Travis Merrigan
Grayl
610 Maple Heights Rd
Camano Island, WA 98282

Re: Bacterial, viral, and protozoan parasite filtration efficacy testing of the provided Grayl Purifier units: BCS ID 1308140-142.

Dear Mr. Merrigan;

We have conducted the requested filtration efficacy study on the provided water bottle purifier units received on August 21, 2013. The experimental set up and challenge of the water filter was designed to evaluate the filter's initial microbiological contaminant removal efficacy. It is intended to demonstrate its efficacy on the removal of bacterial, viral, and parasitic waterborne contaminants. The contaminant species and water condition parameters selected were based on 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

Page 1 of 4

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FL DOH LABORATORY #E82924, EPA# FL01147

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FILE: GRAYL PURIFIERS MICROBIAL REMOVAL EFFICACY STUDY REPORT BCS 1308140-42.DOC



Test Articles: Grayl Bottle Purifiers; Purifier 1-3 (BCS 1308140-142).
Test: Filtration Efficacy / Pressure
Test Parameter: *Raoultella terrigena* (Bacteria), MS-2 Bacteriophage (virus), and 3.0 µM Fluorescent Microspheres as *Cryptosporidium parvum* Oocyst Surrogate
Performed and Analyzed by: George Lukasik, Ph.D. & Alison Stargel, MPH; August 23, 2013

Water Sample	Percent Removal of Challenge Species		
	3.0 µM Fluorescent microspheres ¹ (Parasite Contaminant Percent Removal)	<i>Raoultella terrigena</i> ² (Bacterial Contaminant Percent Removal)	MS-2 Bacteriophage ³ (Viral Contaminant Percent Removal)
Filter Influent Water*	1.4 x 10 ⁴	2.9 x 10 ⁵	4.7 x 10 ⁵
Average performance of tested Purifiers* BCS1308140-142	>99.999%	>99.9999% ³	>99.9999%

¹ Three micron green fluorescent latex microspheres (Fluoresbrite® YG Microspheres 3.00µm, PolySciences Inc. PA, 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 SingleSpot Slides (IDEXX, USA) and viewing by UV fluorescence microscopy.

² *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 for bacterial removal efficacy. The bacteria were enumerated as colony forming units (cfu) following incubation at 36.5°C for 24 hours.

³ 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.



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* Filtration Challenge Study Description: Prior to each filter challenge, 1 liter of laboratory grade reagent water was passed through each unit using the provided bottle and the application of approximately 5 lbs. of downward force. Reagent water at pH 7.5-7.9 was seeded with *Raoultella terrigena*, bacteriophage MS-2, and latex microspheres. This solution was stirred till homogenous and 500 ml was added to each of the Grayl metal bottles. The purifier connected reservoir was steadily pushed down using approximately 5 lbs. downward force. The filtered water was then decanted into a sterile container and assayed for the respective species as per standard methods and Lab Standard Operating Procedures (SOP F-1). A sample of the influent was removed prior to the beginning of the challenge study and at the end. All analysis was conducted in duplicate at minimum. The number of microorganisms and microspheres was determined in each sample. Three filters were tested. The respective percent reductions were determined based on the concentration obtained in the filter influent and effluent.

Page 3 of 4

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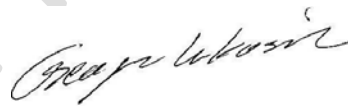
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Study data are summarized in the provided table(s). The results presented pertain only to the study conducted on the test articles/samples provided by the client (or client representative). The study was authorized and commissioned by the client. The 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 to 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.



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Signature of Laboratory Director/Authorized Rep. _____ Date: _____

Page 4 of 4

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