BIOLOGICAL CONSULTING SERVICES
OF NORTH FLORIDA, INC.

October 30, 2014

Jim Giorgi
NANOLAVA - NANOBLASTER

RE: Study report of the virucidal efficacy testing of NANOLAVA - NANOBLASTER ™ NTS concentrate; BCS 1409054

Dear Mr. Giorgi,

We have conducted the virucidal efficacy testing on the provided NANOLAVA - NANOBLASTER NTS concentrate. The testing was conducted as per ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces," and ASTM E2197-11
"Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."

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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AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants (2005)

Human poliovirus 1- strain CHAT virus (ATCC VR-1562) was propagated and enumerated as infectious units (iu) using EPA ICR Methodology (EPA 600/R-95/178). Viruses were harvested by infecting cell monolayers and incubating at 36.5°C and 5% CO_2 until 90–95% of the cells demonstrated a cytopathic effect. The cells were frozen and thawed twice, followed by high speed centrifugation and filtration through a 0.1 µm filter. The supernatant was aliquoted as test virus suspension and stored at -80°C. For enumeration, aliquots containing herpes virus were inoculated on freshly prepared monolayers of Buffalo Green Monkey (BGM) kidney cells. The cells were supplemented with growth media and incubated at 36.5ºC and 5% carbon dioxide and monitored for cytopathic effect development over a 10 day period. Infectious foci and cytopathic effects (CPE) was determined as per methodology outlined in EPA 600/R9-95/178 and a Most Probable Number (MPN) assay was used to determine the infectious units/ml in the samples analyzed.

For challenge studies, frozen viral stock (typically 1 x 10^8 iu/ml) was thawed rapidly in a 35°C water bath on the day of the study. The virus stock was titered by performing

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serial ten-fold dilutions in PBS and was inoculated onto the respective cells as described above.

Test Article/Product: NANOLAVA - NANOBLASTER NTS Concentrate

On September 09, 2014, a bottle of NANOLAVA - NANOBLASTER™ NTS concentrate solution was delivered to BCS Laboratories. The solution was issued BCS identifier 1409054. The solution's directions were followed based on the client's recommendations. The concentrate was diluted with distilled or demineralized water to desired strength. Using 50ml of the concentrate, a 1/1 dilution was made with 50ml of laboratory grade reagent water. The 100ml diluted solution was again diluted 1/1 with an additional 100ml of laboratory grade reagent water. The final diluted solution was placed in a sterile spray bottle and used within 15 minutes of preparation for the microbial spray disinfection studies. The temperature of the diluted solution prior to application and during disinfection efficacy testing was maintained at 20-22°C. All tests were conducted in a Class II biological cabinet.

Study Date for Human poliovirus 1- strain CHATt virus:

Study initiated October 17, 2014 and completed October 29, 2014.

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Test Methodology Narrative for Human poliovirus 1- strain CHAT virus:

On the day of study, viral stock concentrate was removed from -80º C, and thawed rapidly immediately prior to the study. Viral concentrate typically contain approximately 10^8 infectious units/ ml.

Twenty-microliters of the homogenized microbial suspensions were placed and spread onto sterile 24x26 mm glass slides (Propper Scientific, NY). Seven slides were used for the viral study. Five of the slides were inoculated and allowed to dry in a covered chamber at 37°C for 50-60 minutes. Three of the five inoculated slides were then sprayed for 5 seconds from a distance of approximately 12 inches with the diluted concentrate. The spray consisted of a fine steady mist. The glass slides were saturated with sprayed solution. The slides were then incubated at 20-22°C for 10 minutes. Additionally, un-inoculated slides were sprayed and incubated to serve as negative and neutralization controls. The additional remaining inoculated slides that were not exposed to the spray disinfectant were used as a positive control. Immediately following 10 minutes, each of the slides were picked up with sterile forceps, the excess liquid was allowed to run off and the slides were placed into sterile 50ml tubes

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(Corning,USA) containing 10 ml of D/E neutralization broth w/ tween. The recovered microorganisms from the positive control slide were used to determine the initial titer of the microorganisms and thus the percent reductions. The tubes were placed onto a rotary shaker for 15 minutes to elute the viral particles.

The eluates were assayed for the presence of infectious virus particles using a MPN based assay onto BGM cell monolayers. Positive, negative, cytotoxicity, and neutralization controls were performed as per ASTM recommendations and validated the test results. The samples were diluted 1/100 and inoculated onto BGM cells in sets of five at 1.0ml, 0.1ml, and 0.01ml. The positive controls were diluted 1/1000 in phosphate buffered saline (Weber) and inoculated onto BGM cells also in sets of five at 1.0, 0.1, and 0.1 ml. Cells were incubated at 36.5º C for 2 weeks.

Study data are summarized in the provided table(s). Positive, negative, and process 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 express or implied warranty regarding the ownership, merchantability, safety or fitness for a particular purpose of any such property or

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product. 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 it's (their) condition and homogeneity when received and at the time of test. Thus, the data may not be representative of the lot or batch number or other samples. Consequently, the data may not necessarily justify the acceptance or rejection of a lot or batch, a product recall, or support legal proceedings. It is the responsibility of the client to provide all information relevant to the analysis requested. The study and data obtained under the laboratory conditions may not be representative or indicative of a real-life process and/or application. This report does not imply that BCS Laboratories has been engaged to consult upon the consequences of the analysis and for any action that should be taken as a result of the analysis.

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Table 1. The virucidal efficacy of a spray application of diluted NANOLAVA - NANOBLASTER™ NTS concentrate against Human poliovirus 1 (ATCC VR 1562) inoculated onto non porous surfaces. Test was conducted as per adaptation of ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces" and ASTM E2197-11 "Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals."

Microorganism	Number of Replicates Tested	Contact Time (Minutes)	Average Infectious Units Control Slides [#]	Average Recovered From Each Sprayed Slide*	Percent Reduction	Average Percent Reduction	Log_{10} reduction
Human Poliovirus 1	3	10	1.6×10^4 iu/ml	790 iu/mL	95.1%	95.9%	1.4
				940 iu/mL	94.1%		
				230 iu/mL	98.6%		

[#] This number represents the average number of infectious virus particles (units) recovered from glass slides inoculated, dried, and not exposed to treatment (positive control).

* Glass slides were inoculated and allowed to dry. Slides were sprayed to saturation with the solution and allowed to incubate for 10 minutes. Slides were eluted and enumerated for infectious viral particles on Buffalo Green Monkey cell monolayers as described.

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