Biological Consulting Services
of North Florida, Inc.

February 04, 2013

Peter Coleman
Invisible Shield Technology
3365 Pine Walk Drive North, #203
Margate, FLORIDA, 33063

RE: Disinfection efficacy study of the provided liquid formula (BCS ID 1301010) against Murine Norovirus (ATCC# PTA 5935).

Dear Mr. Coleman,

We have conducted the antiviral efficacy testing on the submitted solution received on January 15, 2013. The testing was conducted according to a protocol developed to evaluate virucidal efficacy of spray disinfectants, in particular on the inactivation of Nororovirus. The protocol is based on methodology described in ASTM E1053-97 (Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces).

In the following pages, you will find a summary of the methodology used and the results of our study. Should you have any questions or concerns, please do not hesitate to contact me.

Best Regards,

George Lukasik, Ph.D.
Laboratory Director

pcoleman@ishieldtech.com

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FL DOH Laboratory #E82924, EPA# FL01147

Microbial Cultures:

Murine Norovirus (ATCC# PTA 5935, MNV-1) was used as a model for human Norovirus. MNV-1 was propagated on RAW 264.7 cells (ATCC# TIB-71). Viruses were harvested by infecting cell monolayers and incubating at 37°C and 5% CO2 until 70–95% of the cells showed 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. Viral enumeration was performed using infection of cell monolayers and observation for cytopathic effect development. Infected monolayers in cell culture treated flasks were incubated at 36.5°C and in 5% CO_2 for 5-7 days. A most probable number (MPN) calculation (EPA 600/R-95/178) was used to calculate the number of infectious viral units.

For challenge experiments, frozen viral stock (typically 1 x 10^8 iu/ml) was thawed rapidly in a 35°C water bath on the day of the experiment. The virus stock was tittered by performing serial ten-fold dilutions in PBS and was inoculated onto the respective cells as described above.

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Supplied disinfectant:

On January 15, 2013, a container of a clear liquid labeled “IS-SNP-1” was received by BCS Laboratories Inc. from Invisible Shield Technology. The sample was assigned the BCS identification number 1301010.

Spray Disinfection Study: Initiated January 29, 2013

To 68 milliliters of the supplied IS-SNP-1 disinfectant solution, 2.2mL of House Hold General Bleach Solution (5.25% Sodium Hypochlorite) and 0.54mL of IS-DP 1:128 stock solution (BCS ID 1211073) was added. The temperature of the disinfectant prior to application and during disinfection efficacy testing was maintained at 21-22°C. The prepared test solution was then placed in a trigger aerosol sprayer and used within 30 minutes of preparation.

Twenty five microliter aliquots of the viral suspension were dispensed onto four sterile 25 cm^2 glass slides (Propper scientific, USA). Three slides were used for the spray disinfection (treated) and one slide was used as positive survival control (untreated) that was not exposed to the test solution. Additionally, one uninoculated slide was used as a negative control. The inoculum was allowed to dry on the slides at 22°C. The three inoculated slides and the negative control slide were then sprayed for approximately 10

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seconds from a distance of 10" with the test solution. The slides were evenly and thoroughly saturated with the solution. The slides were allowed to incubate at 22°C for 10 minutes. Immediately following incubation, each slide was aseptically removed, and eluted in a sterile 50 mL tube (Corning, USA) containing 10mL of Neutralizing Broth (BD). The sprayed, uninoculated negative control slide and the inoculated, unsprayed positive control slide were treated in the same manner as described above. The tubes were placed on a rotary shaker for 10 minutes and then diluted in Phosphate Buffered Water (Remel, USA). Each of the solutions were assayed for the presence of infectious virus units (IU) using a MPN based assay onto the RAW 264.7. Positive, negative, cytotoxicity, and neutralization controls were performed as per Good Lab Practices and validated the test results.

Study data are summarized in the following table(s). The results presented pertain only to the study conducted on the test articles 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. 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

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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 product.

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Table 1. The efficacy of the Invisible Shield Technology formulated solution (BCS ID 1301010)* on the inactivation of Murine Norovirus (MNV-1)

Sample ID	Recovered Infectious Units of MNV-1/mL**	Average Recovered IU/mL	Percent Reduction	Log_{10} Reduction
Untreated	4.4×10^4	4.4×10^4	99.98%	3.6
Treated A	8.0	1.0×10^1		
Treated B	8.0			
Treated C	14.0			

*the solution was prepared by mixing 68 milliliters of the supplied IS-SNP-1 disinfectant solution, 2.2mL of House Hold General Bleach Solution (5.25% Sodium Hypochlorite), and 0.54mL of IS-DP 1:128 stock solution (BCS ID 1211073). The prepared test solution was then placed in a trigger aerosol sprayer and used within 30 minutes of preparation.

**MNV-1 (ATCC# PTA 5935) was inoculated onto four sterile glass slides. Three slides were used for the spray disinfection (treated) and one slide was used as positive survival control (untreated) that was not exposed to the test solution. Additionally, one uninoculated slide was used as a negative control. The inoculum was dried and the slides were sprayed for approximately 10 seconds from a distance of 10" with the test solution. The slides were evenly and thoroughly saturated with the solution. The slides were allowed to incubate at 22°C for 10 minutes. Immediately following incubation, each slide was aseptically removed, and eluted in a sterile 50 ml tube (Corning, USA) containing 10mL of Neutralizing Broth (BD). The sprayed uninoculated negative control slide and the inoculated, unsprayed positive control slide were treated in the same manner as described above. Each of the solutions was then assayed for the presence of infectious virus units (IU) using a MPN based assay onto the RAW 264.7.

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