Biological Consulting Services
of North Florida, Inc.

July 21, 2010

Walter Warning
President
Viking Ind Inc
501 Pullman Rd.
Edgewater, Fl.32132
386-428-9800

RE: Viking Pure 1000 antiviral disinfection efficacy report

Dear Mr. Warning,

We have conducted the antiviral efficacy testing on the Viking Pure 1000 disinfectant delivered to our Laboratory. The testing was done according to the direct inoculation protocol. The protocol used is comparable to AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants (2005) and also ASTM E 1053-97 (Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Surfaces). Poliovirus Lsc 1 and Murine Norovirus (MNV-1) were used to determine antiviral efficacy of disinfection. The sample of the disinfectant liquid that you provided exhibited excellent antiviral efficacy.

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

Best Regards,

Dr. George Lukasik

Digitally signed by Dr. George Lukasik
DN: cn=Dr. George Lukasik, o=BCS Laboratories-Gainesville, ou=Laboratory Director, email=Lukasik@gator.net, c=US
Date: 2011.05.10 11:56:24 -04'00'

George Lukasik, Ph.D.
Laboratory Director

BCS Laboratories, Inc. -Gainesville
4609-A NW 6th Street, Gainesville, Florida 32609,
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

Poliovirus Lsc1 Chat strain (ATCC VR-1562) was propagated and enumerated as plaque forming units (pfu) using EPA ICR Methodology (EPA 600/R-95/178). Stock cultures of Poliovirus were obtained from ATCC and propagated to a high titer on BGM cells. Viral stocks were maintained at -80°C. For enumeration, aliquots containing poliovirus were inoculated on freshly prepared monolayers of Buffalo Green Monkey (BGM) kidney cells. Plaque assays were performed using 2X dMEM (MediaTech, USA) and 2X Bacto Agar containing 0.0001 % Neutral Red as per methodology outlined in EPA 600/R9-95/178. Cell flasks were incubated at 36.5°C and in 5% CO_2 for 72-96 hours. Plaques on the respective flasks were counted following additional Neutral Red staining.

Murine Norovirus (MNV-1) was propagated on RAW 264.7 cells (ATCC TIB-71). The tissue culture flask was incubated at 37°C and 5% CO_2 until 70–95% of the cells showed a cytopathic effect (after 1–2 days). The cells were frozen and thawed twice, followed by high speed centrifugation and filtration through a 0.1 µm filter. The supernatant was aliquotted as test virus suspension and stored at -80°C. Viral enumeration was performed using infection of cell monolayers and observation for

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cytopathic development. A most probable number (MPN) calculation (EPA 600/R-95/178) was the 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 prior to the experiment. Stock was then diluted 1/10 in Phosphate Buffered Saline (PBS) supplemented with 2% Bovine Serum Albumin (BSA) and used for the viral challenge experiment below. The diluted virus stock was tittered by performing serial ten fold dilutions in PBS and was inoculated onto the respective cells as described above. All analysis was performed in accordance to NELAC accreditation standards that are equivalent to ISO 17025.

On June 15, 2010, a 1-gallon bottle labeled “Viking Pure 1000; Lot. 0001” was delivered to BCS Laboratories-Gainesville. The bottle was opened and used the within 5 hours for the microbial spray disinfection studies. Upon opening the total chlorine residual was measured by the use of DPD test. Total chlorine concentration was measured by a calibrated hand held colorimeter (Series 942 Mini-Analyst, Orbeco-Hellige Inc., USA). The temperature of the disinfectant prior to application and during disinfection efficacy testing was maintained at 20-22°C. The Viking Pure 1000 liquid was placed into handheld spray bottle (Fisher Scientific). The test was conducted in a biological

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containment hood. The diluted viral stocks described above were tittered and contained approximately 10^7 infectious units/ml. Ten microliters of the viral suspension were placed and spread onto sterile 25x25 mm glass slides (Fisher Scientific, PA). Eleven slides of each viral species were prepared and used to test the efficacy of the provided disinfectant. The inoculum was allowed to dry at 37°C for 20 minutes. Ten of the eleven inoculated slides were the sprayed for 10 seconds with the provided solutions; the glass slides were completely covered with spray solution. The slides were allowed to incubate at 21°C for 10 minutes. Each of the slides was picked up with sterile forceps; the excess liquid was shaken off; and each slide was placed into its own sterile glass tube containing 10 ml of Dulbecco's modified Eagle's medium (DMEM, 4.5 g/l glucose, Invitrogen Corp., USA) with 3% fetal calf serum (FCS, low endotoxin, Invitrogen, USA). All tubes were then agitated for 15 minutes to allow for viral elution. Additionally, un-inoculated slides were used as a negative controls and the eleventh remaining slide of the inoculated slides that was not exposed to the spray disinfectant was used as a positive growth control. Following agitation, the media was incubated onto the cell lines respective for the viral inoculum. The inoculated cells were allowed to incubate for 2 hours and then supplemented with fresh DMEM/3%FCS. The cell culture flasks were then incubated at 37°C and 5% CO_2. The flasks were monitored regularly for cytopathic

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effect development throughout an 8 day incubation period. Eluants of the sprayed glass slides did not show any signs of cytotoxicity by direct plating onto the cells.

All data are summarized in the following Tables. The results presented pertain only to the samples analyzed and the batch number indicated. They are not representative nor are they indicative of a process. All analyses were performed in accordance to laboratory practices and procedures governed by our NELAC accreditation standards (ISO 17025) unless otherwise noted.

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Table 1. Inactivation of Poliovirus (Lsc-1) and Murine Norovirus (MNV-1) by the Viking Pure 1000 Spray (Batch # 0001; 284 ppm total chlorine; pH 2.45) Test was conducted as per AOAC Official Method 961.02; Germicidal Spray Products as Disinfectants (2005) and ASTM E 1053-97 (Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Surfaces).

Microorganism	Number of Inoculated Glass Slides Sprayed with Viking Pure 1000	Number of glass slides containing recoverable infectious/viable virus units*	Positive Control (un-sprayed slide)	Negative Control (un-inoculated sprayed slide)
Poliovirus lsc-1	10	None	Positive CPE	No CPE
Murine Norovirus 1	10	None	Positive CPE	No CPE

* treated and untreated inoculated slides were eluted and inoculated onto BGM or RAW 264.7 cell lines for the detection of polio and MNV-1 respectively. The inoculated cells were allowed were then incubated at 37°C and 5% CO_2. The flasks were monitored regularly for cytopathic effect development throughout an 8 day incubation period.

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Table 2. Inactivation of Poliovirus (Lsc-1) and Murine Norovirus (MNV-1) by the Viking Pure 1000 Spray (Batch # 0001; 284 ppm total chlorine; pH 2.45). Test was conducted as per AOAC Official Method 961.02; Germicidal Spray Products as Disinfectants (2005) and ASTM E 1053-97 (Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Surfaces).

Microorganism	Number of inoculated slides inoculated and sprayed with Viking Pure 1000	Average infectious units (iu)/ml inoculated per slide[#]	Average iu/ml recovered from slides sprayed with Viking Pure 1000*	Percent Reduction	Log_{10} reduction
Poliovirus Lsc-1	**10**	**5.6×10^4**	**<1.0**	**>99.995%**	**>4.75**
Murine Norovirus 1	**10**	**9.2×10^4**	**<1.0**	**>99.9989%**	**>4.96**

[#] This number represents the average number of recovered viruses from glass slides inoculated with viruses, dried, and not exposed to disinfection treatment (positive control).

* Glass slides were inoculated with the indicated microorganisms and allowed to dry. Slides were sprayed to saturation with the disinfectant and allowed to incubate at 22.0°C for ten minutes. Slides were eluted as described in the methodology section. Aliquots of the eluant were inoculated onto BGM or RAW 264.7 cell lines for the detection of polio and MNV-1 respectively. The inoculated cells were then incubated at 37°C and 5% CO_2. The flasks were monitored regularly for cytopathic effect development throughout an 8 day incubation period.

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