



inc.

Biological Consulting Services
of North Florida, Inc.

March 8, 2011

Carl Ricciardi
Altapure LLC
N10569 Spring Creek Rd.
P.O. Box 166
Tomahawk WI, 54487

RE: The Virucidal disinfection efficacy of the Altapure cold sterilant fogging system.

Dear Mr. Ricciardi

We have conducted the antimicrobial efficacy testing on the AltaPure cold sterilant fogging system that was delivered to our Laboratory. The testing was conducted as per AOAC Method 961.02 (AOAC Official methods of Analysis; 2005). The disinfectant efficacy was tested against Poliovirus Lsc1. According to observed results, the tested sanitizer system demonstrated excellent antiviral properties.

In the following pages, you will find a summary of the methodology used and the results of our analysis.

Should you have any questions, 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)

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. Most Probable number infectivity assays (MPN) were performed using dMEM (MediaTech, USA) containing 2 % Fetal Bovine Serum (FBS, Invitrogen) as per methodology outlined in EPA 600/R9-95/178. Cell flasks were incubated at 36.5°C and in 5% CO₂ for 72-96 hours. Infectivity foci on the respective flasks were counted following additional Neutral Red staining.

For Challenge experiments, frozen viral stock (approximately 1 x 10⁸ iu/ml) was thawed rapidly in a 35°C water bath on the day prior to the experiment. The diluted virus stock was tittered by performing serial ten fold dilutions in PBS and inoculation onto the respective cells as described above.

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Positive and negative controls were performed as outlined in the method and as per Good Laboratory Practices. All analysis was performed in accordance to NELAC accreditation standards that are equivalent to ISO 17025.

Supplied disinfectant:

On February 23, 2011, a bottle of AltaPure cold sterilant and a fog generation unit was received at our laboratory. The supplied disinfectant was diluted 1:25 in deionized water. A total of 9.0 liters was produced on the day of the challenge.

Challenge Study: Fogging disinfection efficacy study - Initiated March 01, 2011

The diluted AltaPure cold sterilant was placed into the provided unit. The temperature of the disinfectant prior to application and during disinfection efficacy testing was maintained at 125-135°F.

One hundred microliters of the viral suspension was placed onto each of four sterile shallow glass jars (Fisher 02911763, Thermo-Fisher, USA). Three of the jars were used for the disinfection (Challenge) study and one was used as untreated positive

survival control that was not exposed to the disinfectant (initial). Additionally, one uninoculated jar was used as a negative control. The inoculum was allowed to partially dry at 22°C for 30 minutes. The three inoculated slides and the uninoculated control slide were placed into the treatment chamber and the unit was powered on for 2 minutes to allow the complete saturation of the treatment chamber with the generated fog. The unit was then powered off and the samples were allowed to expose in the chamber for an additional 8 minutes. Following, the chamber was opened and evacuated. The exposed samples were removed. The samples appeared dry to sight and touch. To each jar, 30 ml of a dMEM (MediaTech, USA) supplemented with 2% FBS was added. The treated uninoculated negative control and the inoculated and untreated positive control were treated as described above. The media contained the pH indicator phenol red that demonstrated that the pH remained in the neutral range following elution. The jars were then covered and placed onto an orbital shaker. The jars were agitated on medium-low speed for 10 minutes. The media in the tubes was then assayed for the presence of infectious virus particles by inoculation onto BGM monolayers and the development of Cytopathic Effects (CPE) as described above.

The results of the above study are summarized in the following Table. The results presented pertain only to the samples analyzed and the disinfectant batch number

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indicated in the condition at the time of testing. They are not representative nor are they indicative of a process. All analyses were performed in accordance to NELAC accreditation standards (ISO 17025) unless otherwise noted.

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Table 1. Inactivation of Poliovirus Lsc1 (VR-1562) by the AltaPure Cold Sterilant Fogging Systems. Test was conducted as per AOAC Official Method 961.02; Germicidal Spray Products as Disinfectants (2005)

Microorganism	Number of inoculated carriers treated by the system	Number of carriers demonstrating presence of infectious virus particles***	Positive Control (un-sprayed slide)	Negative Control (un-inoculated slide)
Poliovirus	3*	None** <1.0 virus particle detected (>99.999% Inactivation)	CPE Positive*** Recovered inoculum 1.0 x 10 ⁵	No Viral CPE

* Glass shallow jars were inoculated with approximately 10⁶ viral infectious units of poliovirus Lsc 1 and allowed to dry. The carriers were exposed to the saturated fog generated by the system for a total of 10 minutes at room temperature. The glass carriers were then removed and the viruses were eluted with cell culture media containing 2% FBS. Viral presence and titer was determined using MPN cytopathic effect (CPE) assay on BGM cell monolayers.

**percent inactivation was determined based on the absence of growth; >99.99% inactivation is equivalent to > 5 log₁₀ reduction

*** CPE: Cytopathic effects

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