



BIOLOGICAL CONSULTING SERVICES
OF NORTH FLORIDA, INC.

May 19, 2014

Omni-Lyte Enviro Inc
Robert Hofer
P.O. Box 328
Wawanasa, MB ROK 2GO, Canada
Tel: 204 761 7751

RE: Study report on the efficacy of the provided "Omni-Lyte Enviro Neutral Anolyte Biocide" solution against Porcine Epidemic Diarrhea [PED] virus. BCS1405

Dear Mr. Hofer,

We have conducted the efficacy testing on the solution you shipped to our facility. The testing was conducted as per AOAC Method 961.02 (AOAC Official Methods of Analysis; 2005) in conjunction with ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces". The "Omni-Lyte Enviro Neutral Anolyte Biocide" labeled solution was tested against the Porcine Epidemic Diarrhea (PED) Virus strain that was diagnosed in the United States in mid-May 2013. The solution was >99.9999% effective in inactivating the virus on the contaminated surfaces described in the study.

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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FL DOH LABORATORY #E82924, EPA# FLO1 147
FILE: OMNI-LYTE PED EFFICACY STUDY BCS 1403344.DOC

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Virus Propagation

Porcine epidemic diarrhea virus (PED, CO isolate) was obtained from USDA, National Veterinary Services Laboratories (NVSL, AMES, IA). The virus was propagated in Vero cells. Virus infectivity was measured by the production of Cytopathic Effects (CPE). Quantification was conducted using a Most Probable Number (MPN) analysis. Cell culture enumeration was conducted according an adaptation of “The culturable quantal virus assay” described in the US EPA Manual of Methods for Virology (EPA/600/4-84/013) and documentation from USDA/NVSL laboratory staff.

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

On April 30, 2014, two bottles of a clear solution were delivered to BCS Laboratories from Omni-Lyte Enviro Inc. The solution was issued BCS identifier 1404344. The total chlorine residual was measured by the use of a commercial colometric test. Total chlorine concentration was measured to be 395 ppm (Orbeco Hellige MC500-10 colorimeter). The solution was used within 15 minutes of opening. The temperature of the liquid prior to application and during disinfection efficacy testing was maintained at 20-23°C. The liquid was dispensed as a mist using a handheld spray bottle.

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Study Date: May 8, 2012

One hundred microliters of the viral suspension was placed and spread onto each of 7 sterile 22 mm² glass slides (Propper Scientific, NY). The inoculum was allowed to dry in a covered chamber at 37°C for 30-40 minutes. Five of the seven inoculated slides and an uninoculated slide (neutralization control) were then sprayed for 10 seconds from a distance of approximately 12" with the test solution. The glass slides were completely saturated with sprayed solution. The slides were allowed to incubate at 20-23°C for 30 seconds. Following the contact time, each of the slides were picked up using sterile forceps, the excess liquid was allowed to run off, then was placed into a sterile tube containing 10 ml of buffered peptone water with 0.05% sodium thiosulfate. The two additional slides that were not sprayed were eluted similarly and served as the positive recovery controls (Initial). One hundred microliters of the virus stock suspension was added to the Neutralization control. The tubes were agitated on a tabletop shaker at a medium speed for 10 minutes.

The solution in the tubes was assayed for viral species as previously described. Only 3 of the 5 inoculated and sprayed slides were analyzed. Each sample was diluted (serial 100-fold dilution) and analyzed by plating 5 replicates of 0.1 ml and 1.0 ml samples of the direct,

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1 x 10⁻², and 1 x 10⁻⁴ dilution of each slide eluent. Aliquots were inoculated onto confluent Vero cells cultivated in T-25 (Corning USA) adherent culture flasks. Positive and neutralization controls were plated similarly at 1 x 10⁻² and 1 x 10⁻⁴ dilution. The inoculated flasks were incubated at 36.5°C ±1. The flasks were examined routinely for CPE development and results were recorded. Neutralization control recoveries confirmed the efficient neutralization of the disinfectant residual. The summary data of the study are presented in Table 1.

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 obtained under the laboratory conditions 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

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Table 1. The viral inactivation efficacy of the “Omni-Lyte Enviro Neutral Analyte Biocide” solution (BCS1403344); Germicidal Spray Products as Disinfectants (2005) using a 30 second contact time.

Microorganism	Number of Sprayed Inoculated Slides (number of replicates tested)	Average infectious units (iu) / inoculated per slide[#]	Average iu recovered from each of the slides sprayed*	Percent Reduction	Log₁₀ reduction
Porcine Epidemic Diarrhea virus (PED, CO isolate)	3	2.5 x 10 ⁵	None recovered < .2	>99.9999%	>6.0

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

* Glass slides were inoculated with the indicated microorganisms and allowed to dry. Slides were sprayed to saturation with the solution and allowed to incubate at 20-23.0°C for the indicated time. Slides were eluted and examined for the respective species as described in the methodology section.

