



Biological Consulting Services
of North Florida, Inc.

July 14, 2010

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

RE: Viking Pure 1000 spray disinfection efficacy report.

Dear Mr. Warning,

We have conducted the antimicrobial efficacy testing on the Viking Pure 1000 disinfectant delivered to our Laboratory. The testing was conducted as per AOAC Method 961.02 (AOAC Official methods of Analysis; 2005). The sample of the disinfectant liquid that you provided exhibited excellent antibacterial 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,

George Lukasik, Ph.D.
Laboratory Director

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BCS Laboratories, Inc. -Gainesville
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FL DOH Laboratory #E82924, EPA# FL01147

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

Staphylococcus aureus (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella choleraesuis* (ATCC 10708), *E. coli* O157:H7 (ATCC 43895), *Tricophyton mentagrophytes* (ATCC 9533), and *Listeria monocytogenes* (ATCC 4428) stock cultures were obtained from American Type Culture Collection and maintained as per the described methodology and in AOAC 961.02 and the referenced related AOAC protocols. For Challenge experiments, cultures were grown and purified as per the protocols, incubation temperature and times specified in the specified method. Fungal spores of *Tricophyton mentagrophytes* were produced and purified as outlined in Method 955.17. All media used for microbial growth were manufactured by Beckton Dickinson (Sparks, MD) and were purchased from ThermoFisher Scientific (Waltham, MA). All media and consumables used for this study were new and were purchased specifically for this study. 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. 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 within 5 hours for the microbial spray disinfection studies. Upon opening, the total chlorine residual was

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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 a handheld spray bottle (Fisher Scientific). The test was conducted in a biological laminar flow hood. Cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Listeria monocytogenes*, and *Salmonella choleraesuis* were grown as specified in Method 961.02. The bacterial population in each of the cultures was determined to be greater than 10^7 cfu/ml. Spore suspension of *Tricophyton mentagrophytes* was prepared as per 955.17 and was determined to be 10^6 cfu/ml. Ten-microliters of one of the bacterial or spore suspensions was placed and spread onto sterile 25x25 mm glass slides (Fisher Scientific, PA). Eleven slides of each bacterial or fungal 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 11 inoculated slides were then sprayed for 10 seconds with the provided solutions; the glass slides were completely covered with spray solution. They were then 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 sterile glass tubes containing 20 ml of appropriate growth media. The tubes were then incubated as per method requirements.

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Additionally, un-inoculated slides were used as 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 incubation, the tubes were examined for microbial growth at 48 and 72 hour intervals. Sub cultures were also removed and examined for growth as described in the method. Selected tubes demonstrating “no growth” following the incubation period were inoculated with 10-100 cfu of the respective microorganisms and were observed for growth after 24 hours; this was done to ensure the absence of residual antimicrobial residual effect.

All data is summarized in the following Table. The results presented pertain only to the samples analyzed and 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 bacterial and fungal species 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)

Microorganism	Number of Inoculated Glass Slides Sprayed with Viking Pure 1000	Number of Tubes Demonstrating Growth	Positive Control (un-sprayed slide)	Negative Control (un-inoculated slide)
<i>Staphylococcus aureus</i>	10	None	Growth	No-Growth
<i>Pseudomonas aeruginosa</i>	10	None	Growth	No-Growth
<i>Salmonella choleraesuis</i>	10	None	Growth	No-Growth
<i>Tricophyton mentagrophytes</i>	10	None	Growth	No-Growth
<i>Listeria monocytogenes</i>	10	None	Growth	No-Growth
<i>E. coli</i> O157:H7	10	None	Growth	No-Growth

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Table 2. Inactivation of bacterial and fungal species 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)

Microorganism	Number of inoculated slides inoculated and sprayed with Viking Pure 1000	Average microorganism cfu/ml inoculated per slide[#]	Average cfu/ml recovered from slides sprayed with Viking Pure 1000*	Percent Reduction	Log₁₀ reduction
<i>Staphylococcus aureus</i>	10	>1.0 x 10 ⁵	<1.0	>99.999%	>5.0
<i>Pseudomonas aeruginosa</i>	10	>1.0 x 10 ⁵	<1.0	>99.999%	>5.0
<i>Salmonella choleraesuis</i>	10	>1.0 x 10 ⁵	<1.0	>99.999%	>5.0
<i>Listeria monocytogenes</i>	10	>1.0 x 10 ⁵	<1.0	>99.999%	>5.0
<i>E. coli</i> O157:H7	10	>1.0 x 10 ⁵	<1.0	>99.999%	>5.0
<i>Tricophyton mentagrophtes</i>	10	>1.0 x 10 ⁴	<1.0	>99.99%	>4.0

[#] This number represents the average number of microorganisms from glass slides inoculated, 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 and examined for growth as described in the methodology section.