Biological Consulting Services
of North Florida, Inc.

February 09, 2012

Syd Williams
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16026 University Oak
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RE: Trio Spray Disinfection Efficacy Study Report.,

We have conducted the antimicrobial efficacy testing on the liquid produced by the provided Trio system. The testing was conducted as per AOAC Method 961.02 (AOAC Official Methods of Analysis; 2005), ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces", and ASTM E2111-00 "Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal and Sporicidal Potencies of Liquid Chemical Germicides". Based on the observed results, the generated disinfectant liquid exhibited excellent antibacterial, sporocidal, and antiviral efficacy.

Additionally, the disinfestation efficacy rate of the generated disinfectant was tested using ASTM 2315-08 "Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure". The generated disinfectant inactivated *E. coli* O157:H7 and *Listeria monocytogenes* by >99.999% within 10 seconds of contact.

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,

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Laboratory Director

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Table 1. The bacterial disinfection efficacy of the disinfectant liquid generated by the provided Trio unit. Test was conducted as per AOAC Official Method 961.02; Germicidal Spray Products as Disinfectants (2005)

Microorganism	Number of Sprayed Inoculated Slides	Number of Tubes Demonstrating Growth	Positive Control (un-sprayed slide)	Negative Control (un-inoculated slide)
Staphylococcus aureus (MRSA)	**10**	**None**	**Growth**	**No-Growth**
Salmonella enterica	**10**	**None**	**Growth**	**No-Growth**
Listeria monocytogenes	**10**	**None**	**Growth**	**No-Growth**
Pseudomonas aeruginosa	**10**	**None**	**Growth**	**No-Growth**
***E. coli* O157:H7**	**10**	**None**	**Growth**	**No-Growth**

* 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 20-22.0°C for ten minutes. Slides were eluted and examined for growth as described in the methodology section.

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Table 2. The bacterial disinfection efficacy of the disinfectant liquid generated by the provided Trio unit; Germicidal Spray Products as Disinfectants (2005)

Microorganism	Number of Sprayed Inoculated Slides (number of replicates tested)	Average microorganism cfu/ml inoculated per slide[#]	Average cfu/ml recovered from each of slides sprayed*	Percent Reduction	Log_{10} reduction
Staphylococcus aureus (MRSA)	**10**	**$>1.0 \times 10^5$**	**<1.0**	**>99.999%**	**>5.0**
Salmonella enterica	**10**	**$>1.0 \times 10^5$**	**<1.0**	**>99.999%**	**>5.0**
Listeria monocytogenes	**10**	**$>1.0 \times 10^5$**	**<1.0**	**>99.999%**	**>5.0**
Pseudomonas aeruginosa	**10**	**$>1.0 \times 10^5$**	**<1.0**	**>99.999%**	**>5.0**
***E. coli* O157:H7**	**10**	**$>1.0 \times 10^5$**	**<1.0**	**>99.999%**	**>5.0**

[#] This number represents the average number of microorganisms recovered 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 solution and allowed to incubate at 20-22.0°C for ten minutes. Slides were eluted and examined for growth as described in the methodology section.

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Table 3. The viral disinfection efficacy of the disinfectant liquid generated by the provided Trio unit. Test was conducted as per AOAC Official Method 961.02; Germicidal Spray Products as Disinfectants (2005) and ASTM E1053 "Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces"

Microorganism	Number of Sprayed Inoculated Slides (number of replicates tested)	Average infectious particles (iu) /ml inoculated per slide[#]	Average iu/ml recovered from each of slides sprayed*	Percent Reduction	Log_{10} reduction
Murine Norovirus MNV-1 (Human Norovirus Surrogate)	5	4.6×10^4	<0.5	>99.999%	>5.0
Poliovirus CHAT Lsc1	5	1.3×10^5	<0.5	>99.9999%	>6.0

[#] This number represents the average number of infectious virus particles recovered 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 solution and allowed to incubate at 20-22.0°C for ten minutes. Slides were eluted and enumerated for infectious viral particles on respective cell monolayers as described in the methodology section.

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Table 4. Inactivation of *E. coli* O157:H7 and *Listeria monocytogenes* at the indicated time points following introduction to the disinfectant liquid produced by the Trio unit.

Microorganism	Bacterial cfu/ml at the indicated time points following study start[1]								
	0 (control)	10 seconds	30 seconds	60 seconds	90 seconds	120 seconds	180 seconds	5 minutes	Control (Final)
***E. coli* O157:H7**	2.9×10^6	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log_{10} reduction)	**2.9×10^6**
Listeria monocytogenes	1.1×10^6	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log_{10} reduction)	<1.0 (>99.9999% or >6 Log	**8.9×10^5**

[1] Aliquots of the above bacteria were added to 200 ml of Class I ASTM water (Control) and 200 ml of the liquid generated by the Trio unit. The flasks containing the liquids were agitated on an orbital shaker at a medium speed. At each of the indicated time points following the start of the study, samples were removed, neutralized and assayed for the bacterial species by spread plating onto TSA and incubation at 36.5°C for 24-36 hours.

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