



inc. **Biological Consulting Services**
of North Florida, Inc.

March 6, 2007

RE: Antibacterial Efficacy Testing of Formula 080 (sold as Enviro-One Multi-Use Green Ceaner.) Liquid Soap (Lot# 011102-05).

Dear Ms. DiAire:

Greetings.

We have conducted the antimicrobial efficacy study that we proposed previously. Following, you will find our report on the analysis that was conducted.

Should you have any questions please do not hesitate to contact me.

Sincerely,

George Lukasik, Ph.D.
Sr. Laboratory Director

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Analysis Parameter: Bacterial killing efficacy of Formula 080 (sold as Enviro-One Multi-Use Green Cleaner) liquid soap (Lot# 011102-05).

Four bottles of Formula 080 (sold as **Enviro-One Multi-Use Green Cleaner**) liquid soap (Lot# 011102-05) were delivered to our laboratory on February 23rd, 2007

Methodology: Analysis was conducted as per the proposal submitted on February 20th, 2007 and the following phone conversations with Ms. D'Áire. Briefly, the bacteria *Escherichia coli* C3000 (ATCC 15597), and *Salmonella typhimurium* were grown up overnight in Tryptic Soy Broth (Beckton Dickinson, MD) at 37°C the day prior to the experiment. Dilutions of the supplied soap stock solution were prepared in standard reagent grade water as follows: 1:5, 1:10, 1:100, and reagent water was used as the negative control. Aliquot (0.01 ml) of each of the bacterial overnight cultures was added to 100 ml of the above prepared solutions. The solutions were agitated on an orbital shaker on low speed for 5 minutes and one hour. At the previously specified time intervals, aliquots of the solutions were removed and the number of bacteria (as colony forming units; cfu) present in each solution was determined by spread plating onto MacConkey Agar (Beckton Dickinson, MD). All assays were performed in duplicates. In The following table you will find the summary of the results

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Table 1. Efficacy of Formula 080 (sold as Enviro-One Multi-Use Green Cleaner) liquid soap (Lot# 011102-05) on the inactivation of *Escherichia coli* C3000 (ATCC 15597), and *Salmonella typhimurium* after 5 and 60 minutes of agitation.

Treatment	<i>Escherichia coli</i> C3000		<i>Salmonella typhimurium</i>	
	Average cfu/ml*	Efficacy (percent reduction as compared to water control)	Average cfu/ml*	Efficacy (percent reduction as compared to water control)
1:5 5 minutes	$<5.0 \times 10^2$	>99%	$<5.0 \times 10^2$	>99%
1:10 5 minutes	$<5.0 \times 10^2$	>99%	$<5.0 \times 10^2$	>99%
1:100 5 minutes	1.9×10^4	74.3%	5.6×10^4	70.5%
Water (Control) 5 Minutes	7.4×10^4	Control	1.9×10^5	Control
1:5 60 minutes	$<1.0 \times 10^2$	>99%	$<1.0 \times 10^2$	>99%
1:10 60 minutes	$<1.0 \times 10^2$	>99%	$<1.0 \times 10^2$	>99%
1:100 60 minutes	3.1×10^4	54.4%	4.1×10^4	65.8%
Water (Control) 60 Minutes	6.8×10^4	Control	1.2×10^5	Control

*cfu/ml = Colony Forming units of bacteria per milliliter of seeded soap/water solution

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