Biological Consulting Services
of North Florida, Inc.

April 16, 2009

Aphex BioCleanse Systems, Inc.

Dear Sirs,

Greetings.

We have completed antimicrobial efficacy study on the supplied Multi-Purpose Solution. The testing was done according to the protocol we briefly discussed and have used previously in disinfectant studies. According to the observed results Multi- Purpose Solution exhibited exceptional antimicrobial properties. 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,

Dr. George Lukasik

1);g!1dy-byO. G<o<g<l..lik
ON:cn_Or.Gtofoclub:sik.o.o:BCSLaboRt0t nemlle.ou::La.boritor)l»cc1ew.
t:!niihlID5ikep,or t. US
1Ri 2009.o6..3009-.S2:10 YX1

George Lukasik, Ph.D.
Laboratory Director

BCS Laboratories, Inc. -Gainesville
4641 NW 6th Street, Suite C, Gainesville, Florida 32609,
Tel. (352) 377-9272, Fax. (352) 377-5630
www .microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

Bacterial Culture Preparation

E.coli (ATCC 15597), *Salmonella enterica* (ATCC BAA-711), and Methicillin Resistant *Staphylococcus aureus* (MRSA; BAA-44) stock cultures were obtained from American Type Culture Collection and are maintained at -80°C. For Challenge experiments, overnight cultures from colony purified frozen stocks were grown in 10 ml of Tryptic Soy Broth (TSB, Beckton Dickinson, MD) at 36 °C prior to the date of the experiments. At the Day of Challenge, the broth cultures were centrifuged at 3K x G for 5 minutes and suspended in 10 ml of phosphate buffered saline (PBS, Fisher scientific, PA). This was repeated and the pellet was suspended in 10 ml PBS. A 1/100 dilution of the suspended bacteria was then performed in PBS supplemented with 1% fetal bovine serum (FBS, Atlanta Biologicals, GA). The *Salmonella* and *E. coli* cultures were mixed together and the MRSA diluted culture was kept separate.

Challenge Study; April 09, 2009

Two hundred and fifty microliters of either of the above culture dilution was evenly spread on the surface of 50 mm plastic Petri dishes. Each plate was inoculated either with the MRSA culture or the *Salmonella* and *E. coli* culture. The Petri dishes were then allowed to incubate for 20 minutes at 25° C. Following, 500 µl of Multi-Purpose Solution was spread evenly onto the inoculated plates and allowed to incubate for a total contact time of 3 minutes. Four milliliters of Neutralizing Buffer

BCS Laboratories, Inc. -Gainesville
4641 NW 6th Street, Suite C, Gainesville, Florida 32609,
Tel. (352) 377-9272, Fax. {352) 377-5630
www.microbioservices .com
FL DOH Laboratory #E82924, EPA# FL01147

(Beckton Dickinson, MD) was added to each plate at the end of the 3 minute contact time. The liquid on each plate was agitated by repeated pipetting. The liquid was removed from each plate and placed in a sterile 50 ml centrifuge tube (Fisher scientific, PA) containing 15 ml sterile Neutralizing Buffer. Ten fold dilutions of the bacterial suspensions were performed in PBS. The number of viable microorganisms in each of the tubes was enumerated by spread plating onto Brain Heart Infusion Agar (Beckton Dickinson, MD) and incubating at 37°C for 24-48 hours. All analysis was conducted in triplicates. Plates containing the bacterial cultures and no Multi-Purpose Solution treatment were used as negative controls. The recovered bacterial count from the negative control plates was used to calculate challenge concentration and log10 reduction. Table 1 below presents the results of the above-mentioned test.

BCS Laboratories, Inc. -Gainesville
4641 NW 6th Street, Suite C, Gainesville, Florida 32609,
Tel. (352) 377-9272, Fax. (352) 377-5630
www .microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

Table 1. The efficacy of pathogen inactivation by the Multi-Purpose Solution following 3 minute contact time.

Treatment	Microorganism (cfu/ml)		
	MRSA	*E.coli*	S. *enterica*
Untreated Control (initial)	1.0×10^6	9.3×10^5	1.1×10^6
Multi-Purpose Solution (3 min contact)	<0.5	<0.5	<0.5
Lg10 Reduction	>610910	>610910	>610910

BCS Laboratories, Inc.-Gainesville
4641 NW 6th Street, Suite C, Gainesville, Florida 32609,
Tel. (352) 377-9272, Fax. (352) 377-5630

www .microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147