



**Biological Consulting Services**  
*of North Florida/ Inc.*

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May 13, 2009

Aphex BioCleanse Systems, Inc.

Dear Sirs,

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. We compared the efficacy of the provided Multi- Purpose Solution against the efficacy of commercially available alcohol based hand sanitizers as per your request. The efficacy was determined against **Methicillin- resistant Staphylococcus aureus (MRSA)**. MRSA is a bacterium responsible for difficult- to-treat infections in humans. MRSA was chosen for this study due to its relative resistance to common sanitizers and its reputation as a "super bug". MRSA strains have rapidly become the most common cause of cultured skin infections among individuals seeking emergency medical care for these infections in urban areas of the United States.

According to the observed results Multi-Purpose Solution exhibited exceptional antimicrobial properties that out performed top selling alcohol based hand sanitizers. 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 George Lukasik, Ph.D. Laboratory Director

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## **Bacterial Culture Preparation**

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 culture from colony purified frozen stock was 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 5 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).

## **Hand Sanitizers**

Purell® and Germ-X® were purchased at a local supply store on May 11, 2009. Multi- Purpose Solution was provided by Aphex BioCleanse Systems on May 01, 2009.

## **Challenge Study; May 11, 2009**

One hundred microliters of the above culture dilution was evenly spread on the surface of 50 mm plastic Petri dishes. The Petri dishes were then allowed to incubate for 30

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minutes at 25° C. Following, 200 µl of one of the following sanitizers: Multi-Purpose Solution, Purell® or Germ-X® was added to the MRSA bacteria inoculated plates. Immediately following addition the sanitizer was spread evenly onto the inoculated plates to ensure full contact. Sterile cell scrapers (Nunc, USA) were used to spread the sanitizer onto the plates. The plates were then allowed to incubate for a total contact time of either 30 seconds or 180 seconds. Four milliliters of Neutralizing Buffer (Beckton Dickinson, MD) was added to each plate immediately at the end of the 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. Additionally plates containing the bacterial cultures and no antimicrobial hand sanitizer treatment were used as negative controls. 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. The recovered bacterial count from the negative control plates was used to calculate challenge concentration and percent reductions. Table 1 below presents the results of the above- mentioned test.

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Table 1. The efficacy of Multi-Purpose Solution, Purell®, and Germ-X® hand sanitizers on the inactivation of MRSA pathogen following 30 seconds of contact time and 3 minutes (180 seconds).

Treatment	Average MRSA bacterial count (cfu/ml)		
	Purell®	Germ-X®	Multi-Purpose Solution
Untreated Control (initial)	$1.7 \times 10^5$		
30 second contact time	$4.0 \times 10^2$	$4.5 \times 10^2$	$3.3 \times 10^0$
Percent reduction	99.8%	99.7%	99.998%
180 second contact time	$2.0 \times 10^1$	$2.3 \times 10^1$	$<1.0 \times 10^0$ (none detected)
Percent reduction	99.99% <sup>1</sup>	99.99% <sup>1</sup>	> 99.999%

\*Data represents an average of three trials for each test point. Colony forming units (cfu) of MRSA (ATCC BAA-44) were enumerated by spread plating onto BHI agar and overnight incubation at 37°C.

<sup>1</sup>Typically, alcohol based sanitizers do not last more than 30 seconds on the skin of human hands due to the effects of temperature and evaporation. Thus, the results indicated with alcohol sanitizers at the 180 second contact time would unlikely be attained.

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