Biological Consulting Services
of North Florida, Inc.

August 29,2010

Aphex BioCleanse Systems

Dear Sirs,

We have completed the antimicrobial efficacy study on the supplied Dermaphex sanitizer. The testing was done according to the protocol we regularly use to assess antimicrobial efficacy of spray disinfectants . The protocol is based on test methods described in AOAC Official Method 961.02 (Germicidal Spray Products as Disinfectants) and from ASTM E2111-00 (Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal and Sporicidal Potencies of Liquid Chemical Germicides).

The disinfectant efficacy was tested against the NDM-1 carbapenem antibiotic resistant strain of *Klebsiella pneumoniae.* According to observed results, the tested sanitizer demonstrated 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 RegardsDr. **George Lukasik**

Digitally signed by Dr. George Lukasik
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Challenge Bacterial Culture Preparation and Enumeration

Klebsiella pneumoniae (ATCC BAA-1705; Lot 58521529) stock culture was obtained from ATCC was maintained at -70°C. Working cultures were kept and propagated on Tryptic Soy Agar (TSA, Beckton Dickinson, MD). For challenge experiments, an overnight culture from colony purified plate 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 culture was centrifuged at 3K x G for 5 minutes and suspended in 10 ml of phosphate buffered saline (Pl3S, Fisher scientific, PA).

The number of viable bacterial species was enumerated as colony forming units (cfu) using spread plating onto Tryptic Soy Agar. Plates were incubated at 36.5° C for 24 hours.

Supplied disinfectant:

On July 21, 2010 a container labeled Dermaphex Foam Lot. 1001301 was received at our laboratory from Aphex Biocleanse Systems, Inc. The sample was assigned BCS ID# 1008036.

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***K/ebsiella pneumoniae* Challenge Study: Spray disinfection efficacy study - Initiated August 27, 2010**

The supplied Aphex BioCleanse Systems' DermAphex Disinfectant was placed into a clean handheld sprayer. The temperature of the disinfectant prior to application and during disinfection efficacy testing was maintained at 21-22°C. Forty-microliters of the bacterial suspension was placed onto sterile 25 cm^2 glass slides (Fisher Scientific, PA). A total of 7 slides were inoculated; 5 slides were used for the spray disinfection (Challenge) and 2 slides were used as untreated positive growth control that was not exposed to the spray wash (initial). Additionally, one un-inoculated slide was used as a negative growth control. The inoculum was allowed to partially dry at 22°c for 30 minutes. Five of the seven inoculated slides and the uninoculated control slide were sprayed for 1O seconds from a distance of 12" with the SanAphex sanitizer. The slides were evenly saturated with the disinfectant. The slides were allowed to incubate at 22°C for 3 minutes. Immediately following incubation, each slide was aseptically removed, the excess fluid was shaken off, and the slide was placed into a sterile 50 ml tube (Fisher scientific, PA) containing 10 milliliters of Letheen Broth (Beckton Dickinson, MD). The tubes were agitated for 15 minutes on a horizontal plate mixer at a medium speed. The sprayed uninoculated negative control slide and the inoculated and unsprayed positive control slide were treated as described above. Ten fold dilutions of the recovered microbial suspensions were

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performed in PBS. The number of viable bacterial species in each of the tubes was enumerated by spread plating onto TSA as described. All analysis for each sample was conducted in duplicates. Table 1 presents the results of the above-mentioned test.

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Table 1. The disinfection efficacy of Aphex BioCleanse Systems' DermAphex Disinfectant Spray on the inactivation of *Klebsiella pneumoniae* (NDM-1) inoculated onto glass slides; Testing was conducted as per AOAC Official Method 961.02 (Testing of Germicidal Spray Products as Disinfectants) using an exposure time of 3 minutes.

Treatment	Average number of recovered *Klebsiella pneumoniae* NDM-1 (cfu/ml)	Percent Reduction
Untreated Control (initial)***	9.6×10^5	Not Applicable
Trial 1	<5.0**	>99.9995%
Trial 2	<5.0	>99.9995%
Trial 3	<5.0	>99.9995%
Trial 4	<5.0	>99.9995%
Trial 5	<5.0	>99.9995%

*The number of viable bacterial colonies was determined by spread plating onto Tryptic Soy Agar (TSA, Beckton Dickinson, MD). Plates were incubated at 36.5° C for 24 hours.
** No bacterial colonies were observed on any of the plates. None detected for all 5 spray disinfected slides when 0.2 ml inoculum was assayed.
***Untreated Controls represent the microorganisms recovered from glass slides unexposed to the disinfectant treatment (positive controls). Uninoculated negative control plates did not demonstrate any bacterial growth when plated.