



Biological Consulting Services
of North Florida, Inc.

October 14, 2010

Aphex BioCleanse Systems, Inc.

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 Sporocidal Potencies of Liquid Chemical Germicides).

The disinfectant efficacy was tested against *Clostridium difficile* (ATCC 9689). The test results indicate that 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 Regards,

George Lukasik, Ph.D.
Laboratory Director

- Page 1 of 5-

BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Building A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

Challenge Bacterial Culture Preparation and Enumeration

Clostridium difficile (ATCC 9689) stock culture was obtained from ATCC was maintained at -80°C. Working cultures were kept and propagated on Brain Heart Infusion (BHI) Agar containing 10% sheep blood (Beckton Dickinson, MD) anaerobically. For challenge experiments, a 48 hour culture from colony purified plate stock was grown in 10 ml of Brain Heart Infusion (BHI) Broth (Beckton Dickinson, MD) at 36 °C anaerobically. 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 (PBS, Fisher scientific, PA).

The number of viable bacterial species was enumerated as colony forming units (cfu) using spread plating onto BHI Agar containing 10% Sheep Blood (Beckton Dickinson, MD). Plates were incubated in an anaerobic atmosphere at 36.5° C for 96 hours.

Supplied disinfectant:

On August 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.

Challenge Study: Spray disinfection efficacy study - Initiated October 05, 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 cm² 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 10 seconds from a distance of 12" with the disinfectant. 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 Lethen 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 performed in PBS. The

- Page 3 of 5-

BCS Laboratories, Inc. -Gainesville
4609 NW 6th Street, Building A, Gainesville, Florida 32609
Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

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

Table 1. The disinfection efficacy of Apex BioCleanse Systems' Dermaphex Disinfectant Spray on the inactivation of *Clostridium difficile* 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 <i>Clostridium difficile</i>	Percent Reduction
Untreated Control (initial)***	3.3 x 10 ³	Not Applicable
Trial 1	<1.0**	>99.97%
Trial 2	<1.0	>99.97%
Trial 3	<1.0	>99.97%
Trial 4	<1.0	>99.97%
Trial 5	<1.0	>99.97%

*The number of viable bacterial colonies was determined by spread plating onto BHI Agar (Beckton Dickinson, MD). Plates were incubated at 36.5° C anaerobically for 96 hours.

** No bacterial colonies were observed on any of the plates. None detected for all 5 spray disinfected slides when 0.5 ml inoculum was assayed induplicate.

*** 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.