



Biological Consulting Services
of North Florida, Inc.

May 5, 2009

Aphex BioCleanse Systems, Inc.

Dear Sirs,

We have completed the antiviral efficacy study on the supplied Dermaphex hand Sanitizer. The testing was done according to the protocol we briefly discussed and have used previously in disinfectant studies. The protocol used is comparable to ASTM E 1053-97 (Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Surfaces). Rhinovirus 39 (ATCC VR-340) was used as the challenge organism. According to the observed results the supplied Dermaphex Hand Sanitizer exhibited significant antiviral 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,

George Lukasik, Ph.D.
Laboratory Director

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Stock Virus and Cell Culture Preparation

Rhinovirus 39 (ATCC VR-340) stock cultures were obtained from American Type Culture Collection and are maintained at -80°C. Rhinovirus was propagated and enumerated as most probable number (MPN) on HeLa cell monolayers (ATCC CCL-2). Cells were grown in 6 well cell culture plates. For enumeration, aliquots of a sample containing Rhinovirus are inoculated on freshly prepared monolayers of HeLa monolayers. The cells are then incubated in dMEM (MediaTech, USA) media at 33°C and 5% CO₂ for 3-7 days. Cells are monitored routinely microscopically for signs of degeneration. Cells in wells demonstrating signs of infectivity (Cytopathic effects; CPE) are recorded as positive (+) and one that do not demonstrate any CPE are recorded as negative (-). The most probable number of infectious virus in a sample is then calculated using MPNCALC software (version 0.0.0.23). For Challenge experiments, virus stocks (typically 5×10^7 pfu/ml) are thawed at the day of experiment. They are then diluted 1/100 in Class 1 ASTM reagent water supplemented with 1% fetal bovine serum (FBS, Atlanta Biologicals, GA).

Challenge Study; April 28, 2009

The protocol used is comparable to ASTM E 1053-97 (Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Surfaces). Briefly, two hundred and fifty microliters of the above virus dilution was evenly spread on the surface of 50 mm

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plastic Petri dishes. The Petri dishes were then allowed to incubate for 10 minutes at 25° C. Following, 500 µl of Dermaphex was spread evenly onto the inoculated plates using a cell scraper/spreader. The plates were then allowed to incubate for a total contact time of 3 minutes while being agitated. Four milliliters of Neutralizing Buffer (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 viral suspensions were performed in PBS. The number of viable (infectious) Rhinovirus in each of the tubes was enumerated by MPN procedure described above. All analysis was conducted in triplicates. Plates containing viral inoculums and no Dermaphex treatment were used as negative controls. The recovered viable viral mpn from the negative control plates were used to calculate challenge concentration and percent reduction. Table 1 below presents the results of the above-mentioned test.

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Table 1. The efficacy of Rhinovirus 39 (ATCC VR-340) inactivation by the Dermaphex hand sanitizer during a 3 minute contact time.

Treatment	Rhinovirus 39 Average mpn/ml*
Untreated (Negative Control)	6.7 x 10⁵
Dermaphex	4.8 x 10⁰
Percent Reduction	99.993 %

*Data represents an average of three trials for each test point. Most Probable Number (MPN) of rhinovirus were enumerated using EPA ICR comparable Methodology (EPA 600/R-95/178, 1998). For enumeration, aliquots were inoculated on freshly prepared monolayers of CCL-2 cells and CPE was checked during a 7 day incubation period Cells were incubated at 33°C in a 5% CO₂ atmosphere.

Table 2. Raw data used to calculate the MPN data presented in Table 1. Data represents the analysis performed on the samples as described in the laboratory procedure section. Analysis conducted for Dermaphex following 3 minute incubation on rhinovirus 39

Sample	Number of positive (infected) wells / total number of wells inoculated at each of the dilutions below (0.1 mL sample inoculated in each well)				
	Direct	1/10	1/100	1/1000	1/10000
Control	ND	ND	5/5	5/5	2/5
Control	ND	ND	5/5	5/5	2/5
Control	ND	ND	5/5	5/5	3/5
Treated	3/5	0/5	0/5	ND	ND
Treated	1/5	0/5	0/5	ND	ND
Treated	2/5	0/5	0/5	ND	ND