Biological Consulting Services
of North Florida, Inc.

May 20, 2009

Aphex BioCleanse Systems, Inc.

Dear Sirs,

We have completed the antiviral efficacy study on the supplied Multi-Purpose Solution with Hy-IQ. 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). Influenza A (H1N1) virus was used to determine antiviral efficacy of disinfection.

According to the observed results the supplied Multi-Purpose Solution with Hy-IQ exhibited significant antiviral properties. It completely killed the exposed viral inoculums on the treated surfaces. 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

BCS Laboratories, Inc. -Gainesville
4641 NW 5th Street, Suite C, Gainesville, Florida 32609,

Tel. (352) 377-9272, Fax. (352) 377-5630
www.microbioservices.com
FL DOH Laboratory #E82924, EPA# FL01147

Stock Virus and Cell Culture Preparation

Influenza A (H1N1; ATCC VR-1469) virus was propagated and enumerated as Most Probable Numbers (MPN) using MDCK cell monolayers (ATCC CCL-34) as the host. Cells were grown in 6 well cell culture plates. For enumeration, aliquots of a sample containing the virus are inoculated on freshly prepared monolayers of MDCK monolayers. The cells are then incubated in dMEM (MediaTech, USA) media containing trypsin at 35°C and 5% C02 for 3-8 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 ones 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 2 x 10^6 pfu/ml) are thawed at the day of experiment. They are then diluted 1/10 in Class 1ASTM reagent water supplemented with 1% Bovine Serum Albumin (BSA).

Challenge Study; May 12, 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 plastic Petri dishes. The Petri dishes were then allowed to incubate for 30 minutes at 25° C. The plates were semi-dry following the incubation. Following, 500 µl of Multi-

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Purpose Solution with Hy-IQ 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) Influenza A in each of the tubes was enumerated by MPN procedure described above. All analysis was conducted in triplicates. Plates containing viral inoculums and no Multi-Purpose Solution with Hy-IQ treatment were used as positive controls. The recovered viable viral mpn from the positive controlplates were used to calculate challenge concentration and percent reduction. Table 1below presents the results of the above-mentioned test.

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4641 NW 5th Street, Suite C, Gainesville, Florida 32609,
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Table 1. The efficacy of Multi-Purpose Solution with Hy-IQ Sanitizer on the inactivation of Influenza A (H1N1; ATCC VR-1469) during a 3 minute contact time.

Treatment	Influenza A Average mpn/ml*
Untreated (Negative Control)	3.1×10^4
Multi-Purpose Solution with Hy-IQ	<2.2
Percent Reduction	>99.993 %

*Data represents an average of three trials for each test point. Most Probable Number (MPN) of Influenza A virus was enumerated using EPA ICR comparable Methodology (EPA 600/R-95/178, 1998). For enumeration, aliquots were inoculated on freshly prepared monolayers of MOCK (CCL-34) cells and CPE was checked during a 7 day incubation period Cells were incubated at 35°C in a 5% C02 atmosphere.

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