



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

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September 17, 2010

PetAirapy, LLC  
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Re: Antimicrobial efficacy testing of the PetAirapy surface and air disinfection system (PA-260).

We have completed the antimicrobial efficacy study on the PA-260 UVGI system. The testing was done according to the protocol we regularly use to assess antimicrobial efficacy of 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 various animal and human pathogens that are transmissible through contaminated surfaces or air. According to observed results, the tested system demonstrated excellent antibacterial and antiviral efficacy. It should effectively reduce and control the transmission of pathogens when used as directed. We are currently testing the efficacy of the system on the inactivation of parvovirus. The results should be comparable to the reported results. We will provide that information and update the report as the data becomes available.

In the following pages, you will find a summary of the methodology used and the results of our analysis. Should you have any questions please do not hesitate to contact me.

Best Regards,  
George Lukasik, Ph.D.  
Director

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## **Challenge Bacterial Culture Preparation and Enumeration**

*Bordetella bronchiseptica* (ATCC 10580) and Methicillin resistant *Staphylococcus aureus* (MRSA; BAA-44) stock cultures were obtained from American Type Culture Collection (ATCC) and was maintained at -80°C. Working cultures were both 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. On 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 Tryptic Soy Agar. Plates were incubated at 36.5° C for 24 hours.

## **Stock Virus and Cell Culture Preparation**

Stock cultures of the viruses below were obtained from ATCC and propagated to a high titer on their respective indicated cell lines. Viral stocks were maintained at -80°C. Poliovirus Lsc1 Chat strain (ATCC VR-1562) was propagated and enumerated as plaque forming units (pfu) using EPA ICR Methodology (EPA 600/R-95/178). Canine Influenza A (H3N8; ATCC VR-1469) virus was propagated and enumerated as Most Probable Numbers (MPN) using Madin-Darby Canine Kidney type I (MDCK) cell

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monolayers (ATCC CCL-34) as the host. Simian Rotavirus SA-11 (ATCC VR-1565) was propagated and enumerated as Most Probable Numbers (MPN) using MA104 cell monolayers (ATCC CRL-2378) as the host. Feline Calicivirus; Strain: F-9 (ATCC VR-782) virus was propagated and enumerated as Most Probable Numbers (MPN) using a feline (*Felis catus*) renal cell line (CRFK) cell monolayers (ATCC CCL-94) as the host. The Most Probable Number of infectious virus in a sample was calculated using MPNCALC software (version 0.0.0.23).

For Challenge experiments, frozen viral stock (typically  $1 \times 10^8$  iu/ml) was thawed rapidly in a 35°C water bath on the day prior to the experiment. Stock was then diluted 1/10 in Phosphate Buffered Saline (PBS) supplemented with 2% Bovine Serum Albumin (BSA) and used for the viral challenge experiment below. The diluted virus stock was tittered by performing serial ten fold dilutions in PBS and inoculation onto the respective cells as described above.

**Supplied disinfection system:**

The PA-260 UVGI system was received at our laboratory from PetAirapy, Inc. The system was assembled using the supplied lamps.

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### **Challenge Study:**

The supplied PA-260 UVGI unit was mounted onto a vertical support beam. The unit was located 50 cm between the horizontal plane of the UVGI lamps and the exposure surface. Temperature was maintained at 21-22°C. Ten-microliters of the microbial suspension were placed onto each sterile 20 x 26 mm glass slides (Fisher Scientific, PA). Four slides were inoculated for each microbial species; 3 slides were used for the UVGI disinfection (Challenge) and one as untreated positive growth control (not exposed to the UV; initial). Inoculated glass slides were dried at 22°C for 30 minutes. Three of the four inoculated slides were placed onto the surface below the UVGI lamps. The slides were placed in the center between the lamps 50 cm away from the horizontal plane of the lamps. The slides were exposed to the UVGI for 60 seconds. Following, the slides was aseptically removed and placed into a sterile 50 ml tube (Fisher scientific, PA) containing 5.0 milliliters of TSB (Beckton Dickinson, MD). The tubes were agitated for 15 minutes on a horizontal plate mixer at a medium speed. The inoculated positive control slide was treated also as described above. Ten fold dilutions of the recovered microbial suspensions were performed in PBS. The number of viable bacterial species in each of the tubes was enumerated by spread plating onto TSA as described. Viral analysis was conducted as described above. Analysis for each sample was conducted in duplicate. All analysis was performed in accordance to NELAC accreditation standards that are equivalent to ISO 17025. Table 1 presents the results of the above-mentioned tests.

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**Table 1. Inactivation of human and animal pathogens by the PetAirapy PA-260 UVGI surface and air disinfection system. Test was conducted as per AOAC Official Method 961.02; Germicidal Spray Products as Disinfectants (2005) and ASTM E1053-97 (Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Surfaces).**

Microorganism	Number of inoculated slides exposed to UVGI	Average infectious units (iu)/ml inoculated per slide <sup>#</sup>	Average iu/ml recovered from UVGI Treated slides*	Percent Reduction
Enterovirus (Poliovirus)	3	5.6 x 10 <sup>4</sup>	<1.0	>99.998%
Rotavirus SA11	3	1.6 x 10 <sup>4</sup>	<1.0	>99.994%
Feline Calicivirus F-9	3	2.3 x 10 <sup>4</sup>	<1.0	>99.996%
Canine Influenza H3N8	3	1.6 x 10 <sup>4</sup>	<1.0	>99.994%
Methicillin Resistant <i>Staphylococcus aureus</i> (MRSA)	3	2.9 x 10 <sup>5</sup>	<5.0	>99.998%
<i>Bordetella bronchiseptica</i>	3	3.1 x 10 <sup>4</sup>	1.8 x 10 <sup>1</sup>	99.94%

<sup>#</sup> This number represents the average number of recovered microorganisms from glass slides inoculated with pathogens, dried, and not exposed to disinfection treatment (positive control).

\* Glass slides were inoculated with the indicated microorganisms and allowed to dry. Slides were exposed to the UVGI radiation at 22.0°C for 60 seconds. Slides were eluted as described in the methodology section. Aliquots of the eluant were inoculated onto respective media or cell culture lines for the detection and enumeration of pathogens. All analysis was conducted in duplicate.

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