



February 21, 2020

SanUVAire, LLC  
6435 W. Quaker St.  
Orchard Park, NY 14127

Re: Evaluation of the antimicrobial efficacy of the **Breathe-Safe & Surface-Safe Systems** utilizing Ultra-Violet Germicidal Irradiation (UVGI) installed on the subject Transit Agency transit bus 2511, February 7, 2020 for air purification and surface disinfection testing for, COVID-19 Human Coronavirus and its various strands, H1N1 Influenza A, Methicillin Resistant *Staphylococcus aureus* (MRSA), Polio Virus and six different pathogenic strains of Salmonella. Final Report; 20 Pages.

Dear SanUVAire:

We have completed our antimicrobial efficacy study of the **Breathe-Safe & Surface-Safe Systems** installed on the subject Transport Authority transit bus 2511. These pathogens represent pathogens that would be prevalent or found on contaminated transit vehicles and indoor transit environments. The pathogens are transmitted to humans through contact with contaminated surfaces or airborne circulation by the vehicle's air conditioning system to all passengers and drivers.

**COVID-19 Human Coronavirus**, was selected because it represents a major current public threat. It is a virus that is spread by both surfaces and airborne into the air that infects humans and has a longer life expectancy on surfaces and in the air, thus increasing the exposure and spread.

**H1N1 Influenza A**, was selected because it represents a public threat, but also represents Influenza in general that yearly threaten the transit public. It is a virus that is spread by both surfaces and airborne into the air that infects humans. CDC estimates 36,000 lives are lost each year to general influenza.

**MRSA** typically is a skin-borne bacterium responsible for difficult-to-treat infections in humans. MRSA was chosen for this study due to its relative resistance to common sanitizers and its reputation as a "super bug." MRSA strains have rapidly become the most common cause of cultured skin infections among individuals seeking emergency medical care for skin infections in urban areas of the United States.

**Polio Virus** was chosen as a model for enteric human viruses. This group of viruses is transmitted by the fecal-oral route, survives for extended periods in the environment, is

fairly resistant to disinfectant, and has a very low infectious dose.

**Salmonella** bacteria are a widespread concern for both humans and animals. In humans, usually these cases are food-borne; however, the infection of salmonella can also be transmitted during contact with animals, contaminated water, or the environment.

In this study, plates containing the above pathogens were placed onto the interior surfaces of a bus equipped with the UVGI system. The system was activated and the survival of the pathogens was determined following 15 and 30 minutes.

Based on the results obtained from the study, the **Breathe-Safe & Surface-Safe Systems** successfully eliminated viral and bacterial contamination on the surfaces exposed to the UV radiation throughout the bus interior.

Should you have any concerns, please do not hesitate to contact me.

Best Regards,  
George Piacante, Ph.D.

## Executive Summary

This study evaluated the surface disinfection efficacy of the **Breathe-Safe & Surface-Safe Systems**, utilizing Ultraviolet Germicidal Irradiation (UVGI) in a transit bus. Disinfection efficacy was determined against **COVID-19 Human Coronavirus**, **Influenza A H1N1 virus**, **Methicillin resistant *Staphylococcus aureus* (MRSA)**, **Poliovirus LSc 1** and six different ***Salmonella* species**. The study was commissioned by SanUVAire LLC and was by conducted using **UVGI Breathe-Safe & Surface-Safe Systems** installed on the subject Transit Authority Bus Number 2511 on February 7, 2020 at the Central Garage.

### Test Results:

1. **COVID-19 Human Coronavirus - 99.999% reduction.**
2. **Influenza A H1N1- 99.999% reduction.**
3. **Methicillian Resistant *Staphylococcus Aureus* - 99.99% reduction**
4. **Polio Virus LSc 1 - 99.99% reduction.**
5. **Six *Salmonella* Species - 99.99% reduction.**

These represent a variety of infective human pathogens that have been implicated in numerous disease outbreaks. The interior surfaces of highly used public transportation system vehicles contain elevated levels of microbiological contamination. This is due to the frequent exposure of these surfaces to microorganisms present in the environment and carried on by passengers. The spread of harmful pathogenic microorganisms in transit vehicles is high because of the numbers and close proximity of passengers. Their complete inactivation is typically very challenging. The selection of these microorganisms for this study was done to illustrate the broad-spectrum capabilities and very high efficacy of the **Breathe-Safe & Surface-Safe Systems** on transit vehicles. Based on the data collected and presented in this report, the pathogens were eliminated from exposed surfaces. The high level of inactivation obtained in the study followed 15 and 30-minutes of exposure to UVGI. This study concludes that 15-minutes exposure is adequate for routine or daily disinfection and 30 minutes exposure would be recommended in cases of extensive microbial contamination. This translates in drastically improved microbiological surface and air quality for the public transit system and ultimately its rider's health.

**Breathe-Safe & Surface-Safe Systems** are environmentally friendly solution to combating microbial pollution and ensuring public transit health. In addition, it eliminates many other harmful bacteria, fungi, viruses, and parasites not tested here, but found in a transit environment. The system does not have any noxious emissions, residual contamination, uses no chemicals and is not labor intensive. **Breathe-Safe & Surface-Safe Systems** UVGI neutralizes airborne irritants, odors, and toxins in an eco-friendly non invasive manner.

## **Breathe-Safe Systems: Airborne Disinfection Transit Units**

BCS Laboratories has previously evaluated the effectiveness of the **Breathe-Safe Systems for Airborne Disinfection** in transit vehicles and in a six month long National Science Foundation, Transportation Research Board Study completed December 2008 at Houston Metro. Virus reduction such as (H1N1 Influenza A) would be represented by the reduction of MS-2 bacteriophage. This study also demonstrated the inactivation of other harmful pathogens.

**MS – 2: Log Reduction > 99.997%**

***Pseudomonas Aeruginosa*, Log Reduction >99.998%**

***Legionella*: Log Reduction > 99.991%**

**PRD – 1: Log Reduction > 99.999%**

**Vancomycin-resistant, *Eterococcus* (VRE): Log Reduction > 99.999%**

**Mold: Log Reduction > 99.999%**

## **EPA-Homeland Security Testing**

Independent laboratory testing funded by the EPA Office of Research and Development; National Homeland Security Research Center and conducted by RTI International (Research Triangle Park, NC) concluded similarly on the use of UVGI disinfection system in large scale HVAC system (EPA 600-R-06-xxx). The tests were conducted using three organisms, two bacteria (*Bacillus atrophaeus* and *Serratia marcescens*) and one bacterial virus (MS2). These organisms were selected because their sizes, shapes and susceptibility to UVGI inactivation make them reasonable surrogates for biological warfare agents (BWAs). The bioaerosol inactivation efficiencies calculated for the three organisms were 96% for *B. atrophaeus*, 99.96% for *S. marcescens* and 99% for MS2.

## **Transit Vehicle Environment**

Enclosed environments like a transit vehicle, respiratory disease is believed to be spread predominantly by airborne contamination rather than surface contamination as infected passengers continuously release infective airborne droplets. These droplets are spread to other passengers by the vehicle's air handler system. Most transit air handler systems move approximately one cabin volume of air once every five (5) minutes. Surface contamination also should be addressed for increased safety of the passengers. UVGI is considered a very cost-effective method of eliminating harmful pathogens.

# **Testing Program**

## **Breathe-Safe & Surface-Safe Systems: Surface Disinfection Description**

The **Surface-Safe System** for surface disinfection was installed in the subject TA Bus 2511 passenger compartment. It was composed of three ceiling mounted fixtures. One lamp was placed in the entrance and driver's area, one was placed in the middle of the bus, and one in the rear middle section of the passenger compartment. Each fixture incorporated a 42" High Output Ultraviolet Lamp, 24 VDC Ballast with special battery protection and end of lamp life circuits. Fixture bodies were constructed of 18-8 Stainless. UV lamp covers are constructed of perforated aluminum with 63% open area. See Attachments #1, #2, #3

## **Study Location and Date**

The study was conducted at the subject Transit Authority Central Garage, on February 7, 2020.

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

COVID-19 Human Coronavirus was propagated and enumerated as Most Probable Numbers (MPN) using Madin-Darby Canine Kidney type I (MDCK) cell monolayers (ATCC CCL-34) as the host. Cells were grown in 6-well cell-culture plates (Corning, USA). For enumeration, aliquots of a sample containing the virus were inoculated on freshly prepared monolayers of MDCK cells. The cells were incubated in dMEM (MediaTech, USA) media containing trypsin at 35°C and 5% CO<sub>2</sub> for 5-7 days. Cells were monitored routinely microscopically for signs of degeneration. The diluted virus stock was tittered by performing serial tenfold dilutions in PBS and inoculation onto MDCK cells as described above.

Influenza A H1N1 (ATCC VR-1469) virus was propagated and enumerated as Most Probable Numbers (MPN) using Madin-Darby Canine Kidney type I (MDCK) cell monolayers (ATCC CCL-34) as the host. Cells were grown in 6-well cell-culture plates (Corning, USA). For enumeration, aliquots of a sample containing the virus were inoculated on freshly prepared monolayers of MDCK cells. The cells were incubated in dMEM (MediaTech, USA) media containing trypsin at 35°C and 5% CO<sub>2</sub> for 5-7 days. Cells were monitored routinely microscopically for signs of degeneration. Cells in wells demonstrating signs of infectivity (Cytopathic effects; CPE) were recorded as positive (+) and ones that did not demonstrate any CPE were recorded as negative (-). The most probable number of infectious virus in a sample was then calculated using MPNCALC software (version 0.0.0.23). For Challenge experiments, frozen viral stock (typically 1 x 10<sup>8</sup> iu/ml) was thawed rapidly in a 35°C water bath on the day prior to the experiment. Bovine Serum Albumin (BSA) and used for the viral challenge experiment below. The diluted virus stock was tittered by performing serial tenfold dilutions in PBS and inoculation onto MDCK cells as described above.

Poliovirus Lsc1 Chat strain (ATCC VR-1562) was propagated and enumerated as

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plaque forming units (pfu) using EPA ICR Methodology (EPA 600/R-95/178, 1998). For enumeration, aliquots containing poliovirus were inoculated on freshly prepared monolayers of Buffalo Green Monkey (BGM) kidney cells and plaque assays were performed using 2X dMEM (MediaTech, USA) and 2X Bacto Agar containing 0.0001 % Neutral Red as per methodology outlined in EPA 600/R9-95/178. Cell flasks were incubated at 36.5°C and 5% CO<sub>2</sub> for 72-96 hours. Plaques on the respective flasks were counted following additional Neutral Red staining. For Challenge experiments, virus stocks (approximate titer 1 x 10<sup>8</sup> pfu/ml) were thawed at the day of experiment. They were then diluted 1/100 in PBS containing 1% FBS and the above-mentioned bacterial cocktail suspension.

### **Stock Bacterial Species and Strains**

The following strains were obtained from American Type Culture Collection: *Salmonella enterica*, serovar *Agona* (ATCC BAA-707), *Gaminara* (ATCC BAA-711), *Michigan* (ATCC BAA-709), *Montevideo* (ATCC BAA-710), *Poona* (BAA-1673), *Salmonella saintpaul* (ATCC 9712), and Methicillin Resistant *Staphylococcus aureus* (MRSA; BAA-44). All bacterial stocks are maintained at -80°C. The day prior to the study, overnight culture from colony purified frozen stock were grown in Tryptic Soy Broth (TSB, Beckton Dickinson, MD) at 36 °C. Each strain of the *Salmonella* was grown in a separate 5 ml of TSB and the MRSA was grown in 25 ml of TSB. At the day of the study, the broth cultures were centrifuged at 3K x G for 5 minutes and suspended in 10 ml of phosphate buffered saline (PBS, Fisher scientific, PA). This was repeated and the pellet was suspended in 10ml PBS. All the different suspended bacteria cultures were combined and then diluted 1/100 in PBS supplemented with 1% fetal bovine serum (FBS, Atlanta Biologicals, GA). This dilute bacterial suspension was inoculated with poliovirus described below.

### **Challenge Study**

During the day of the study, the above described diluted bacterial and viral cultures were transferred to the subject TA station in a double enclosed insulated carrier containing frozen ice packs. The protocol used is comparable to ASTM E 1053-97 (Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Surfaces). Briefly, 100 µl of the diluted bacterial and poliovirus cocktail was placed into the center of each of three wells of a 6-well cell-culture plate (Corning, USA). Into the remaining three wells, 100 µl of the COVID-19 virus was placed. This was repeated for a total of 5 plates. One plate was placed in the front area of the bus atop the fare box, one was placed mid section atop the disabled passenger seating area, one was placed in the rear area atop a seat, and the last one served as the positive (initial) control and was placed in the front area but was covered with light impermeable plastic cover. The plastic cover atop each of the first three plates was removed. The bus was then exited and the UV light system was activated for a 15-minute time period. Upon completion, the first of the three above described plates were removed. To each well, 1.0 ml of PBS containing 2% BSA was added to stabilize the microorganisms. The plates were

covered, sealed and placed into the insulated cold carrier and kept cold and dark till transport and analysis at the lab (4 hours). The above study was then repeated with another set of freshly inoculated plates (as mentioned above) for a UV Lamp exposure time of 30 minutes. Upon completion, all the plates were removed, including the control. Then 1.0 of PBS with 2% BSA was added, and the plates were sealed and stored alongside the 15-minute study plates in the insulated cooler.

Collected samples were maintained cold (4°-10° C) and were transported in a double enclosed carrier to BCS Laboratories-Gainesville. The liquid in each of the wells was enumerated for the challenge microorganisms. Plates were analyzed within 6 hours of collection. Tenfold dilutions of the microbial suspensions in the control plates were performed in PBS. The number of viable bacterial colonies was determined by spread plating onto Tryptic Soy Agar (TSA, Beckton Dickinson, MD) supplanted with ferric ammonium citrate and sodium thiosulfate. Plates were incubated at 36.5° C for 48 hours. The colonies on the plates were enumerated and the colony forming units (cfu) per well was determined.

The number of viable (infectious) Influenza A in each of the tubes was enumerated by the MPN procedure described above using Madin-Darby Canine Kidney type I (MDCK) cell monolayers (ATCC CCL-34). All analysis was conducted in triplicates.

Poliovirus was enumerated by the plaque assay procedure using Buffalo Green Monkey (BGM) kidney as per EPA 600/R9-95/178. Cell flasks were incubated at 36.5°C and 5% CO<sub>2</sub> for 72-96 h. Plaques on the respective flasks were counted following additional Neutral Red staining.

Table 1 through 10 present the results

**Table 1. The efficacy of UVGI Breathe-Safe & Surface-Safe Systems on the inactivation of COVID-19 Human Coronavirus in the interior of a passenger bus during a 15-minute exposure.**

<b>Sample</b>	<b>COVID-19 Coronavirus Calculated mpn *</b>	<b>Average Percent Reduction</b>
<b>Inoculated Viral Infectious Units (UV unexposed positive control)</b>	<b>4.8 x 10<sup>5</sup></b>	<b>NA</b>
<b>Front Driver Area (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Front Driver Area (B)</b>	<b>&lt;3.0</b>	
<b>Front Driver Area (C)</b>	<b>&lt;3.0</b>	
<b>Middle Seat (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Middle Seat (B)</b>	<b>&lt;3.0</b>	
<b>Middle Seat (C)</b>	<b>&lt;3.0</b>	
<b>Rear Seat (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Rear Seat (B)</b>	<b>&lt;3.0</b>	
<b>Rear Seat (C)</b>	<b>&lt;3.0</b>	

\*Most Probable Number (MPN) of Viral Infectious units was calculated using the MPNCalc Software as per EPA 600/R95/178. Viruses were enumerated by inoculation onto MDCK (CCL-34) cells and monitored for Cytopathic Effect (CPE) development during a 5-7-day incubation period. Cells were incubated at 35°C in

**Table 2. The efficacy of UVGI Breathe-Safe & Surface-Safe Systems on the inactivation of H1N1 Influenza A; ATCC VR-1469) in the interior of a passenger bus during a 15-minute exposure.**

<b>Sample</b>	<b>Influenza A H1N1 Calculated mpn *</b>	<b>Average Percent Reduction</b>
<b>Inoculated Viral Infectious Units (UV unexposed positive control)</b>	<b>4.8 x 10<sup>5</sup></b>	<b>NA</b>
<b>Front Driver Area (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Front Driver Area (B)</b>	<b>&lt;3.0</b>	
<b>Front Driver Area (C)</b>	<b>&lt;3.0</b>	
<b>Middle Seat (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Middle Seat (B)</b>	<b>&lt;3.0</b>	
<b>Middle Seat (C)</b>	<b>&lt;3.0</b>	
<b>Rear Seat (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Rear Seat (B)</b>	<b>&lt;3.0</b>	
<b>Rear Seat (C)</b>	<b>&lt;3.0</b>	

\*Most Probable Number (MPN) of Viral Infectious units was calculated using the MPNCalc Software as per EPA 600/R95/178. Viruses were enumerated by inoculation onto MDCK (CCL-34) cells and monitored for Cytopathic Effect (CPE) development during a 5-7-day incubation period. Cells were incubated at 35°C in a 5% CO<sub>2</sub> atmosphere.

**Table 3. The efficacy of UVGI Breathe-Safe & Surface-Safe Systems on the inactivation of six *Salmonella* species in the interior of a passenger bus during a 15-minute exposure.**

<b>Sample</b>	<b><i>Salmonella</i> cfu/ml</b>	<b>Average Percent Reduction</b>
<b>Inoculated bacterial colonies (UV unexposed positive control)</b>	<b>4.3 x 10<sup>5</sup></b>	<b>NA</b>
<b>Front Driver Area (A)</b>	<b>&lt;5.0</b>	<b>&gt;99.999%</b>
<b>Front Driver Area (B)</b>	<b>&lt;5.0</b>	
<b>Front Driver Area (C)</b>	<b>5.0</b>	
<b>Middle Seat (A)</b>	<b>3.0 x 10<sup>1</sup></b>	<b>99.98%</b>
<b>Middle Seat (B)</b>	<b>5.0 x 10<sup>1</sup></b>	
<b>Middle Seat (C)</b>	<b>1.4 x 10<sup>2</sup></b>	
<b>Rear Seat (A)</b>	<b>&lt;5.0</b>	<b>&gt;99.999%</b>
<b>Rear Seat (B)</b>	<b>1.0 x 10<sup>1</sup></b>	
<b>Rear Seat (C)</b>	<b>&lt;5.0</b>	

\* The number of viable bacterial colonies was determined by spread plating onto Tryptic Soy Agar (TSA, Beckton Dickinson, MD) supplanted with ferric ammonium citrate and sodium thiosulfate. Plates were incubated at 36.5° C for 48 hours.

**Table 4. The efficacy of UVGI Breathe-Safe & Surface-Safe Systems on the inactivation of Methicillin Resistant *Staphylococcus aureus* (MRSA) in the interior of a passenger bus during a 15-minute exposure.**

<b>Sample</b>	<b>MRSA cfu/ml</b>	<b>Average Percent Reduction</b>
<b>Inoculated bacterial colonies (UV unexposed positive control)</b>	<b>1.1 x 10<sup>4</sup></b>	<b>NA</b>
<b>Front Driver Area (A)</b>	<b>&lt;5.0</b>	<b>&gt;99.99%</b>
<b>Front Driver Area (B)</b>	<b>&lt;5.0</b>	
<b>Front Driver Area (C)</b>	<b>&lt;5.0</b>	
<b>Middle Seat (A)</b>	<b>&lt;5.0</b>	<b>&gt;99.99%</b>
<b>Middle Seat (B)</b>	<b>&lt;5.0</b>	
<b>Middle Seat (C)</b>	<b>&lt;5.0</b>	
<b>Rear Seat (A)</b>	<b>&lt;5.0</b>	<b>&gt;99.99%</b>
<b>Rear Seat (B)</b>	<b>&lt;5.0</b>	
<b>Rear Seat (C)</b>	<b>&lt;5.0</b>	

\* The number of viable bacterial colonies was determined by spread plating onto Tryptic Soy Agar (TSA, Beckton Dickinson, MD) supplemented with ferric ammonium citrate and sodium thiosulfate. Plates were incubated at 36.5° C for 48 hours.

**Table 5. The efficacy of UVGI Breathe-Safe & Surface-Safe System on the inactivation of Poliovirus LSc Chat strain (VR-1562) in the interior of a passenger bus during a 15-minute exposure.**

<b>Sample</b>	<b>Polio LSc 1 Chat cfu/ml</b>	<b>Average Percent Reduction</b>
<b>Inoculated Viral Load (UV unexposed positive control)</b>	<b>1.3 x 10<sup>4</sup></b>	<b>NA</b>
<b>Front Driver Area (A)</b>	<b>&lt;1.0</b>	<b>&gt;99.99%</b>
<b>Front Driver Area (B)</b>	<b>&lt;1.0</b>	
<b>Front Driver Area (C)</b>	<b>&lt;1.0</b>	
<b>Middle Seat (A)</b>	<b>&lt;1.0</b>	<b>&gt;99.99%</b>
<b>Middle Seat (B)</b>	<b>&lt;1.0</b>	
<b>Middle Seat (C)</b>	<b>&lt;1.0</b>	
<b>Rear Seat (A)</b>	<b>&lt;1.0</b>	<b>&gt;99.99%</b>
<b>Rear Seat (B)</b>	<b>&lt;1.0</b>	
<b>Rear Seat (C)</b>	<b>&lt;1.0</b>	

\* Poliovirus was enumerated by the plaque assay procedure using Buffalo Green Monkey (BGM) kidney as per EPA 600/R9-95/178. Cell flasks were incubated at 36.5°C and 5% CO<sub>2</sub> for 72-96 hours.

**Table 6. The efficacy of UVGI Breathe-Safe & Surface-Safe Systems on the inactivation of COVID-19 Human Coronavirus in the interior of a passenger bus during a 30-minute exposure.**

<b>Sample</b>	<b>Influenza A H1N1 Calculated mpn *</b>	<b>Average Percent Reduction</b>
<b>Inoculated Viral Infectious Units (UV unexposed positive control)</b>	<b>4.8 x 10<sup>5</sup></b>	<b>NA</b>
<b>Front Driver Area (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Front Driver Area (B)</b>	<b>&lt;3.0</b>	
<b>Front Driver Area (C)</b>	<b>&lt;3.0</b>	
<b>Middle Seat (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Middle Seat (B)</b>	<b>&lt;3.0</b>	
<b>Middle Seat (C)</b>	<b>&lt;3.0</b>	
<b>Rear Seat (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Rear Seat (B)</b>	<b>&lt;3.0</b>	
<b>Rear Seat (C)</b>	<b>&lt;3.0</b>	

\*Most Probable Number (MPN) of Viral Infectious units was calculated using the MPNCalc Software as per EPA 600/R95/178. Viruses were enumerated by inoculation onto MDCK (CCL-34) cells and monitored for Cytopathic Effect (CPE) development during the incubation period. Cells were incubated at 35°C in a 5% CO<sub>2</sub> atmosphere.

**Table 7. The efficacy of UVGI Breathe-Safe & Surface-Safe Systems on the inactivation of Influenza A (H1N1; ATCC VR-1469) in the interior of a passenger bus during a 30-minute exposure.**

<b>Sample</b>	<b>Influenza A H1N1 Calculated mpn *</b>	<b>Average Percent Reduction</b>
<b>Inoculated Viral Infectious Units (UV unexposed positive control)</b>	<b>4.8 x 10<sup>5</sup></b>	<b>NA</b>
<b>Front Driver Area (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Front Driver Area (B)</b>	<b>&lt;3.0</b>	
<b>Front Driver Area (C)</b>	<b>&lt;3.0</b>	
<b>Middle Seat (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Middle Seat (B)</b>	<b>&lt;3.0</b>	
<b>Middle Seat (C)</b>	<b>&lt;3.0</b>	
<b>Rear Seat (A)</b>	<b>&lt;3.0</b>	<b>&gt;99.999%</b>
<b>Rear Seat (B)</b>	<b>&lt;3.0</b>	
<b>Rear Seat (C)</b>	<b>&lt;3.0</b>	

\*Most Probable Number (MPN) of Viral Infectious units was calculated using the MPNCalc Software as per EPA 600/R95/178. Viruses were enumerated by inoculation onto MDCK (CCL-34) cells and monitored for Cytopathic Effect (CPE) development during the incubation period. Cells were incubated at 35°C in a 5% CO<sub>2</sub> atmosphere.

**Table 8. The efficacy of UVGI Breathe-Safe & Surface-Safe Systems on the inactivation of six *Salmonella* species in the interior of a passenger bus during a 30-minute exposure.**

<b>Sample</b>	<b><i>Salmonella</i> cfu/ml</b>	<b>Average Percent Reduction</b>
<b>Inoculated bacterial colonies (UV unexposed positive control)</b>	<b>4.3 x 10<sup>5</sup></b>	<b>NA</b>
<b>Front Driver Area (A)</b>	<b>&lt;5.0</b>	<b>&gt;99.999%</b>
<b>Front Driver Area (B)</b>	<b>&lt;5.0</b>	
<b>Front Driver Area (C)</b>	<b>&lt;5.0</b>	
<b>Middle Seat (A)</b>	<b>&lt;5.0</b>	<b>&gt;99.999%</b>
<b>Middle Seat (B)</b>	<b>&lt;5.0</b>	
<b>Middle Seat (C)</b>	<b>&lt;5.0</b>	
<b>Rear Seat (A)</b>	<b>&lt;5.0</b>	<b>&gt;99.999%</b>
<b>Rear Seat (B)</b>	<b>&lt;5.0</b>	
<b>Rear Seat (C)</b>	<b>&lt;5.0</b>	

\* The number of viable bacterial colonies was determined by spread plating onto Tryptic Soy Agar (TSA, Beckton Dickinson, MD) supplanted with ferric ammonium citrate and sodium thiosulfate. Plates were incubated at 36.5° C for 48 hours.

**Table 9. The efficacy of UVGI Breathe-Safe & Surface-Safe Systems on the inactivation of Methicillin Resistant *Staphylococcus aureus* (MRSA) in the interior of a passenger bus during a 30-minute exposure.**

<b>Sample</b>	<b>MRSA cfu/ml</b>	<b>Average Percent Reduction</b>
<b>Inoculated bacterial colonies (UV unexposed positive control)</b>	<b>1.1 x 10<sup>4</sup></b>	<b>NA</b>
<b>Front Driver Area (A)</b>	<b>&lt;5.0</b>	<b>&gt;99.99%</b>
<b>Front Driver Area (B)</b>	<b>&lt;5.0</b>	
<b>Front Driver Area (C)</b>	<b>&lt;5.0</b>	
<b>Middle Seat (A)</b>	<b>&lt;5.0</b>	<b>&gt;99.99%</b>
<b>Middle Seat (B)</b>	<b>&lt;5.0</b>	
<b>Middle Seat (C)</b>	<b>&lt;5.0</b>	
<b>Rear Seat (A)</b>	<b>&lt;5.0</b>	<b>&gt;99.99%</b>
<b>Rear Seat (B)</b>	<b>&lt;5.0</b>	
<b>Rear Seat (C)</b>	<b>&lt;5.0</b>	

\* The number of viable bacterial colonies was determined by spread plating onto Tryptic Soy Agar (TSA, Beckton Dickinson, MD) supplemented with ferric ammonium citrate and sodium thiosulfate. Plates were incubated at 36.5° C for 48 hours.

**Table 10. The efficacy of UVGI Breathe-Safe & Surface-Safe Systems on the inactivation of Poliovirus LSc Chat strain (VR-1562) in the interior of a passenger bus during a 30-minute exposure.**

<b>Sample</b>	<b>Polio LSc 1 Chat cfu/m</b>	<b>Average Percent Reduction</b>
<b>Inoculated Viral Load (UV unexposed positive control)</b>	<b>1.3 x 10<sup>4</sup></b>	<b>NA</b>
<b>Front Driver Area (A)</b>	<b>&lt;1.0</b>	<b>&gt;99.99%</b>
<b>Front Driver Area (B)</b>	<b>&lt;1.0</b>	
<b>Front Driver Area (C)</b>	<b>&lt;1.0</b>	
<b>Middle Seat (A)</b>	<b>&lt;1.0</b>	<b>&gt;99.99%</b>
<b>Middle Seat (B)</b>	<b>&lt;1.0</b>	
<b>Middle Seat (C)</b>	<b>&lt;1.0</b>	
<b>Rear Seat (A)</b>	<b>&lt;1.0</b>	<b>&gt;99.99%</b>
<b>Rear Seat (B)</b>	<b>&lt;1.0</b>	
<b>Rear Seat (C)</b>	<b>&lt;1.0</b>	

\* Poliovirus was enumerated by the plaque assay procedure using Buffalo Green Monkey (BGM) kidney as per EPA 600/R9-95/178. Cell flasks were incubated at 36.5°C and 5% CO<sub>2</sub> for 72-96 hours.

## **Results and Conclusions**

Tables 1-10 present the data of the antimicrobial efficacy study conducted on The UVGI Breathe-Safe & Surface-Safe Systems Installed in a Bus. Tables 1-5 indicate that a 15-minute UV exposure is adequate to obtain an effective inactivation of pathogens on the interior surfaces of the evaluated bus. The installed system efficacy was high even when the plates containing the pathogens were placed diagonally from the UV radiation source as indicated in the results presented for the middle seat scenario. Both bacterial and viral pathogens were inactivated equally well. Tables 6-10 demonstrate the complete elimination of the challenge microorganisms from the exposed surfaces of the bus interior following a 30-minute UV radiation exposure. This extended exposure period eradicated all pathogens.

The obtained results indicated that 15 minutes exposure to the installed UV system was adequate for routine or daily inactivation of microorganisms on the surfaces of the bus interior. This would lead to reduced microbial contamination and would increase public health and perception of the transit system. In cases when a suspected major biological contamination would occur, then the 30-minute exposure would be recommended. The 30-minute exposure was suitable for a thorough and extensive disinfection of the surfaces.

Attachment #1. Photos of the UVGI Surface-Safe System installed onto the ceiling of the bus interior. Three UV lamps were installed in the tested bus.



Attachment #2. Salmonella, MRSA, Poliovirus, Influenza A H1N1 and COVID-19 virus were inoculated into the center of the well plates and subjected to UV radiation for 15 and 30 minutes at various location throughout the bus interior cabin. Microorganisms were enumerated in plates that were subjected to the UVGI and in ones that were not to determine UV system inactivation efficacy.



Attachment #3. Plates containing the challenge microorganisms were exposed to UVGI Breathe-Safe & Surface-Safe Systems installed in the bus. UV exposure was verified by UV radiometer.



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