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EFFICACY OF THE GREENFAN® BIPOLAR IONIZER AGAINST AEROSOLIZED SARS-COV-2 DELTA VARIANT

PROJECT: GREENFAN® BIPOLAR IONIZER AEROSOL SARS-COV-2

PRODUCT: GREENFAN® BIPOLAR IONIZER 1600-01

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM(S):

SARS-CoV-2 DELTA VARIANT

Dana Yee, M.D.

Medical Director

Study Completion Date

09/01/2021

Testing Facility

Innovative Bioanalysis, Inc.

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Laboratory Project Number

1112

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Efficacy Study Summary

Study Title	EFFICACY OF THE GREENFAN® BIPOLAR IONIZER AGAINST AEROSOLIZED SARS-COV-2 DELTA VARIANT
Laboratory Project #	1112
Guideline:	Modified ISO standards as no international standards exist.
Testing Facility	Innovative Bioanalysis, Inc.
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Delta Variant virus which causes the Coronavirus Disease 2019 (COVID-19).
Description	The GreenFan® Bipolar Ionizer Model 1600-01 unit is designed to be integrated into an air circulation, movement, ventilation, purification, and/or management system to disinfect the air. This in-vitro study was sought to determine the efficacy of the GreenFan® Ionizer on aerosolized SARS-CoV-2 Delta Variant virus when operating.
Test Conditions	Testing was conducted in a sealed 20'x8'x8' chamber that complied with BSL-3 standards. The temperature during testing was approximately 74 ±2°F, with a relative humidity of 29%. A 4.03×10^6 Median Tissue Culture Infectious Dose per milliliter (TCID50/mL) of the SARS-CoV-2 Delta Variant in a Fetal Bovine Serum (FBS) based virus suspension media was nebulized into the chamber. Air samples were collected at 15, 30, and 60 minutes after exposure.
Test Results	The GreenFan® Bipolar Ionizer results displayed a higher reduction of collectible SARS-CoV-2 Delta in the air over 60 minutes compared to the controls. Against the SARS-CoV-2 Delta variant, the GreenFan® Ionizer reduced collectible active concentrations to 9.85×10^4 TCID50/mL after 60 minutes of exposure, indicative of a 97.55% reduction.
Control Results	A control test was conducted with only the fan operating, and samples were taken at the corresponding time points used for the challenge. Control test showed a natural viability loss over of collectible SARS-CoV-2 Delta variant decreasing from 4.03×10^6 TCID50/mL to approximately 1.68×10^6 TCID50/mL after 60 minutes. The data was used as a comparative baseline to calculate net virus reduction.
Conclusion	Overall, the GreenFan® Bipolar Ionizer Model 1600-01 unit demonstrated the ability to significantly reduce the SARS-CoV-2 Delta Variant virus concentration in the air to 9.85×10^6 TCID50/mL over 60 minutes of device operation, indicating a 97.55% reduction.

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Study Report:

Study Title: EFFICACY OF THE GREENFAN® BIPOLAR IONIZER AGAINST AEROSOLIZED SARS-COV-2 DELTA VARIANT

Sponsor: GreenFan® Inc.

Test Facility: Innovative Bioanalysis, Inc. 3188 Airway Ave Suite D, Costa Mesa, CA 92626

Device Testing: GreenFan® Bipolar Ionizer

Study Report Date: 08/31/2021

Experimental Start Date: 05/27/2021

Experimental End Date: 08/05/2021

Study Completion Date: 09/01/2021

Study Objective:

GreenFan® supplied a Bipolar Ionizer Model 1600-01 unit for testing purposes to determine the efficacy against pathogens in the air specifically the SARS0CoV-2 virus. This study evaluated the Ionizer's capabilities to inactivate the genetic strain referred to as the SARS-CoV-2 Delta Variant.

Test Method:

Bioaerosol Generation:

Nebulization occurred using a Blaustein Atomizing Modules (BLAM) with a pre-set PSI and computer controlled liquid delivery system. Prior to testing the nebulizer was checked for proper functionality by nebulizing the FBS solution without the test virus present to confirm average particle size distribution. The nebulizer was filled with 4.03 x 10^6 TCID50/mL of the SARS-CoV-2 Delta Variant virus in FBS-based virus suspension media and nebulized at a flow rate of 1mL/min for twenty minutes with untreated local atmospheric air. After nebulization, the nebulizer's remaining virus stock volume was weighed to confirm that roughly the same amount was nebulized during each run. Bioaerosol procedures for the controls and viral challenges were performed in the same manner with corresponding time points and collection rates.

Figure 1: BLAM Nebulizer

Bioaerosol Sampling:

This study used four probes for air sampling, each connected to a calibrated Gilian 10i vacuum device and set at a standard flow of 5.02L/min with a 0.20% tolerance. Before use, the devices were inspected for functionality and the vacuum system calibration are confirmed using a Gilian Gilibrator-2 NIOSH Primary Standard Air Flow Calibrator. Sample collection volumes were set to 10-minute draws per time point which allowed for approximately 50 liters of air collection per collection port. The air sampler operated with a removable sealed cassette and manually removed after each sampling time point. Cassettes had a delicate internal filtration disc to collect virus samples, which was moistened with a virus suspension media to aid in the collection. Filtration discs from Zefon International, Lot# 24320, were used for testing. At each time point all the sample discs were pooled into on collection tube to provide an average across the 4 sampling locations

Figure 2: Sensidyne 37mm directionnel air flow semple cassette.

Test System Strains: SARS-CoV-2 Delta Variant (NR-55611)

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/PHC658/2021 (Lineage B.1.617.2; Delta Variant)

Study Materials and Equipment:

Equipment Overview: The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. Due to the closed design, no assessment was conducted on the inner components of the device. Before testing, positive, and negative ion generation was confirmed using two Alpha Lab AIC2 ion polarity meters. The device was powered on to ensure functionality before testing.

MANUFACTURER: GreenFan® Inc.

MODEL: Bipolar Ionizer 1600-01

MAKE: GreenFan®

Figure 3. GreenFan® Bipolar Ionizer Model 1600-01 as tested.

Testing Layout:

All testing was conducted in a sealed 20'x8'x8' chamber per BioSafety Level 3 (BSL-3) standards. The GreenFan® Bipolar Ionizer was placed at the outlet of a custom 12-inch diameter ductwork system with fan attached. The airflow velocity of the fan was measured in a grid pattern across the opening of the duct. The average overall velocity of the exiting airspeed was 204 feet per minute. The 204 fpm airflow velocity was requested by the manufacturer based on a 12-inch diameter duct providing a calculated volumetric airflow of approximately 160 cubic feet per minute (cfm) (i.e., 160 cfm/(0.5 ft x 0.5 ft x PI) = 204 fpm). The ductwork system was set on a stainless-steel table 36" above the ground and positioned in the center against the 8' chamber wall. Four variable-speed mixing fans (~30 cfm each) were placed on the floor at a 45-degree angle in each corner to encourage air mixing. Air sampling was collected using two calibrated Gilian 10i programmable vacuum devices located 6 feet off the chamber floor. Before testing, the chamber was pressure tested and visually inspected for leaks and all internal lab systems and equipment were reviewed before testing.

When aerosolizing pathogens and collecting said pathogens, there are variables that cannot be fully accounted for, namely, placement of pathogen, collection volume, collection points, drop rate, surface saturation, virus destruction on collection, virus destruction on nebulization and possibly others. Every effort was made to simulate a real-life situation and address constraints with the experimental design and execution while taking the proper precautions when working with a BSL-3 pathogen. These efforts are reflected in the control data and trial data.

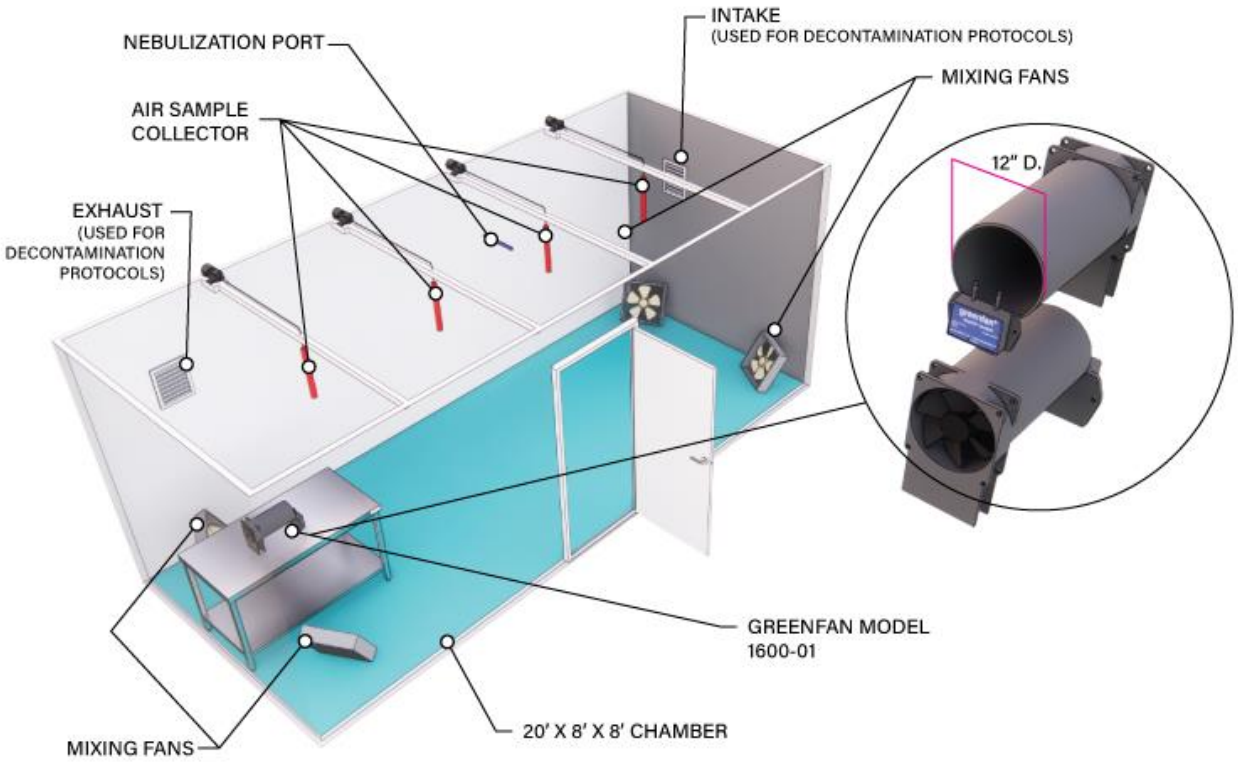

Figure 4. Testing layout for control and experimental trials. Testing chambers exhaust and intake are not used during active virus testing outside of the decontamination function.

Control Protocol:

To accurately assess the GreenFan® Bipolar Ionizer, two control trials were conducted without the device operating in the testing chamber. Control samples were taken at the corresponding time points used for the challenge trial to serve as a comparative baseline to assess the virus reduction when the device was operating.

Test Method:

Exposure Conditions:

1. The temperature during all test runs was approximately 74 ±2°F with a relative humidity of 29%.
2. Samples were collected 10 minutes after nebulization stopped (T-0) at the following time points with T equal to minutes: T-15, T-30, and T-60.
3. Two controls and one viral challenge were conducted using the same methodology.

Experimental Procedures:

1. Before the initial control test and following each trial, the testing area was decontaminated and prepped per internal procedures
2. 10 mL of 4.03 x 10^6 TCID50/mL of SARS-CoV-2 Delta Variant in virus media was nebulized into the room via the dissemination port.
3. After nebulization, the GreenFan® Bi-polar Ionizer was turned on via remote.
4. During the challenges, the device was turned off at the pre-determined time points for sample collection.
5. Air sample collections were set to 10-minute continuous draws at the point of sampling.
6. Sample cassettes were manually removed from the collection system and brought to an adjacent biosafety cabinet for extraction and placement into a virus suspension media.
7. All samples were sealed after collection and provided to lab staff for analysis after study completion.

Post Decontamination:

After each viral challenge test, the UV system inside the testing chamber was activated for 30 minutes. After 30 minutes of UV exposure, there was a 30-minute air purge through the air filtration system. Air purge consisted of outside air being treated through a mechanical filter via the intake of the chamber. Air was extracted through the HEPA filtered exhaust to return the testing chamber to the same ambient conditions for each trial run. All test equipment was cleaned at the end of each day with a 70% alcohol solution. Collection lines were soaked in a bleach bath mixture for 30 minutes then rinsed repeatedly with DI water. The nebulizer and vacuum collection pumps were decontaminated with hydrogen peroxide mixtures.

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Certificate of Analysis: (COA) for Virus used

Preparation of The Pathogen

Virus Stock: SARS-Related Coronavirus 2, Isolate hCoV-19/USA/PHC658/2021 (BEI NR-55611)

Test	Specifications	Results
Identification by Infectivity in Calu-3 cells	Cell Rounding and Detachment	Cell Rounding and Detachment
Next-Generation Sequencing (NGS) of the complete genome using Illumina® iSeq™ 100 Platform	≥ 98% identity with SARS-CoV 2, hCoV-19/USA/PHC658/2021 depositor sequence	99.9% identity with SARS-CoV 2, hCoV-19/USA/PHC658/2021 depositor sequence
Titer by $TCID_{50}$ in Calu-3 Cells by Cytopathic Effect	Report Results	6.5 X 10^5 $TCID_{50}$ per mL^2
Sterility (21-Day Incubation)		
Harpo's HTYE Broth, aerobic	No Growth	No Growth
Trypticase Soy Broth, aerobic	No Growth	No Growth
Sabouraud Broth, aerobic	No Growth	No Growth
Sheep Blood Agar, aerobic	No Growth	No Growth
Sheep Blood Agar, anaerobic	No Growth	No Growth
Thioglycollate Broth, anaerobic	No Growth	No Growth
DMEM with 10% FBS	No Growth	No Growth
Mycoplasma Contamination		
Agar and Broth Culture	None Detected	None Detected
DNA detection by PCR of extracted Test Article nucleic acid	None Detected	None Detected

*The viral titer listed in the Certificate of Analysis is representative of the titer provided by BEI Resources. These viruses are grown on VeroE6 cells either in-house or at a partner lab to the concentrations listed within the experiment design.

Study Results:

The graph below shows the number of collectible SARS-CoV-2 Delta virus with and without the GreenFan® Ionizer operating over 60 minutes. The control plotted shows a natural viability loss over time decreasing to a virus concentration of approximately 1.68 x 10^6 TCID50/mL at 60 minutes. Against SARS-CoV-2 Delta, 2.22 x 10^6 TCID50/mL was collected after 15 minutes of device operation. After 30 minutes, 1.03 x 10^6 TCID50/mL of collectible SARS-CoV-2 Delta indicating a 74.50% reduction. Compared to an initial virus concentration of 4.03 x 10^6 TCID50/mL, the GreenFan® Bipolar Ionizer reduced collectible SARS-CoV-2 Delta variant by 97.55% to 9.85 x 10^4 TCID50/mL at 60 minutes.

Table 1. Collectible SARS-CoV-2 Delta variant with and without the GreenFan® Bipolar Ionizer operating over 60 minutes.

	0 minutes	15 minutes	30 minutes	60 minutes
Control (TCID50/mL)	4.03 x 10^6	2.69 x 10^6	2.13 x 10^6	1.68 x 10^6
% Reduction – Control		33.25%	47.18%	58.16%
Experiment (TCID50/mL)	4.03 x 10^6	2.22 x 10^6	1.03 x 10^6	8.85 x 10^4
% Reduction – Experiment		44.95%	74.50%	97.55%
% Difference – Control vs Experiment		17.5%	51.6%	94.1%

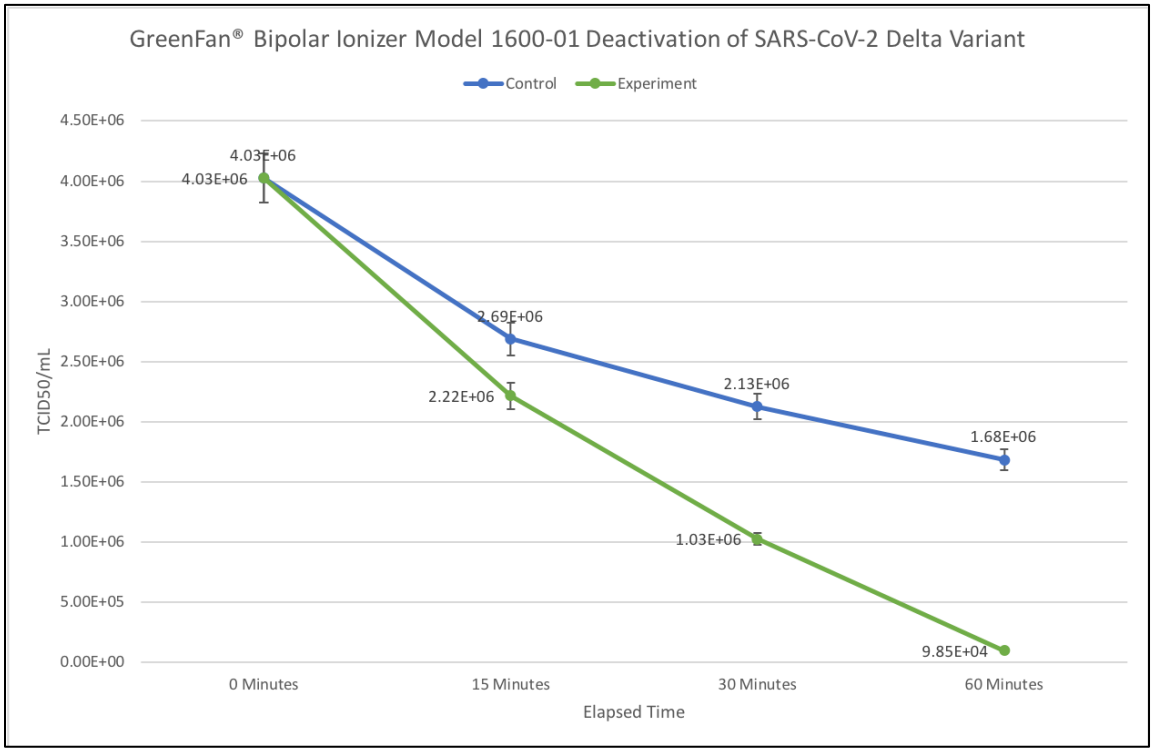

Figure 5. Collectible SARS-CoV-2 Delta variant with and without the GreenFan® Bipolar Ionizer operating over 60 minutes.

***As it pertains to data represented herein, the value of 1.2E+02 indicates a titer that is lower than the specified limit of quantitation (LLOG). The limit of quantitation for this assay is 1.2E+02. The percentage error equates to an average of ±5% of the final concentration.*

Conclusion:

Based on the test data, GreenFan® Bipolar Ionizer Model 1600-01 demonstrated the ability to consistently reduce the aerosolized SARS-CoV-2 Delta virus from the breathable air at all three time points tested. A 97.55% reduction was observed after 60 minutes of exposure based on reducing the initial concentration of collectable active SARS-CoV-2 Delta variant virus from 4.03×10^6 to 9.85×10^4 TCID50/mL after 60 minutes of exposure. Doing a comparative analysis between the control loss and the experiment loss there was an observed reduction difference of 94.1% after 60 minutes. The experiment results are a combination of the effect of natural decay, air movement and ionization present. The study focused on the device's impact on a specific volume of space with a pre-defined velocity of airflow measured in feet per minute interacting with the ionization device. In this testing scenario the air velocity in feet per minute was the force generating and establishing the negative an ion concentration in the testing environment. Altering parameters of the test design such as air velocity or room volume may alter achievable results. Variables such as room size, outdoor airflow, movement, and more will have a direct impact on results. Every effort was made to simulate a real-life situation and address constraints with the experimental design and execution while taking the proper precautions when working with a BSL-3 pathogen. These efforts are reflected in the meaningful recovery of the virus in the control test and the experiment.

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Disclaimer:

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APPENDIX A: Glossary

COA: A Certificate of Analysis refers to an authenticated document that is issued by BEI or ATCC Quality Assurance Department that ascertains that a product has met its predetermined pathogen specifications and preparations.

FBS: Fetal bovine serum (FBS) is derived from the blood drawn from a bovine fetus via a closed system of collection at the slaughterhouse. Fetal bovine serum is the most widely used serum-supplement for the in vitro cell culture of eukaryotic cells. This is due to it having a very low level of antibodies and containing more growth factors, allowing for versatility in many different cell culture applications.

The globular protein, bovine serum albumin (BSA), is a major component of fetal bovine serum. The rich variety of proteins in fetal bovine serum maintains cultured cells in a medium in which they can survive, grow, and divide.

Because it is a biological product, FBS is not a fully defined media component, and as such varies in composition between batches.[1] As a result of this and to minimize the possibility of transfer of adventitious agents, serum-free and chemically defined media (CDM) have been developed. The effectiveness of serum-free media is limited, however as many cell lines still require serum to grow, and many serum-free media formulations can only support the growth of narrowly defined types of cells.[2]

GCLP: Good Clinical Laboratory Practice (GCLP) is a set of standards that provide guidance on implementing Good Laboratory Practice (GLP) and Good Clinical Practice (GCP) principles to the analysis of samples from a clinical trial. GLP is a quality system that covers the organizational process and the conditions under which non-clinical laboratory studies are planned, performed, monitored, recorded, archived, and reported (OECD 1998) By combining the GLP and GCP sets of guidelines, GCLP ensures the quality and reliability of the clinical trial data generated by laboratories. Consequently, GCLP is critical for clinical trial laboratory operations, and it is expected that compliance with the GCLP standards, monitored annually by external audits, will help laboratories to maintain clinical trial data integrity, provide safety to the study participants and produce effective results that are consistent, auditable, and repeatable.

LLOQ: The ULOQ and LLOQ are the highest and lowest standard curve points that can still be used for quantification; they are the values below and above which, respectively, quantitative results may be obtained with a specified degree of confidence, or the highest/lowest concentration of an analyte that can be accurately measured. Together, the ULOQ and LLOQ define the range of quantification for the assay. Limits of quantitation are matrix, method, and analyte-specific, and can be calculated as follows:

Equation 1.

(Calculation used in Q-View): ULOQ & LLOQ = Highest or Lowest Standard, respectively, with a %backfit of 120%-80%, a %CV of < 30%, and a positive mean pixel intensity difference between it and the negative control.

Equation 2.

(Commonly used in science to estimate the LLOQ): LLOQ = (Mean negative control pixel intensity) + 10 * (StDev of negative control pixel intensities).

TCID/50mL: The number of infectious virus particles is frequently quantified by using the Median Tissue Culture Infectious Dose (TCID50) assay. The assay works by adding a serial dilution of the virus sample to cells in a 96 well plate format. The type of cell is specifically selected to show a cytopathic effect (CPE), i.e., morphological changes upon infection with the virus or cell death. After an incubation period, the cells are inspected for CPE or cell death and each well is classified as infected or not infected. Colorimetric or fluorometric readouts are also possible, which can increase assay sensitivity. The dilution, at which 50% of the wells show a CPE, is used to calculate the TCID50 of the virus sample. This calculation can generally be done by a variety of mathematical approaches, e.g., the Spearman-Karber method or the Reed-Muench method. Virus titer is expressed as TCID50 / ml.

VERO/E6: Vero cells are a lineage of cells used in cell cultures. Vero E6, also known as Vero C1008 (ATCC No. CRL-1586) This line is a clone from Vero 76. Vero E6 cells show some contact inhibitions, so are suitable for propagating viruses that replicate slowly.

APPENDIX B: Alternate Graphical View

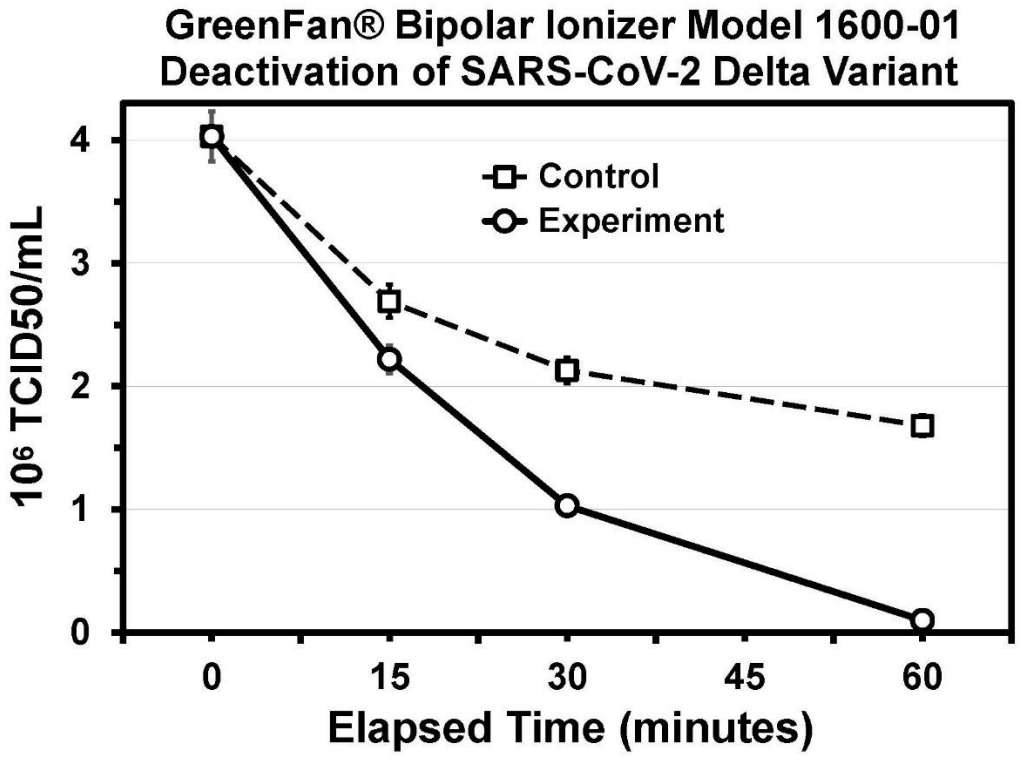

Figure 4. Collectible SARS-CoV-2 Delta Variant with and without GreenFan® Bipolar Ionizer operating over 60 minutes