EFFICACY OF THE REACT AIR DESTROYER AGAINST AEROSOLIZED SARS-COV-2

PROJECT: REACT AIR DESTROYER BIOAEROSOL SARS-COV-2

PRODUCT: REACT AIR DESTROYER

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM(S):

SARS-COV-2 USA-CA1/2020

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Laboratory Project Number

1073

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Efficacy Study Summary

Study Title	EFFICACY OF REACT AIR DESTROYER DEVICE AGAINST AEROSOLIZED SARS-COV-2
Laboratory Project #	1073
Guideline:	Modified ISO standards as no international standards exist.
Testing Facility	Innovative Bioanalysis, Inc.
Study Dates:	
Study Initiation Date:	05/10/2021
Study Completion Date:	06/21/2021
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	SARS-CoV-2 USA-CA1/2020
Description	The Reaction Group React Air Destroyer unit was designed as a portable standalone air purification system for use in commercial environments. This in vitro study is being conducted to determine the efficacy of the React Air Destroyer device in reducing the aerosolized pathogen, SARS-CoV-2 when operating.
Test Conditions	The test was conducted in a large, sealed environment that complied to BSL-3 standards and was inspected for any leaks prior to usage. The temperature during all test runs was approximately 72 ±2°F, with a relative humidity of 35%. Air samplers were calibrated by the manufacturer on September 3, 2020 and set at a standard flow of 5.02L/min. Calibration records indicate a 0.20% tolerance. The nebulizer was filled with the same amount of viral stock (6.32 x 10^6 TCID50 per mL) in FBS-based viral media and nebulized at a constant rate while four mixing fans were running simultaneously to ensure homogenous air.
Test Results	When tested against aerosolized SARS-CoV-2 three times, the device showed an average reduction over the course of three tests runs of 89.27% of active virus after 15 minutes of operation.
Control Results	Two control tests were conducted without the device operating and samples were taken at the corresponding timepoints used for the challenge trial. The results showed the natural viability loss over time in the chamber and served as a comparative baseline in order to calculate viral reduction.
Conclusion	The study showed the React Air Destroyer was able to reduce the concentration of aerosolized SARS-CoV-2 in the specified space after 15 minutes of operating.

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Study Report

Study Title: EFFICACY OF REACT AIR DESTROYER DEVICE AGAINST AEROSOLIZED SARS-COV-2

Sponsor: Reaction Group

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

Device Testing: Testing the efficacy of the React Air Destroyer system against an aerosolized known pathogen, SARS-CoV-2

Study Report Date: 06/22/2021

Experimental Start Date: 06/01/2021

Experimental End Date: 06/21/2021

Study Completion Date: 06/22/2021

Study Objective:

The Reaction Group React Air Destroyer unit was designed as a portable standalone air purification system. This in vitro study was designed to determine the effectiveness of the React Air Destroyer unit against and aerosolized virus and the known pathogen, SARS-CoV-2 was used.

Test Method:

Bioaerosol Generation:

For the control and the viral challenges, the nebulizer was filled with the same amount of viral stock (6.32×10^6 TCID50 per mL) in FBS-based viral media. The solution was nebulized at a flow rate of 1mL/min. The nebulizer was driven by untreated local atmospheric air. After each completion, the nebulizer's remaining viral stock volume was weighed to confirm that the same amount of viral stock was nebulized.

Bioaerosol Sampling:

For air sampling, four Gilian 10i programmable vacuum devices were used. The manufacturer calibrated the air samplers in September 2020, and the certificates were inspected prior to use. Air sample volume collections were confirmed prior to use with a Gilian Gilibrator 2, SN-200700-12 and a high flow bubble generator SN-2009012-H. The air samplers were operated in conjunction with removable sealed cassettes, which were manually removed after each sampling time point. The cassettes had a delicate internal filtration disc coated with a viral suspension media to aid in the collection of viral samples. The low volume mixing fans stayed active throughout all testing scenarios and conditions to encourage bioaerosol suspension and reduction in natural particle descent rates.

Test System Strains: SARS-CoV-2

The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-CA1/2020, NR-52382.

Study Materials and Equipment:

Equipment Overview: The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. All filtration systems and lamps were installed prior to arrival at the laboratory. The device was powered on to confirm functionality prior to testing. A supply voltage of 230V is required to operate the device. As a result, the Instapark ITU-1000 Step Up & Down Transformer was used to provide the necessary power.

MANUFACTURER: Reaction Group

MODEL: React Air Impact/Destroyer

SIZE: 400mm X 400mm X 1210mm

MAKE: N/A

SERIAL #: N/A

Equipment Specifics: The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. All filtration systems and lamps were installed prior to arrival at the laboratory. The manufacturer also provided additional spare parts, in case of any damages upon arrival. The device was powered on to confirm functionality prior to testing. The Instapark ITU-1000 Step Up & Down Transformer was used to provide the required 230V supply voltage power. Upon inspection, the screen was not activated therefore was not used during testing.

Testing Chamber: The test was conducted inside a large, sealed chamber with active monitoring of testing conditions via calibrated wireless devices and air sampling sensor. At each corner of the chamber, low volume mixing fans were positioned to ensure homogenous, bioaerosol concentrations. The testing chamber was set up to allow all exhausted air after the test samples had been taken to be exhausted through a dual HEPA filtration system.

Design Layout:

The testing chamber was a 20'x8'x8', sealed chamber consisting of metal walls and epoxy floor equipped with 4 sealed viewing windows and a lockable chamber door for entry and exit complying with BSL-3 standards. The chamber was designed to maintain a negative pressure environment. The chamber was designed to be completely sealed from the outside to prevent any release of testing media into the atmosphere. A monitoring system is set in place to confirm no loss of pressure occurred during testing. During testing, the ambient temperature was 72°F with a relative humidity of 35%.

The testing chamber had HEPA filtered inlets and exhaust, coupled with an active UV-C system in all ducting lines. Humidity and temperature were monitored inside the chamber using a calibrated wireless device. For air sample testing, the chamber was equipped with 2 probes that were along the centerline of the room and protruded down from the ceiling 24". Each probe tube was connected to a Gilian 10i programmable system with sampling cassettes from lot # 24320 made by Zefon International. A single bioaerosol nebulizing port was in the center of the 10' wall. The dissemination port protruded from the wall 24" and was connected to a programmable compressor nebulizer system. Low volume mixing fans were positioned at 45-degree angles at each corner of the chamber to encourage bioaerosol suspension and reduce natural particle descent rates.

An active air sampling sensor was used to confirm operations of the equipment and O_3 measurements were taken only during safety testing for verification that the system was operating properly. Test scenario captures O_3, but the conditions are not designed to be compared to EPA requirements. Thus, cannot be used for O_3 claims as the sensors and test parameters are not designed to meet O_3 certification requirements. The bioaerosol testing system was constructed to meet internal SOP requirements and all seams were sealed. All sample collection pumps were set to a 10-minute air draw at the point of sampling.

Prior to testing, all internal lab systems were reviewed and determined to be functioning. The chamber was pressure tested for leaks by visual inspections using a colored smoking device. All seals for the chamber were confirmed and all equipment used had a function test done to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

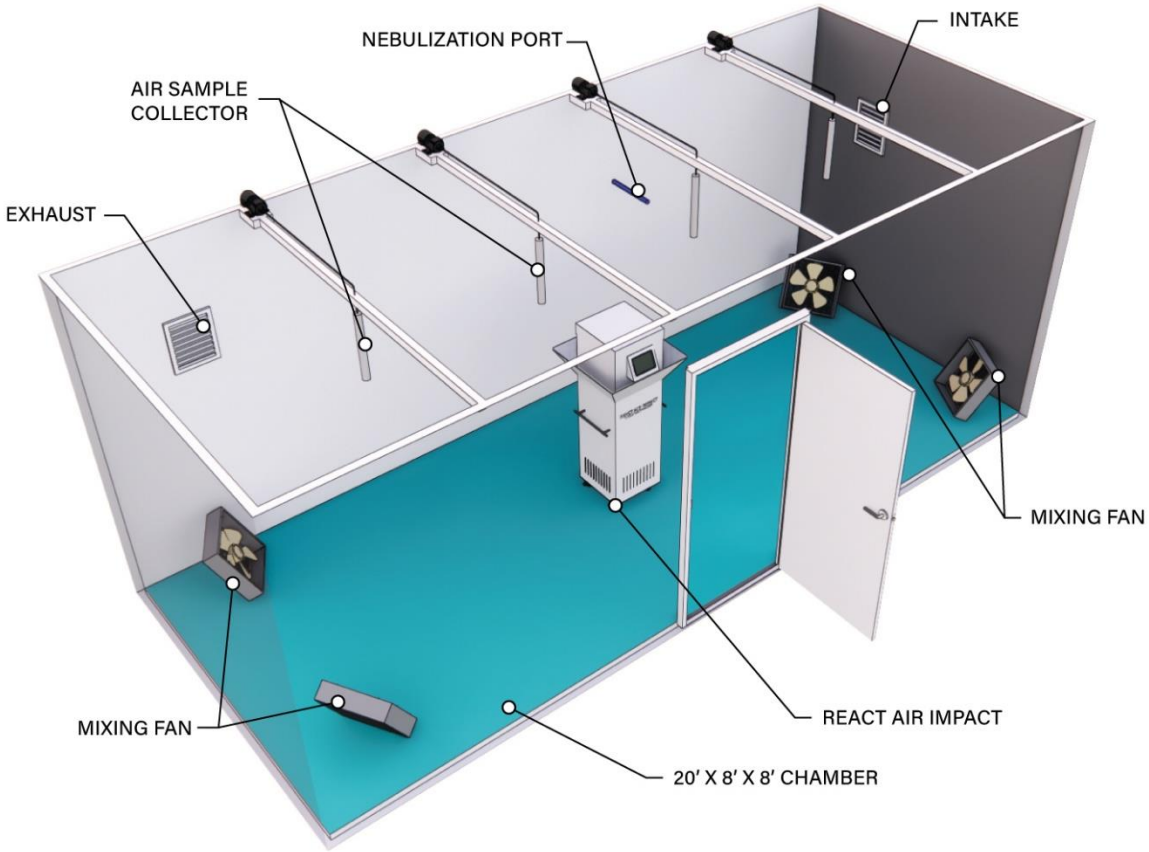


Figure 1. Room layout for control and experimental trial.

Test Method:

Reaction Group supplied the React Air Destroyer for testing purposes to determine efficacy against viral pathogens. This study evaluated the efficacy of the React Air Destroyer in its ability to reduce the viral strain referred to as SARS-CoV-2 within the air.

Exposure Conditions:

1. Prior to the initial control test and following each trial run, the testing area was decontaminated and prepped per internal procedures.
2. The temperature during all test runs was approximately 72°F ±2°F with a relative humidity of 35%.
3. The air samplers were calibrated by the manufacturer on September 3, 2020 and set at a standard flow of 5.02L/min. Calibration records indicate a 0.20% tolerance.
4. The air sample collection volumes were set to 10-minute continual draws at the point of sampling.
5. Low volume mixing fans were placed at each corner of the chamber at a 45-degree angle and turned on prior to nebulization.
6. The test condition had the React Air Destroyer device in the center of the test box.

Nebulization:

1. Nebulization for control and viral test challenges were performed in the same manner.
2. After nebulization of the pathogen, the React Air Destroyer system was turned on via remote control.
3. For the viral challenge, a known quantity of viral media was nebulized into the sealed environment from a dissemination port.
4. The viral media was nebulized at a constant rate for 25 minutes.
5. During the pathogen challenge, the React Air Destroyer was turned off after 15 minutes of exposure for air sample collection to start.
6. Air sampling collection occurred after nebulization ceased for a total of 10 minutes for both the challenge and control tests.
7. Fan speed on the React Air Destroyer was set on high for the test conditions.
8. The sample cassettes were manually removed from the collection system after each control run and each air pass challenge and pooled.
9. Upon cassette removal after each challenge, cassette sets were taken to an adjacent biosafety cabinet for extraction and placement into viral suspension media.
10. Two controls were completed and three viral challenges were conducted using the same methodology.

Post Decontamination:

At the conclusion of each viral challenge test, the UV-C system inside the testing chamber was activated for 30 minutes. After 30 minutes of UV-C exposure there was a 30-minute air purge through the air filtration system. 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.

Preparation of The Pathogen

Viral Stock: SARS-CoV-2 USA-CA1/2020 (BEI NR-52382)

Test	Specifications	Results
Identification by Infectivity in Vero 6 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, isolate USA-CA1/2020 GenBank: MN994467.1	99.9% identity with SARS-CoV 2, isolate USA-CA1/2020 GenBank: MN994467.1
Approx. 940 Nucleotides	≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1	100% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-CA1/2020 GenBank: MT246667.1
Titer by TCID50 in Vero E6 Cells by cytopathic effect	Report Results	2.8 X 10^5 TCID50 per mL in 5 days at 37°C and 5% CO_2
Sterility (21-Day Incubation)		
Harpos HTYE Broth, aerobic	No Growth	No Growth
Trypticase Soy Broth, aerobic	No Growth	No Growth
Sabourad 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.

TCID50 Procedure:

Materials and Equipment:

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips—20uL, 200 uL, 1000uL
- Inverted Microscope
- Tubes for dilution
- Hemocytometer with coverslip
- Cell media for infection
- Growth media appropriate for the cell line
- 0.4 % Trypan Blue Solution
- Lint-free wipes saturated with 70% isopropyl alcohol
- CO_2 Incubator set at 37°C or 34°C, or other temperature as indicated

Procedure:

1. One day before infection, prepare 96 well dishes by seeding each well with Vero E6 cells in DMEM plus 7.5% fetal bovine serum, 4mM Glutamine, and antibiotics.
2. On the day of infection, make dilutions of virus samples in PBS.
3. Make a series of dilutions at 1:10 of the original virus sample. Fill the first tube with 2.0 mL PBS and the subsequent tubes with 1.8mL.
4. Vortex the viral samples, then transfer 20 uL of the virus to the first tube, vortex, discard tip.
5. With a new tip, serial dilute subsequent tips transferring 200 uL.

Additions of virus dilutions to cells

1. Label the lid of a 96-well dish by drawing grid lines to delineate quadruplicates and number each grid to correspond to the virus sample and label the rows of the plate for the dilution, which will be plated.
2. Include four (4) negative wells on each plate which will not be infected.
3. Remove all but 0.1 mL of media from each well by vacuum aspiration.
4. Starting from the most dilute sample, add 0.1 mL of virus dilution to each of the quadruplicate wells for that dilution.
5. Infect four wells per dilution, working backward.

6. Allow the virus to absorb to the cells at 37°C for 2 hours.
7. After absorption, remove the virus inoculum. Start with the most dilute and work backward.
8. Add 0.5 mL infection medium to each well, being careful not to touch the wells with the pipette.
9. Place plates at 37°C and monitor CPE using the inverted microscope over a period of 1 to 4 weeks.
10. Record the number of positive and negative wells.

Protocol Changes:

Protocol Amendments: None

Protocol Deviations: None

Control Protocol

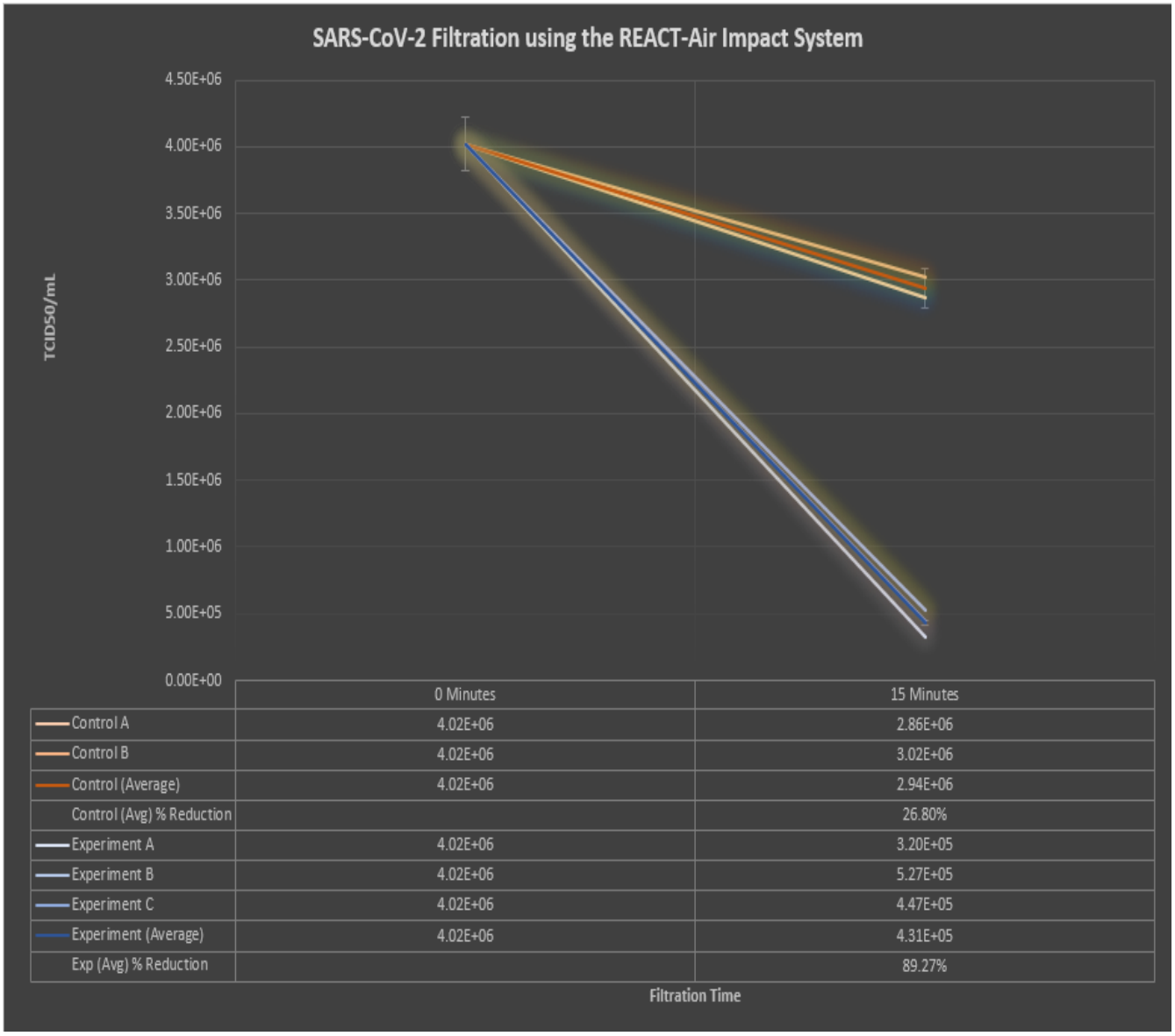
Two control tests were conducted without the React Air Destroyer system operating in the testing chamber. During the control test, four low volume fans were operated in each corner to ensure homogenous mixing of the air. Control samples were taken at the corresponding sample times used for the challenge trial. Nebulization of viral media and collection methods were the same for the control as the viral challenge. Control testing was used for the comparative baseline to assess the viral reduction when the React Air Destroyer device was operated in the challenge trials to enable net reduction calculations to be made. Furthermore, temperature and relative humidity were monitored and confirmed to be in relative range, ±5% compared to control testing conditions prior to running the viral challenges.

Aerosolization of Viral Media:

The control samples were performed in the same manner as the viral test regarding the time points and collection rate. A viral stock of SARS-CoV-2 USA-CA1/2020 with a concentration of 6.32 X 10^6 TCID50/mL* was used for this experiment.

Study Results

RESULTS:



	0 Minutes	15 Minutes
Control A	4.02E+06	2.86E+06
Control B	4.02E+06	3.02E+06
Control (Average)	4.02E+06	2.94E+06
Control (Avg) % Reduction		26.80%
Experiment A	4.02E+06	3.20E+05
Experiment B	4.02E+06	5.27E+05
Experiment C	4.02E+06	4.47E+05
Experiment (Average)	4.02E+06	4.31E+05
Exp (Avg) % Reduction		89.27%

***As it pertains to data represented herein; the percentage error equates tio an average of ±5% of the final concentration.

Conclusion:

The Reaction Group React Air Destroyer demonstrated an overall more rapid reduction of recoverable active virus in the air in relatively a short amount of time as compared to control values. After 15 minutes of operation, results showed an average 89.27% net reduction of viable viruses within the air. Experiment (A) had a 92.03% reduction, experiment (B) had an 86.9% reduction and experiment (C) had an 88.88% reduction. As the test was designed to observe aerosol reduction capabilities of the React Air Destroyer device, it is unknown if any active pathogen remained on the surface areas inside the unit or on the testing chamber walls. Overall, the trials showed how efficient the React Air Destroyer was at reducing the concentration of aerosolized SARS-CoV-2 in the breathable air.

Effort was made to simulate a real-life environment in the chamber while taking into consideration the special precautions needed when working with a Biosafety Level 3 Pathogen. Furthermore, 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, viral destruction on collection, viral destruction on nebulization and possibly others. Every effort was made to address these constraints with the design and execution of the trials. And these efforts are reflected in the meaningful recovery of virus in the control test.

Considerations:

Different air volume environments will create different efficacy reductions. Airflow/ACH/location and multitude of other factors will have effects on overall performance of the equipment.

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Disclaimer

The Innovative Bioanalysis, Inc. ("Innovative Bioanalysis") laboratory is not certified or licensed by the United States Environmental Protection Agency and makes no equipment emissions claims pertaining to ozone or byproduct of any React Air Destroyer device. Innovative Bioanalysis, Inc. makes no claims to the overall efficacy of any Reaction Group devices. The experiment results are solely applicable to the device used in the trial. The results are only representative of the experiment design described in this report. Innovative Bioanalysis, Inc. makes no claims as to the reproducibility of the experiment results given the possible variation of experiment results even with an identical test environment, viral strain, collection method, inoculation, nebulization, viral media, cell type, and culture procedure. Innovative Bioanalysis, Inc. makes no claims to third parties and takes no responsibility for any consequences arising out of the use of, or reliance on, the experiment results by third parties.

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Chief Scientific Officer, Innovative Bioanalysis, Inc.

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Kevin Noble **Date**

Laboratory Director, Innovative Bioanalysis, Inc.