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Rhinovirus Viral Reduction Through Ionization

CLIENT: ACA/IAE

PROJECT: ACA/IAE-ION-VIRAL-02

PRODUCT: ACA-RN-0001 and ACA4800GU-1, Powered by GPS NPBI™ Technology
CAP LIC NO: 886029801
CLIA LIC NO: O5D0955926
STATE ID : CLF 00324630

REPORT DATE: 11/18/2020

CHALLENGE ORGANISIM(S):

- **Rhinovirus - 40, 1794**

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ABSTRACT: EFFICACY OF THE ACA BI-POLAR IONIZATION SYSTEMS AGAINST RHINOVIRUS

Background: This in vitro study was designed to determine the efficacy of the ACA RN-0001 and 4800GU-1 units against knowns pathogens. These products are commercially available and designed for similar purposes. The ACA 4800GU-1 system is engineered as a mobile disinfection device manufactured ACA using GPS NPBI™ technology. The system is designed to be placed free standing in a large room or structure and decrease the concentration of bacteria and pathogens in the air and on surfaces while operational. The ACA RN-0001 system is designed to be installed in the air ducting of an Environmental Control System or other similar air transfer systems. THE ACA RN-0001 is designed to reduce the concentration of bacteria and pathogens in the air and on surfaces while operational. The main difference between the two systems tested was application and how air flow was moved across the needle point ionization pillars. Both units use similar components and have the same process for creating positive and negatively charged ions.

For this challenge, the following viral strain was used.

- **Rhinovirus - 40, 1794**

Rhinovirus is also sometimes referred to as the common cold. According to the CDC common colds are the main reason that children miss school and adults miss work. Each year in the United States, there are millions of cases of the common cold. Adults have an average of 2-3 colds per year, and children have even more. Up to 80% of common cold illnesses may be associated with a documented rhinovirus infection however not all colds are cause by strains of Rhinovirus. According to Oxford academic rhinovirus infection has also been associated with lower respiratory tract symptoms. It is widely accepted that rhinovirus is an important cause of asthma exacerbations in school-aged children. Rhinovirus infection is associated with 60%–70% of the asthma exacerbations in certain age groups. Rhinovirus also appears to play a role in exacerbations of cystic fibrosis in children and of chronic bronchitis in adults.

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EQUIPMENT PROVIEDED:

MANUFACTURER: Aviation Clean Air (ACA)

MODEL: 4800GU-1

SERIAL# 17642

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UNIT 2

MANUFACTURER: Aviation Clean Air (ACA)

MODEL: RN-0001

SERIAL#: TEST UNIT

ACA EUIPMENT:

The equipment arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. Prior to starting the challenge, the 4800GU-1 and RN-0001 were operated separately for 1 hour in a dry run in a sealed bioaerosol to confirm correct operations. Chamber was the same BSL3 chamber used for the viral challenge testing. RKI air monitoring systems continuously sampled air for O3, H202, N20 production which could put staff members at risk. Air monitoring was in place as a safety mechanism for staff and no alarms for unsafe elevated O3 were activated during testing.

TESTING CHAMBER:

The testing chamber was a large, sealed air volume testing chamber consisting of metal walls and epoxy floor which complied with BSL3 standards. The chamber was designed to be completely sealed from the outside environment to prevent outside variables from entering the test chamber. The testing chamber was equipped with 4 sealed viewing windows and a lockable chamber door for entry and exit. The overall dimensions of the test chamber were approximately 8'x8'x20'.

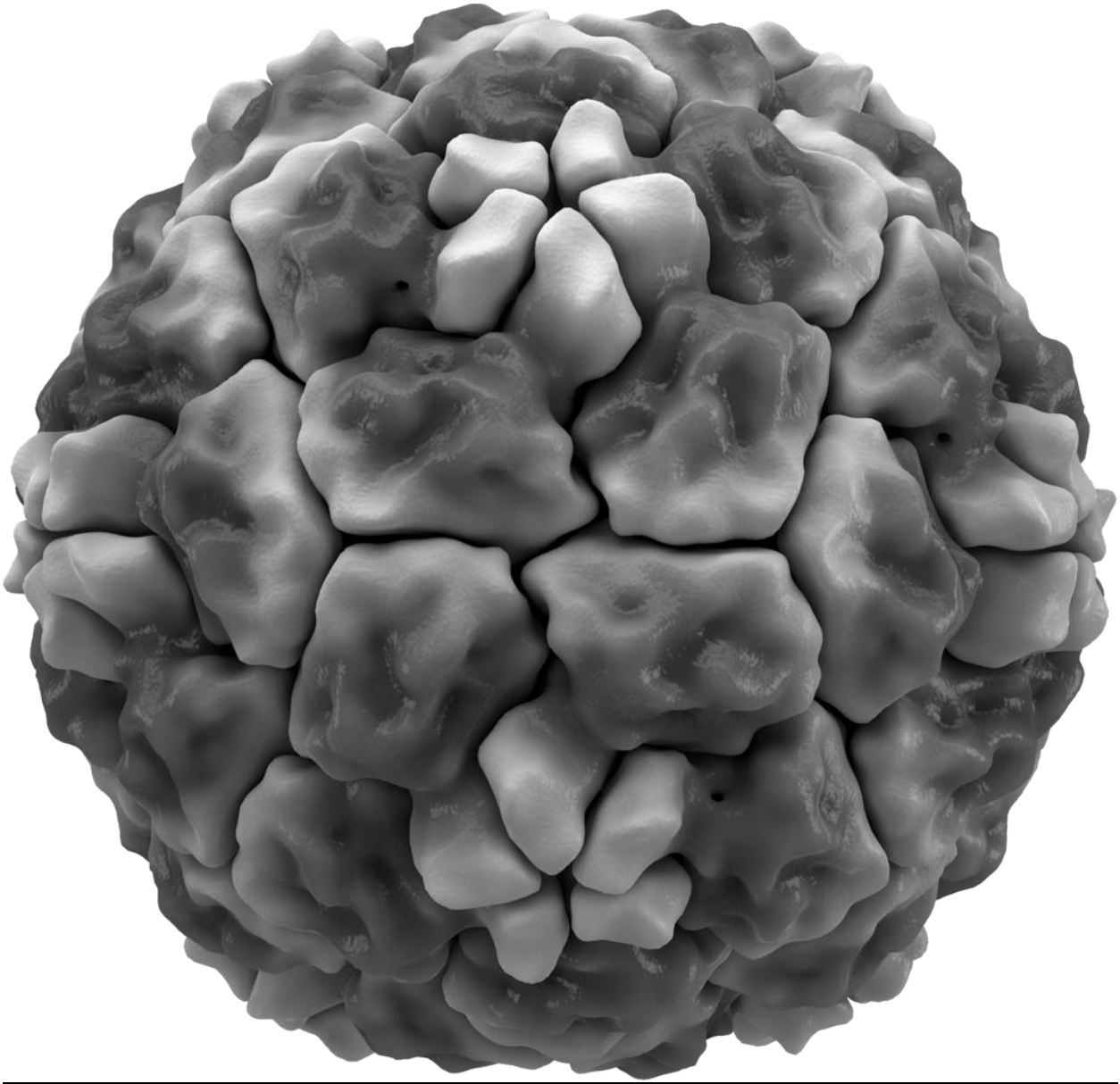
The testing chamber had HEPA filtered inlets and exhaust, coupled with an active UV-C system in all ducting lines. Humidity and temperature were monitored inside the chamber using a calibrated wireless device. Prior to testing, the chamber was pressure tested for leaks and visual inspections were made using a colored smoking device. All seals for the chamber were confirmed and all equipment used had a function test to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

CONTROL SUMMARY:

For the control section one AIC2 Air Ion counter was placed in the center of the of the testing chamber for 5 minutes prior to the control test. The natural state of ions was counted, and little fluctuations were observed. Ion counts were recorded every 0.5 seconds and the average for the duration of the test was 73 ions per cm3 without the ionization unit running.

5 sterile dishes containing viral media were provided by the lab staff, labeled with time point designation and viral strain. Dishes were placed on a table inside the room and the door closed to prevent outside environmental contaminants. Swabs were taken at the pre-defined time points of 0 Minutes, 10 minutes, 20 minutes, 30 minutes, and 60 minutes for three viral pathogens tests and all swabs were sealed after collection and provided to lab staff. The door to the chambers remained closed the entirety of the test and all air entering the test chamber was filtered through a HEPA filter.

RHINOVIRUS:



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EXPERIMENT SUMMARY:

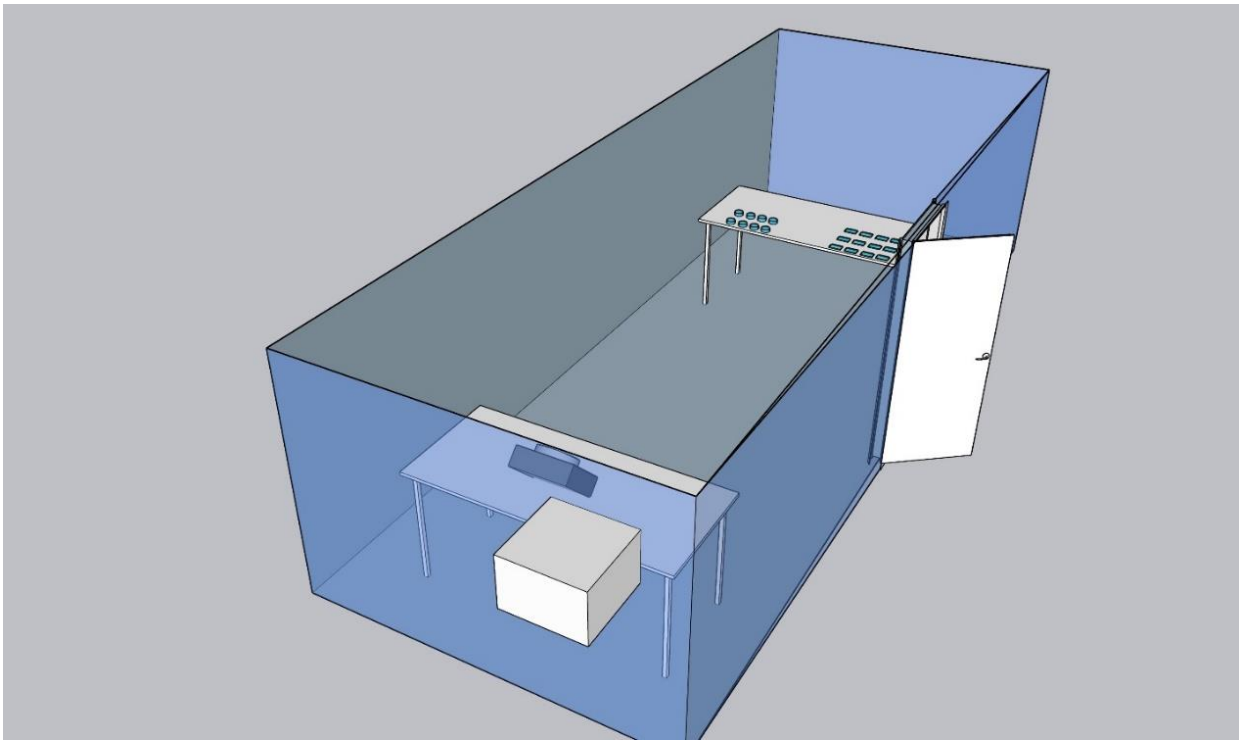
ACA/IAE supplied an **ACA-RN-0001 and ACA4800GU-1** system for testing purposes to determine efficacy against viral pathogens. This study was to evaluate the efficacy of the viral strain referred to as Rhinovirus in a large setting Both the ACA-RN-0001 and AVA4800GU-1 have been tested and evaluated to be consistent in production of equal ions in the test chamber under controlled conditions. Both systems have been used in the following test procedures and the results of each system are identical in performance and ion production depending on airflow and orientation.

- Prior to the initial control test and following each trial run the testing area was decontaminated and prepped per internal procedures.
- Temperature during all test runs was approximately 73F +/- 2F with a relative humidity of 48%.
- Relative humidity and temperature were taken in two sections of the chamber during all tests to confirm there was no more than a 3% deviation from each side.
- Swabs were taken at predefined time points of the following with T=minute
    - T-0
    - T-10
    - T-20
    - T-30
    - T-60
- Testing chamber was interior seal was not breached during the test, all air entering passed through a HEPA filter.
- No drop in humidity was observed and based on previous studies a fluctuation of a few % of humidity change will have negative impact on the challenge study.
- Behind the rowed test site there was an AIC2 Air Ion Counter continually logging the negative ion count.
- Three separate test rows were laid out for each the three separate viral strains.
- All sample dishes were labeled with their virus strain and the time point they were to be used with. 1 sample swab was taken from each dish, as well as a swab collected for residual viral media at 0-minute timepoint, 10-minute time point, 20-minute time point, and 30-minute time point and 60-minute time point.
- The RN-0001 system was attached to a variable speed fan and angled up at a 45-degree angle to allow the ions to cascade down throughout the room
- Fan speeds were adjusted until the desired concentration was reached prior to exposing test samples. All samples were brought into the testing chamber sealed and removed from the testing chamber sealed.
- 4800GU-1 system was placed on a table opposite of bacteria samples and angled upwards at a 45-degree angle.
- Upon testing completion, samples were provided to lab staff for further review.

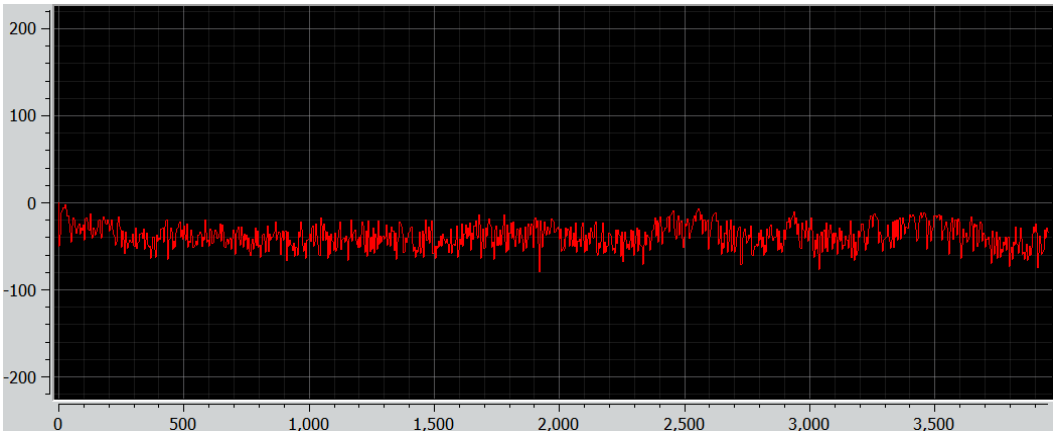
DESIGN LAYOUT:

The ACA RN-0001 and 4800GU-1 needed 2 separate design layouts to create similar operating conditions. For the RN-0001 system which is designed for ECS ducting systems a mock setup was constructed to supply airflow to the ionization portion of the system using a variable speed fan.

When testing the 4800GU-1 series the same environment was used and the system was placed on top of the table in the same location the RN-0001 operated.



TEST AVERAGE RN – 001 (-27K IONS PER CUBIC CENTIMETER)



VIRAL STOCK:
Rhinovirus - 40, 1794,
Starting Concentration: 1.0 X 10^6 TCID50/mL
Item Number: NR-51453

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 cover slip**
- **Cell Media for infection**
- **Growth Media appropriate for cell line**
- **0.4 % Trypan Blue Solution**
- **Lint Free Wipes saturated with 70% isopropyl alcohol**
- **CO2 Incubator set at 37°C or 34°C or other temperature indicated.**

Procedure:

1. **One day previous to infection, prepare 48 well dishes by seeding each well with in DMEM plus 7.5 % fetal bovine serum, 4mM Glutamine, and antibiotics.**
2. **On day of infection, make dilutions of virus sample in PBS.**
3. **Make a series of dilutions at 1:10 of the original virus sample. First tube with 2.0 mL PBS and subsequent tubes with 1.8mL.**
4. **Vortex Virus samples transfer 20 uL of virus to first tube, vortex, discard tip.**
5. **With new tip, serial dilute subsequent tips transferring 200 uL.**

Additions of virus dilutions to cells

1. **Label lid of 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 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 4 wells per dilution, working backward.**
6. **Allow the virus to absorb to cells at 37°C for 2 hours.**
7. **After absorption, remove virus inoculum. Start with the most dilute and work backwards**
8. **Add 0.5 mL infection medium to each well being careful to not 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.**
11. **Calculate TCID50.**

VIRAL TITRATION DETERMINED BY TCID50 ASSAY PROTOCOL

Each of the 10 samples collected were subject to the same TCID50 assay protocol to determine viral concentration. Each collected swab was vortexed for 30 seconds in 2ml viral preservation media prior to serial dilution.

INNOCULATION OF THE TEST CARRIERS:

Each of the 10 testing sites were simultaneously and equally subjected to a 1ml direct inoculation of the pathogens identified above with the respective concentrations.

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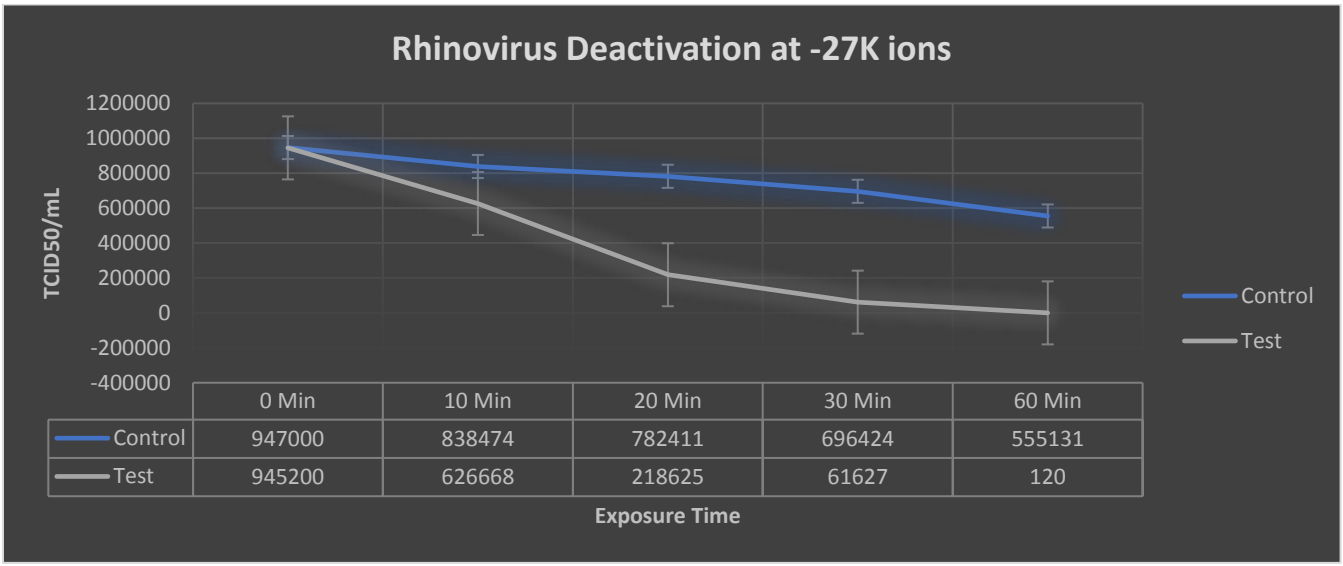
EFFICACY TESTING:

Viral media with a known concentration was applied via direct inoculation to the glass slides in single location in the containment unit and exposed to bipolar ionization for a period of 0 Min, 10 Min, 20 Min, 30 Minutes, and 60 Minutes. Swabs were taken of all material after a rinse with viral media and cultured by the same means as the original viral titration performed on the BEI Resources provided certificates of analysis. Results are as follows.



	0 Min	10 Min	20 Min	30 Min	60 Min
Control	947000	838474	782411	696424	555131
Test	945200	626668	218625	61627	120

Log 10 Reduction:

Control: 0 Min: 0.02 10 Min: 0.05 20 Min: 0.08 30 Min: 0.13 60 Min: 0.23

Test: 0 Min: 0.02 10 Min: 0.18 20 Min: 0.64 30 Min: 1.19 60 Min: >3.9

CONCLUSION:

Given the results displayed above it is understood that the pathogens tested in this environment were neutralized much more rapidly in an environment with an average concentration of 27K ions per cubic centimeter compared to the control samples. As it relates to the individual pathogens: Rhinovirus has an average life of 3 hours on the same type of surfaces. This is clearly indicated in the control line of the respective graphs. Due to the nature of this test being surface, the same efficacy can be theorized for an aerosol but was not directly tested.

CONSIDERATIONS:

- This experiment was conducted in a controlled environment in accordance with all standards set forth by the CDC for handling infectious pathogens.
- The specific viral titers utilized for this experiment are likely much higher than you would come across in a normal mode of transmission.
- The environment, although controlled, did not account for all environmental factors that could be experienced in a real-world scenario.
- All pathogens utilized in this experiment were quality tested prior to use.

DISCLAIMER:

The Innovative Bioanalysis, LLC. ("Innovative Bioanalysis") laboratory is not certified or licensed by the United States Environmental Protection Agency and makes no equipment emissions claims pertaining to ozone, hydrogen peroxide gas, reactive oxygen species, volatile organic compounds, or byproduct of any device. Innovative Bioanalysis makes no claims to the overall efficacy of any ACA products. 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 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, and culture procedure. Innovative Bioanalysis 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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