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ESCHERICHIA COLI Reduction Through Ionization

CLIENT: ACA

PROJECT: ACA/IAE-ION-BACTERIA-02

REPORT NUMBER: ACA/IAE-ION-ECOLI-1

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: 11/19/2020

CHALLENGE ORGANISIM(S):

- **ESCHERICHIA COLI O157:H7**

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ABSTRACT: EFFICACY OF THE ACA BI-POLAR IONIZATION SYSTEMS AGAINST E. COLI O157:H7

Background: This in vitro study was designed to determine the efficacy of the ACA RN-0001 and 4800GU-1 units against a known strain of bacteria. 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 Escherichia Coli O157:H7 **(E. COLI)** bacteria were used. According to public health studies each year in the United States, *E. coli* infections cause approximately 265,000 illnesses and about 100 deaths. Approximately 40 percent of these infections are caused by the strain *E. coli* O157:H7, a strain that is part of the shiga toxin-producing group of *E. coli* bacteria (STEC). The other 60 percent of *E. coli* cases are caused by non-0157:H7 shiga toxin-producing *E. coli* (STEC). There is a demand for disinfectant devices that have a proven ability to reduce the presence of bacteria in the air and on surfaces thereby reducing the risk of human infection and transmission.

Escherichia coli (E. coli) is a bacterium that normally is an important part of the healthy intestinal tracts of humans and animals. However, there are some kinds of *E. coli* that are harmful and can cause disease.

The most common type of *E. coli* infection that causes illness in people is called *E. coli* O157. *E. coli* O157 is naturally found in the intestinal tracts of many farm animals, including healthy cattle, sheep, and goats. Animals can carry *E. coli* O157 and shed the germs in their stool but still appear healthy and clean. The germs can quickly contaminate the animals' skin, fur, feathers, and the areas where they live and roam.

Most people become infected with *E. coli* O157 from contaminated food, such as undercooked ground beef or raw (unpasteurized) milk, but *E. coli* O157 can be passed directly to people from the stool of young calves and adult cattle. *E. coli* O157 *also* can be spread from person to person, particularly in places where frequent and close contact between people occurs, such as day-care facilities. Animals can appear healthy and clean but can spread *E. coli* O157 to humans or other animals.

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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.

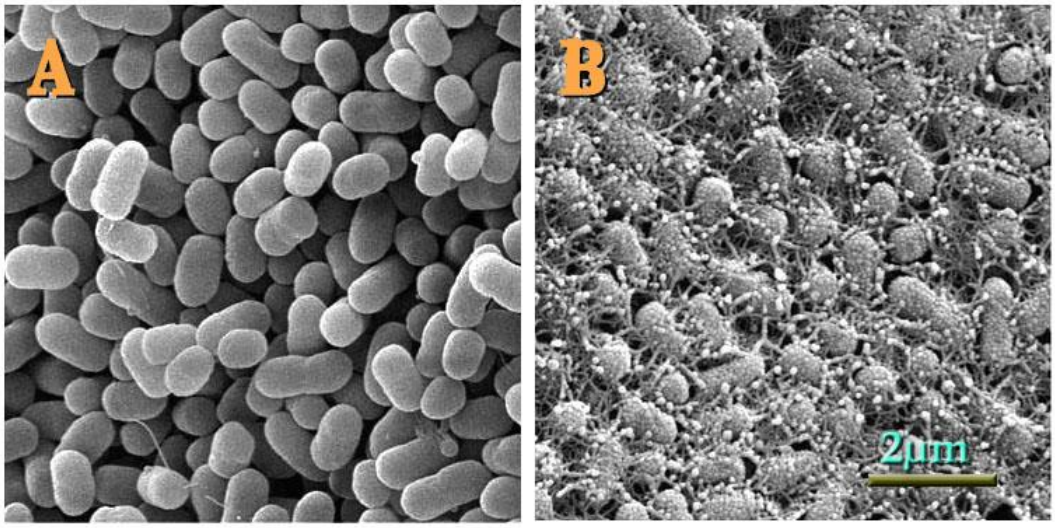
CONTROL SUMMARY:

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 organisms were provided by the lab staff, labeled with time point designation and organism. 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 E. Coli 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.

BACTERIA

BEI Resources Catalog number NR-8 (CoA attached) Escherichia Coli was cultured by plating the thawed broth on Tryptic Soy Agar and allowed to incubate at 32°C with 5% CO2 for 24 hours. A single isolate colony was harvested and introduced to a tryptic soy broth and allowed to incubate at 32°C for an additional 24 hours. Upon completion of the incubation period, bacteria were harvested and rinsed 3 times in phosphate buffered saline. A 1 to 10 dilution was made by removing 1 mL of inoculated tryptic soy broth and adding it 9 mL of phosphate buffered saline. This solution was further diluted to a final concentration of 1:100.



MATERIALS AND EQUIPMENT:

- **Certified Biological Safety Cabinet**
- **Micropipette and sterile disposable aerosol resistant tips – 20uL, 200 uL, 1000uL**
- **Microscope**
- **Tubes for dilution**
- **Hemocytometer with cover slip**
- **Tryptic Soy Broth**
- **Tryptic Soy Agar**
- **10 uL Inoculation Loops**
- **CO2 Incubator set at 34°C**

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EXPERIMENT SUMMARY:

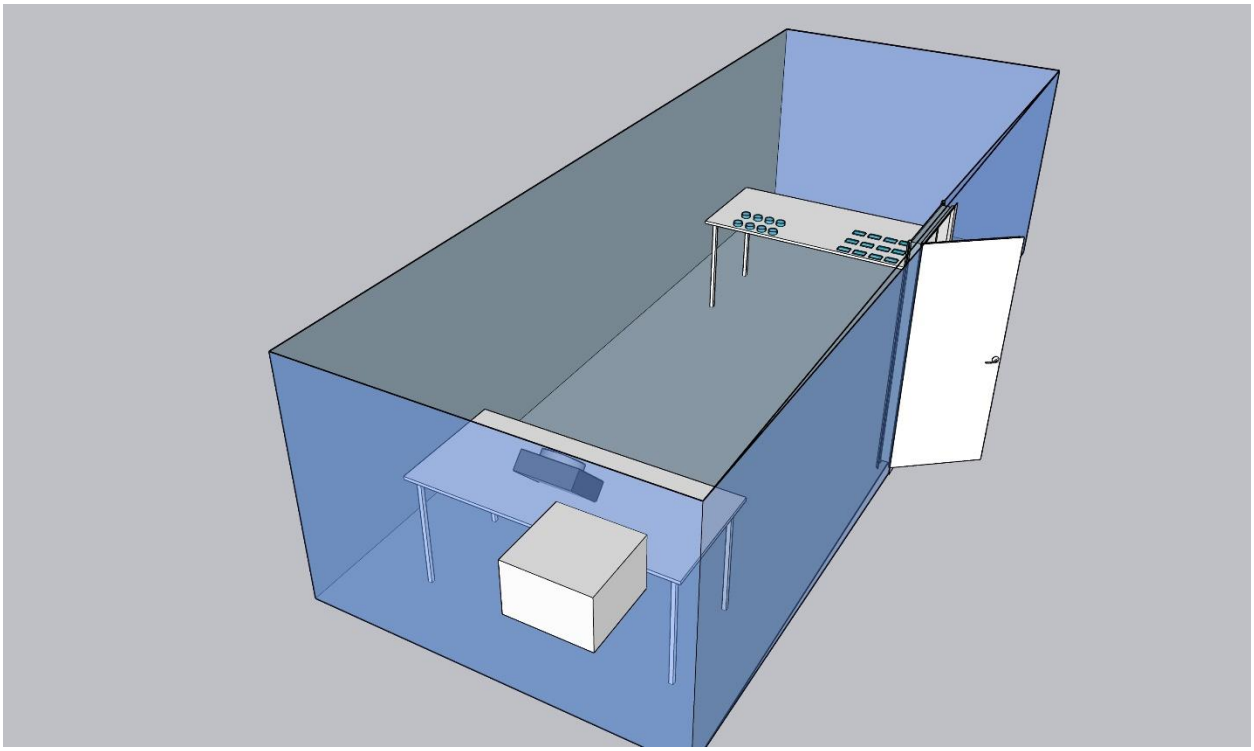
ACA/IAE supplied an **ACA-RN-0001 and ACA4800GU-1** system for testing purposes to determine efficacy against Escherichia Coli. This study was to evaluate the efficacy of one bacterial strain referred to as E. Coli in a large setting. Both the ACA-RN-0001 and AVA4800GU-1 have been tested and evaluated 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.
- Test row contained 5 round petri dishes provided by the lab inoculated with 1mL of E. Coli with a concentration of 31,000 CFU/mL.
- All sample dishes were labeled with their bacterium 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 bacteria 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.



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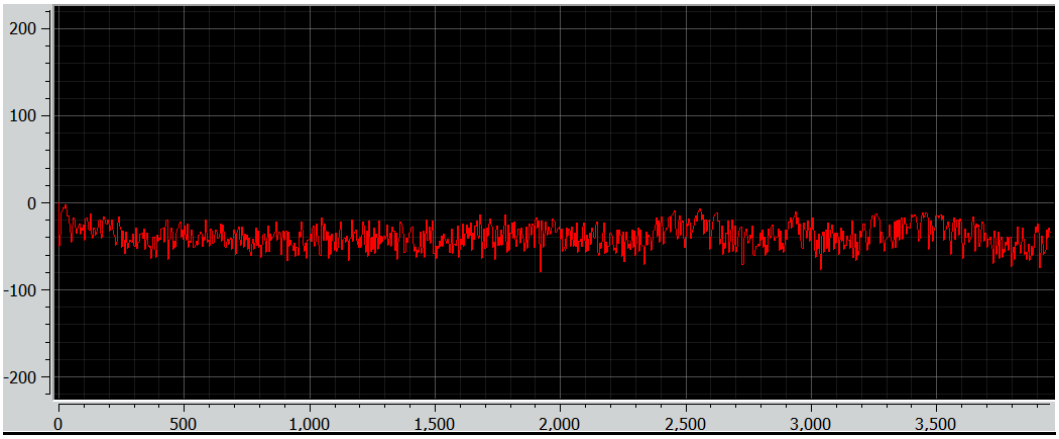
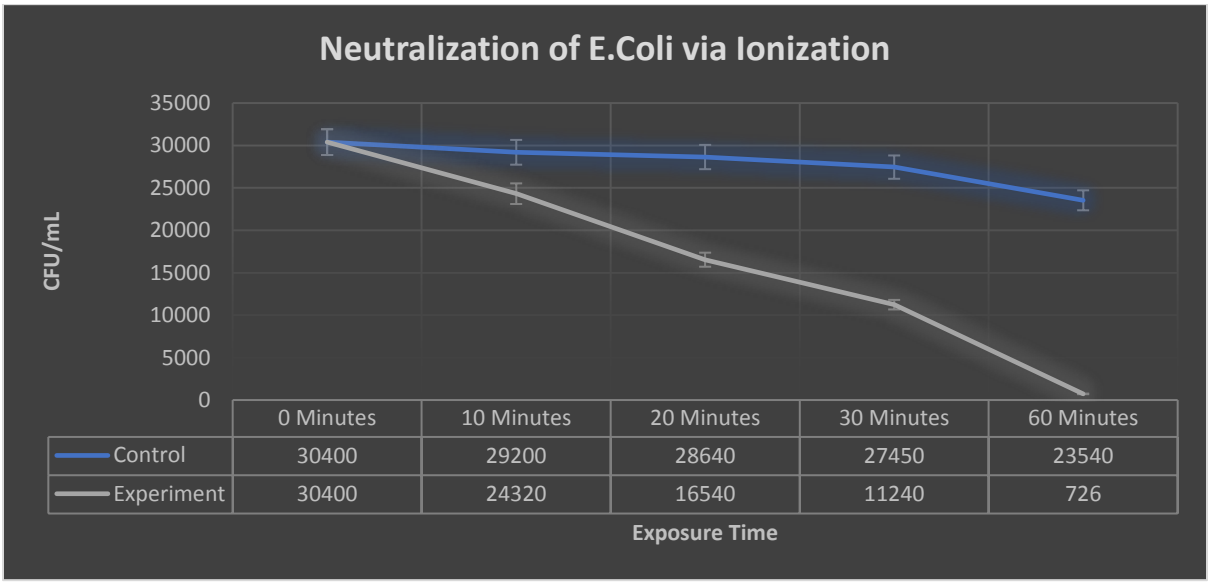
TEST AVERAGE RN – 001 (-27K IONS PER CUBIC CENTIMETER)



RESULTS:



CONCLUSIONS:

The overall reduction of controls (natural degradation) versus experiment shows the increased degradation of E. Coli in an ionized environment. It is important to note the natural degradation at each time point when considering the effectiveness of the ionized environment. In conclusion, utilization of the ACA-RN-0001 and ACA4800GU-1 in the test environment significantly decreased the concentration of E. Coli in a 60-minute time by 97.6 % overall and 75 % better than natural degradation.

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