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ESCHERICHIA COLI SUSPENSION SURFACE EFFICACY TESTING

PROJECT: GPS SURFACE *E. COLI*

TECHNOLOGY: Needle Point Bipolar Ionization (NPBI™)

DEVICE: GPS-FC48-AC™

CAP LIC NO: 8860298

CLIA LIC NO: 05D0955926

STATE ID: CLF 00324630

CHALLENGE ORGANISM:

ESCHERICHIA COLI O157: H7

Dana Yee, M.D.

Medical Director

Study Completion Date:

6/14/2021

Study Revision Date:

3/22/22

Testing Facility:

Innovative Bioanalysis, Inc.

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Laboratory Project Number:

1034-E

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Efficacy Study Summary

Study Title	*ESCHERICHIA COLI* SUSPENSION SURFACE EFFICACY TESTING
Laboratory Project #	1034-E
Guideline:	No standard exists; GCLP and modified ISO standards were used.
Testing Facility	Innovative Bioanalysis, Inc.
GLP Compliance	All internal SOPs and processes follow GCLP guidelines and recommendations.
Test Substance	*ESCHERICHIA COLI* O157: H7
Description	The GPS-FC48-AC™ device housing NPBI™ technology is commercially available and designed to be installed in the ductwork of an HVAC system to reduce the concentration of certain viruses and bacteria on surfaces and potentially in the air while operational. Testing was conducted on the device to evaluate the effectiveness of the NPBI™ technology in reducing a known bacterial strain, *Escherichia coli,* on a surface.
Test Conditions	The test was conducted in an airtight chamber with a redundant negative pressure system connected to HEPA filters and in duct UV-C to be used when needed. The overall dimensions of the test chamber were approximately 8'x8'x20'. The temperature during all test runs ranged from 72°F to 73°F, with a relative humidity of 44%.
Test Results	The GPS-FC48-AC™ device housing NPBI™ technology performed to manufacturer specifications and demonstrated an 89.55% reduction of active *Escherichia coli* on a surface after 60 minutes of exposure. Ion concentrations were measured during testing with an average over the 60 minutes of 14,000 negative ions per cm^3.
Control Results	The natural state of positive and negatively charged ions was counted. *Escherichia coli* neutralization at 14,000 ions/cm^3 was measured and decreased from 63,400 to 48,560 CFU/mL.
Conclusion	Using a GPS-FC48-AC™ device housing NPBI™ technology, *Escherichia coli* pathogens were neutralized much more rapidly than in a controlled setting without the device. There was an 89.55% reduction in active *Escherichia coli* on a surface after 60 minutes of exposure. The *Escherichia coli* decreased from 63,400 to 6,620 CFU/mL.

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Study Report

Study Title: *ESCHERICHIA COLI* SUSPENSION SURFACE EFFICACY TESTING

Sponsor: Global Plasma Solution

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

Technology Tested: NPBI™

Device Tested: GPS-FC48-AC™

Study Dates:

Study Report Date: 06/14/2021
Study Report Revision Date: 03/22/2022
Experimental Start Date: 04/12/2021
Experimental End Date: 04/15/2021
Study Completion Date: 06/14/2021

Study Objective:

This in vitro study was designed to determine the efficacy of the GPS-FC48-AC™ containing NPBI™ technology against a known strain of bacteria, *Escherichia coli* (*E. coli*), on a surface. This product is commercially available and designed to be installed in the ductwork of an HVAC system to reduce the concentration of bacteria and pathogens in the air and on surfaces while operational. *Escherichia coli* is a bacterium that normally is an important part of the healthy intestinal tracts of humans and animals. However, some kinds of *Escherichia coli* are harmful and can cause disease.

E. coli O157 is the most common type of *E. coli* infection to cause illness in people. *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 while appearing 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 can also be spread from person to person, particularly in places where frequent and close contact between people occurs, such as day-care facilities.

According to public health studies, each year in the United States, *Escherichia coli* infections cause approximately 265,000 illnesses and about 100 deaths. Roughly 40 percent of these infections are caused by the strain *E. coli* O157: H7, which 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 bacteria in the air and on surfaces, thereby reducing the risk of human infection and transmission.

Test Method:

Five dishes were inoculated with the prepared *Escherichia coli* for the control and experimental testing. Dishes were placed in the testing chamber on a table. Rinses and swabs were taken at four pre-determined times and the start of the test. All swabs were sealed after collection and analyzed by the staff after the study was completed. The door to the testing chamber remained closed throughout the test.

Test System Strains: *Escherichia coli (E. coli)*

BEI Resources Catalog number NR-8 *Escherichia Coli* O157:H7 (*E. Coli*) bacteria were used for this challenge.

Study Materials and Equipment:

Equipment Overview: GPS-FC48-AC™ device housing NPBI™ technology, manufactured by Global Plasma Solutions, was used along with a testing chamber that complied with BSL3 standards. For the testing, power was supplied through a power-regulated 120v outlet with a surge protector and backup battery system.

MANUFACTURER: Global Plasma Air

MODEL: GPS FC48-AC™

SERIAL #: N/A

Equipment Specifics: The GPS-FC48-AC™ device housing NPBI™ technology arrived at the laboratory pre-packaged from the manufacturer and was inspected for damage upon arrival. Prior to starting the challenge, the device was operated for 1 hour in a dry run in a sealed bioaerosol to confirm correct operations.

Testing Chamber: The testing chamber was a large, sealed air volume testing chamber comprising metal walls and epoxy floor, which complied with Biosafety Level 3 (BSL3) standards. The chamber was airtight and sealed to separate the internal and external environments and therefore prevent the accidental exposure or release of any testing media. The testing chamber had a redundant negative pressure system connected to HEPA filters and an in-duct UV-C to be used when needed. The testing chamber was equipped with four sealed viewing windows and an automatic interlocking chamber door for entry and exit. The overall dimensions of the test chamber were approximately 8'x8'x20'. Humidity and temperature were monitored inside the testing lab using a calibrated wireless device.

Prior to testing, the chamber was pressure tested for leaks, and visual inspections were made. 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.

Design Layout:

Prior to testing, all internal lab systems were reviewed and determined to be functioning. All seals for the chamber were confirmed, and all equipment used had function tests to confirm working conditions. For calibrated equipment, calibration records were checked to confirm operational status.

The air temperature fluctuated slightly through the test and ranged from 72°F to 73°F. The humidity inside the test chamber was 44%. Sample dishes were placed on a stainless-steel table approximately 35 inches above the ground. Two Alpha Labs AIC2 ion meters were used behind the sample dishes to monitor positive and negative ion concentrations. During testing, ion measurements were taken directly above the samples to confirm consistent readings as shown in Figure 2. The FC48 was mounted on a movable scaffolding on the opposite side of the testing lab as the samples and was in an elevated position 74 inches above the ground. A variable-speed fan was placed behind the FC48 to create the necessary airflow to produce the required concentration of negative ions.

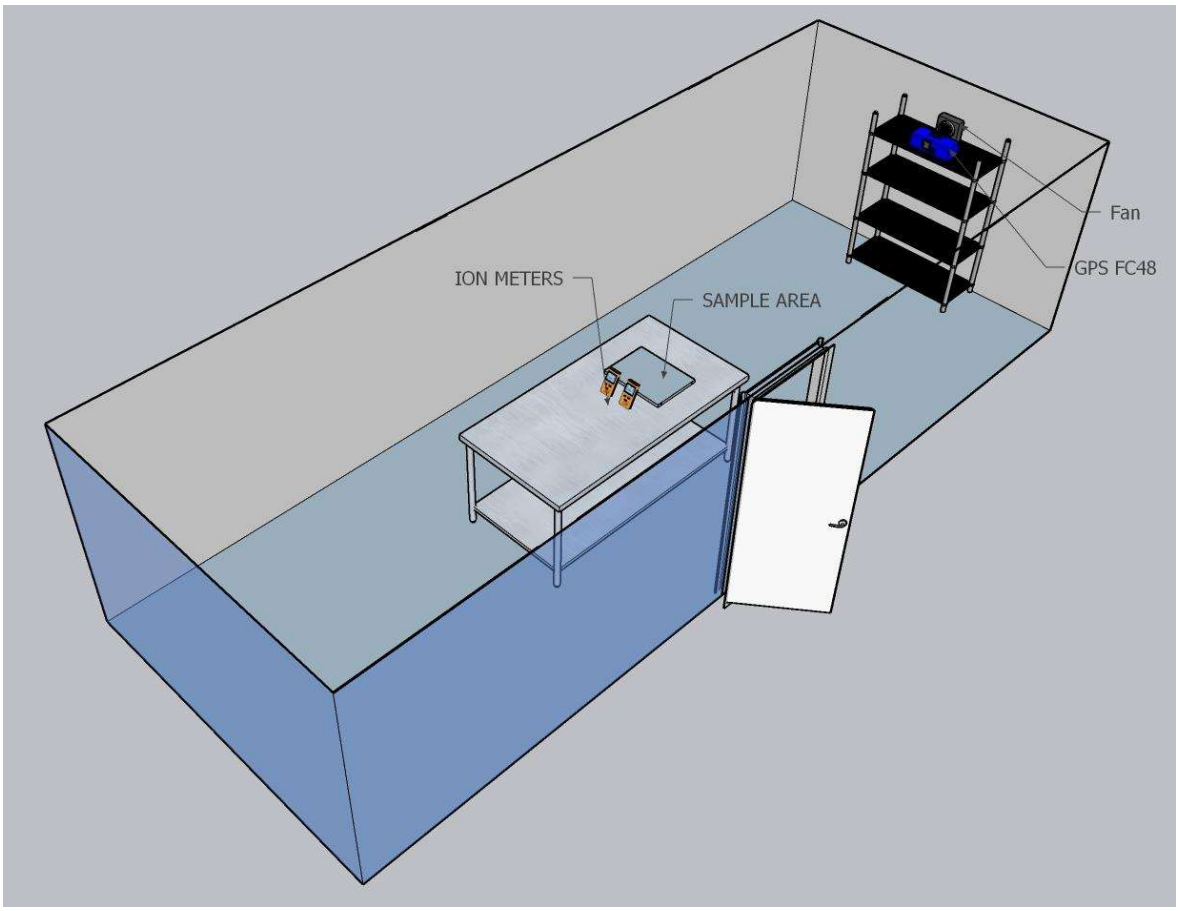


Figure 1. Room layout for the control and experimental trials.

Test Method:

GPS supplied a GPS-FC48-AC™ device housing NPBI™ technology for testing purposes to determine its efficacy against *Escherichia coli (E. coli*). Air ion measurements were taken directly above the samples to confirm that negative and positive ion concentrations were consistent with the readings from the meters behind the samples. A swab and rinse were performed on each sample dish based on the time point and cultured to determine bacteria recovery and overall efficacy.

Exposure Conditions:

In a dry run prior to running the organism challenge, all equipment was tested for functionality, and calibrations were performed as needed. Temperatures taken during all test runs ranged from 72°F to 73°F with a relative humidity of 44%.

Experimental Procedure

1. Prior to the initial control test and following each run, the testing area was decontaminated and prepped per internal procedures.
2. The GPS-FC48-AC™ device housing NPBI™ technology was turned on just prior to the start of testing at the 0-minute time point.
3. One sterile dish was inoculated with the *Escherichia coli* bacteria strain and used for its corresponding time point. A total of five dishes were used in the control and challenge portions of the testing and were provided by lab staff, labeled with time point designation and organism.
4. Dishes were placed on a stainless-steel table inside the room.
5. A swab and rinse were performed on each sample dish based on time point and cultured to determine bacteria recovery and overall efficacy.
6. Surface samples were taken at the following pre-determined time points after exposure.
    - 15 minutes
    - 30 minutes
    - 45 minutes
    - 60 minutes
7. All swabs were sealed after collection and provided to lab staff for analysis after study completion.
8. Air ion measurements were taken directly above the samples to confirm that the negative and positive ion concentrations were consistent with reading from the meters behind the samples.
9. At the conclusion of the testing, the UV system inside the lab was activated for 30 minutes.
10. After 30 minutes of UV exposure, all test equipment was cleaned at the end of each day with a 70% alcohol solution.

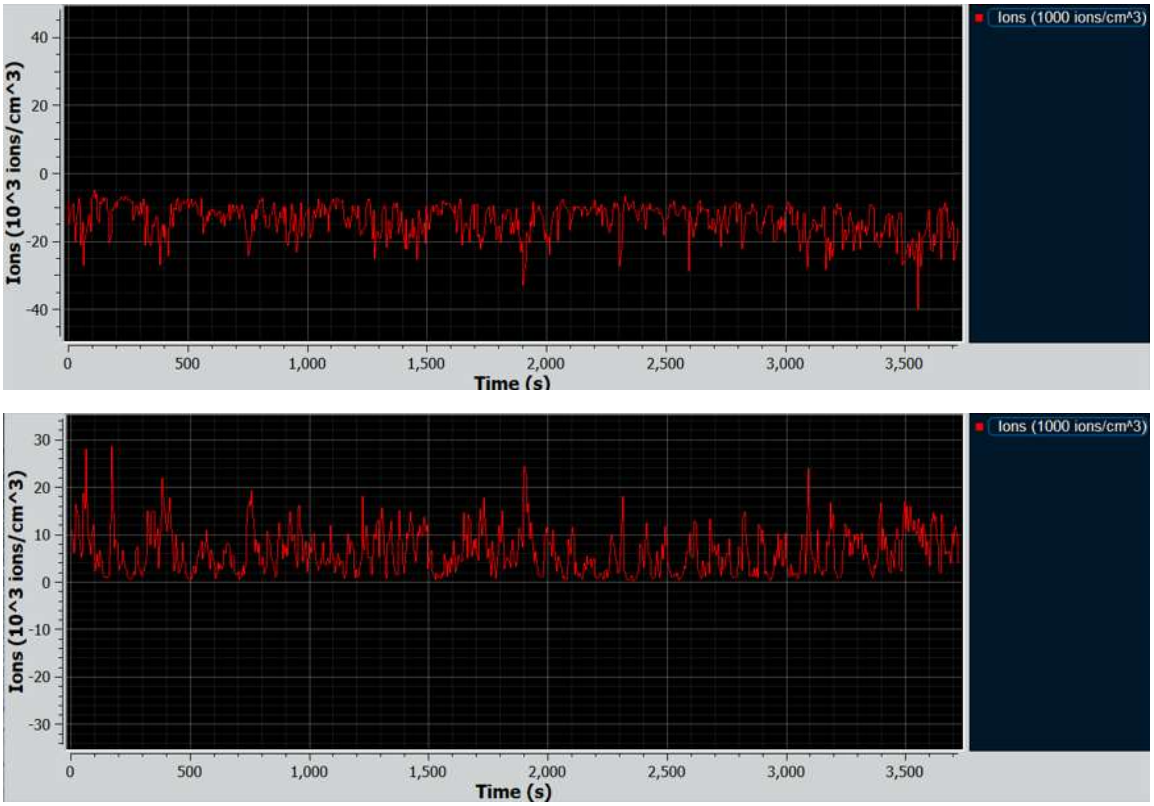


Figure 2. Device ion concentration recordings while in operation during testing.

Preparation of The Pathogen

BEI Resources Catalog number NR-8, *Escherichia coli* was cultured by plating the thawed broth on Tryptic Soy Agar and allowed to incubate at 32°C with 5% CO_2 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 three times in Phosphate Buffered Saline. A 1 to 10 dilution was made by removing 1 mL of inoculated Tryptic Soy Broth and adding it to 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 a coverslip
- Tryptic Soy Broth
- Nutrient Agar
- 10 uL Inoculation Loops
- CO_2 Incubator set at 34°C

Protocol Changes:

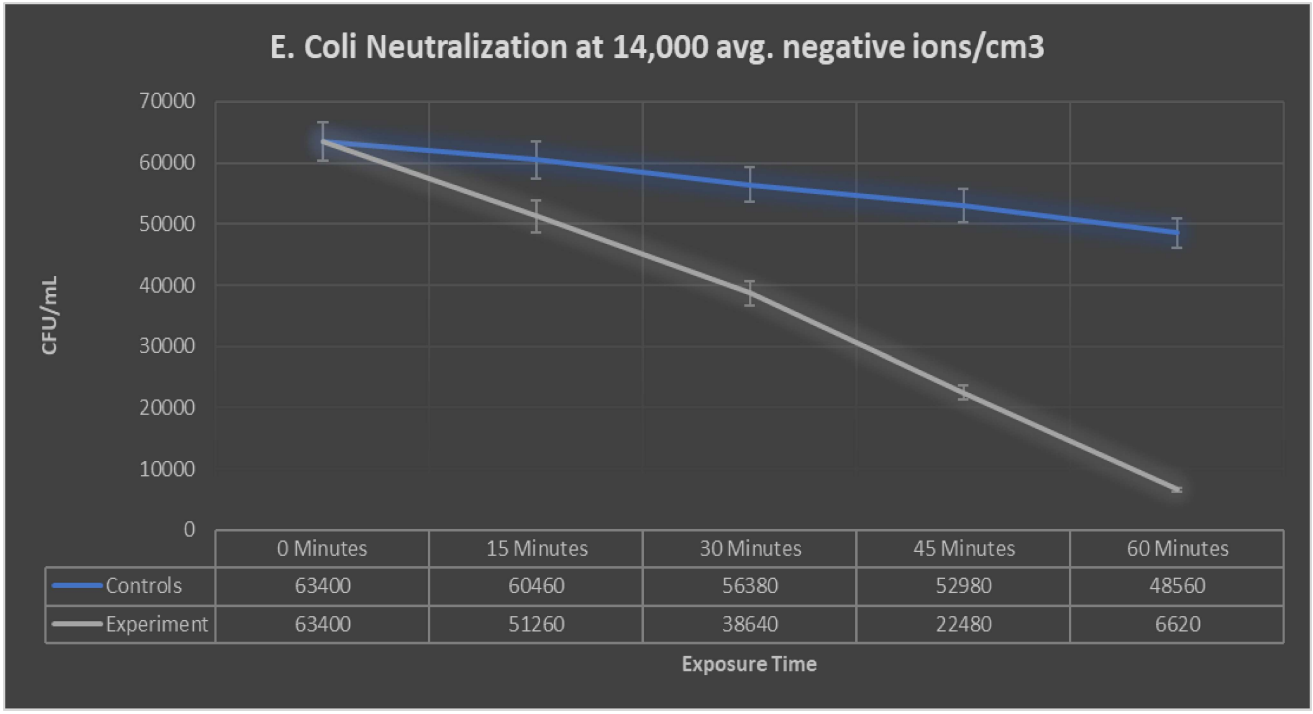
Protocol Amendments: None

Protocol Deviations: None

Control Protocol

The **c**ontrol inoculation and collection used the same methodology as the challenge trial.

Study Results



	0 Minutes	15 Minutes	30 Minutes	45 Minutes	60 Minutes
Controls	63400	60460	56380	52980	48560
Experiment	63400	51260	38640	22480	6620

Conclusion:

The GPS-FC48-AC™ device housing NPBI™ technology performed to manufacturer specifications and demonstrated an 89.55% reduction of active *Escherichia coli* on a surface after 60 minutes of exposure. Ion concentrations were measured during testing with an average over the 60 minutes of 14,000 negative ions per cm^3.

Considerations:

When working with microorganisms and collecting said microorganisms, some variables cannot be fully accounted for, namely, placement of microorganisms, collection volume, collection points, surface saturation, microorganism destruction on collection, 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 microorganisms in the control test.

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