Environmental Diagnostics Laboratory

Study on Efficacy Testing for MCI™ (multi-cluster ionization)

PACS ID#: 07202

Work Order#: 026462

Customer: Best Living Systems, LLC

Dates of Testing: 1/07/2021-1/11/2021

Date Completed: 1/12/2021

Date of Report: 1/15/2021

Environmental Diagnostics Laboratory

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EDL
Environmental Diagnostics Laboratory

PURE AIR
CONTROL SERVICES INC.

January 15, 2021

To
Douglas Hoffman
22174 Prats Road,
Abita Springs, LA 70420

Reference: PACS ID 07202 Work Order 026462
Efficacy Testing for MCI™ (multi-cluster ionization)

Douglas Hoffman,

We appreciate the opportunity to provide you with our professional, environmental microbiology services. EDLab is pleased to submit this report that describes the efficacy testing for MCI™ (multi-cluster ionization).

This report summarizes the findings and other relevant data based on experiments set-up for efficacy evaluation of the above ion generator.

Please call me at 1-800-422-7873, ext. 301 should you have any questions. We look forward in assisting you in future projects.

Respectfully Submitted,

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EXECUTIVE SUMMARY

This report describes the efficacy of the MCI™ (multi-cluster ionization) on microbial flora (bacteria) within an environmental test chamber (ETC) under laboratory conditions. The assessment was completed on January 12, 2021, at the request of Doug Hoffman from Best Living System, LLC. The assays performed in this study have evaluated the antimicrobial efficacy of the above said device against *Staphylococcus aureus* (bacteria) after aerosolizing it.

EDLab's team of microbiologists collected 22 bioaerosol samples while simultaneously recording real time data on temperature and relative humidity during the experimentation. An IAQ Guard Sensor (S/N. 608778) recorded temperature and relative humidity. A total of one (1) bacteria (*S. aureus*) were selected based upon the customer's preference. Traceable culture for the organism was obtained from Microbiologics (*Staphylococcus aureus*- KwikStik™ Ref. 0360P). The viability of this organism was assessed through a pre-experimentation phase. Upon achieving a satisfactory performance, the organisms were used to inoculate the buffer (00267 Lot# 468344 exp. Feb. 27, 2021, Hardy Diagnostics) to prepare a challenge solution containing viable cells of the selected microbial challenge. The concentration of the prepared challenge solutions varied by McFarland's standards and were determined by utilizing serial dilution techniques. The test microbial challenge suspension containing the live inoculum of *S. aureus* was aerosolized by an atomizer. After aerosolization, bioaerosol samples were collected during different treatments as per the scope of work beside a baseline sample that was collected to verify the existing conditions of the ETC without aerosolizing the challenges. The bioaerosol samples were collected in duplicate by utilizing single stage N-6 Andersen Cascade Impactors in the appropriate growth media (TSA) for bacteria challenge. The collected bioaerosol samples were incubated at 30±2°C in an incubator for up to 72 hours.

A chain of custody was prepared for all the collected samples before they were processed by the laboratory. The collected samples were transported to the laboratory using aseptic techniques. All of these samples were processed by Environmental Diagnostics Laboratory (EDLab) at Pure Air Control Services, Inc., an ISO 17025 compliant and A2LA accredited laboratory.

An antimicrobial efficacy has been determined on the microbial challenge *Staphylococcus aureus*. It is evident that MCI™ (multi-cluster ionization) is effective against removing the bacterial challenge (*S. aureus*) within two hours of aerosolization in comparison to the natural decay rate which may take more than three hours under the same simulation.

1. INTRODUCTION

Modern buildings have been designed with the conservation and optimization of energy in mind. Often, when the ventilation of a built environment is controlled by the artificial management of air circulation, avoiding, or minimizing fresh air intake becomes a concern from a health and hygiene point of view along with imbalances in existing environmental parameters such as relative humidity, temperature, pressure, air circulation/flow, etc. Out of many microbiological contaminants, bacteria and fungi are identified as potential indoor contaminants which influence health and hygiene. This study has been undertaken to comprehend the ability of the ion generator technology in removing selected organisms and chemicals under controlled, laboratory conditions.

2. DEFINITIONS AND TERMINOLOGY

The following are definitions and terminology used in this report:

Bioaerosol: Aerosol generated by atomizing a bacterial suspension.

Culture Plate: Petri plate containing culture media for microbial growth.

Control Sample: Sample containing a known concentration of each method analyte derived from externally prepared test materials.

Inoculating Loop: A tool for inoculating and transferring microorganisms to cultivate under microbiological conditions and can also be referred to as a sterile loop.

3. ABBREVIATIONS and ACRONYMS

ATCC: American Type Culture Collection

CFU: Colony Forming Units

CFM: Cubic Feet per Minute

°F: Degrees Fahrenheit

HEPA: High-Efficiency Particulate Arrestance

HP: Horsepower

L: Liter

lpm: Liters per minute

m^3: Cubic meters

mL: Milliliters

mm: Millimeter

MCS Microbial Cell Suspension

mL: Milliliters

Pa: Pascal

psi: Pounds per square inch

SOP: Standard Operating Procedure

TSA: Tryptic Soy Agar

WC: Water Column

µl: Microliter

4. MATERIALS and METHODS[1, 2, 3 and 4]

4.1. Test Facility

The various stages of this experiment were performed by Environmental Diagnostics Laboratory (EDLab). This laboratory is located at Clearwater, Florida 33760. Geographically, it is situated at 27.89 latitude and -82.70 longitude. The average annual temperature is 73.65°F (avg. max 81.9°F, avg. min. 65.4°F). The average yearly relative humidity is about 74%, rain is 54.73 inches and the height from sea level is 29.5 feet.

Air samples for this test were collected from inside the environmental test chamber of EDLab (***Figure 1A***). The dimensions of the test chamber are 14.60 feet (length) x 10.00 feet (width) x 8.00 feet (height). This chamber consists of an experiment room 10.00' x 10.00' x 8.00' and monitoring station 10.00' x 4.60' x 8.00'. The experiment room is connected to the monitoring station with a semi-automatic door (air-sealed, mounted central glass on 7.00" fiberglass material panel and spring hinges) with restricted access (code required to gain entry). This space is ventilated through a grilled duct located at the northwest ceiling and connected to an air scrubber (mounted on top of the ceiling) with an adjustable airflow switch (display mounted on monitoring station) fitted with HEPA by fiberglass insulated flexible duct before being exhausted through a sheet metal duct (9.00" in diameter) through the roof. Typically, this chamber is negatively pressurized in comparison to the monitoring station; however, in this study, the negative pressurization was minimized to restrict the airflow within the testing chamber. All four walls of the experimental room are provided with a fixed, see-through glass/picture window 4.00' x 4.00' mounted in the center of each of the walls. The monitoring room is accessed through a semi-automatic door (air-sealed, mounted central glass on 7.00" fiberglass material panel and spring hinges) from the north side with restricted entry (code is required for gaining entry). This space is positively pressurized in comparison to the warehouse. The ventilation of this space is managed by a closed loop system connected to an air scrubber fitted with HEPA and an air control switch mounted outside on the east wall of the experiment room facing the monitoring center. The return and supply

discharge of air in the monitoring station is connected to grills fitted between south (east and west ceiling center) and north (east and west ceiling center) respectively. On the right wall (entrance side) at 4.00' just before the entrance door to the experiment room, a master control panel/area is located; it is provided with connections, tubing, displays and switches necessary to control the experimentation process. The monitoring station is also provided with a mobile, stainless steel station carriage under the master control panel.

4.2. Equipment to be Tested

4.2.1. An MCI™ (multi-cluster ionization) (***Figure 1C***) was placed within the test chamber as per the experimentation design and "Scope of Work".

4.3. Experimental Design

4.3.1. This experiment is designed to determine the efficacy of the equipment MCI™ (multi-cluster ionization) by performing different trials on the selected microorganism *Staphylococcus aureus* which was determined by comparing the natural decay rate with that of the decay induced by the device. These trials were designed after discussing the needs of the customer. An outline is presented under the "Scope of Work".

4.3.2. A uniquely identified atomizer (***Figure 1D***) was positioned approximately in the center of the ETC and 3'.25" from west wall of the test chamber at 6'.0" height on a PVC pole. This atomizer is designated for aerosolization of challenge microorganisms in the experiment room.

4.3.3. One end of this atomizer was connected to a 0.50" plastic tubing that extended from the compressed air pump (Husky BS 1003 # 20140828179) while the other end was connected to a 0.30" plastic tubing that extended from a challenge suspension holder reservoir glass test tube (SKC 2767).

4.3.4. The reservoir glass test tube was calibrated with markings used to determine a specific volume of suspension.

4.3.5. Two PVC pipes running from the center of the testing chamber into the monitoring room were used for collecting air samples using two single stage N-6 Andersen Cascade Impactors (Aerotech 6™ Microbial Sampler, Lot # 2003-651 (I), Lot # 2003-647 (II) which were placed on top of a stainless-steel table (2'.0"X1'.5"X3'.0") in the

monitoring room. Each impinger and their corresponding air pump were marked with a unique identification.

4.3.6. A probe (IAQ Guard Sensor (S/N. 608778)) for monitoring the temperature and relative humidity was placed inside of the experiment compartment on the south side of the west wall at 5'10" from the surface level.

4.3.7. Specialized tubing was placed on the monitoring station, which joins the elbow penetrating the wall between the experiment chamber and the monitoring room about 4.0' from the floor and 12.00" from the door of the environmental chamber towards the west side next to the PVC pipes. The total length of this tubing is between 18.00"-20.00" and the inner diameter of this tube is 1.00".

4.3.8. The other uniquely identified atomizer (similar as described in section 4.3.2) was placed at the southwest corner of the experiment chamber at a height of 6'.7" (approximately). A decontaminant solution, containing 5% hydrogen peroxide (H_2O_2 CAS# 7722-84-1) and 0.01% silver (CAS# 7440-22-4) was loaded in the atomizer reservoir /controlling unit. This unit connects to the atomizer through a plastic tubing.

4.4. Test/Challenge Microorganisms

One challenge microorganism (***Table 1***) was selected for this study.

Table 1- Organisms Selected for This Experiment

Sl. Number	Organism Description	Source
1	*Staphylococcus aureus*	KwikStik™ Ref. 0360P

The above-mentioned organism was received by EDLab as a KWIK-STIK™ in special packaging delivered by express courier service. These organisms were shipped to this facility in accordance with the United States Export Administration Regulations as declared by the supplier. The supplier assures that all elements of FDA Quality System Regulation are met with purity specifications of the supplied organisms.

4.5. Culturing of Microorganisms

KWIK-STIK™ swab for certified *Staphylococcus aureus* was taken out from the shipping container. The swab was allowed to equilibrate to room temperature (78°F). Simultaneously, plates (media containing plate with a diameter of 88 mm and lid of the plate with diameter

of 92 mm) containing TSA, from the EDLab media stock were taken and allowed to equilibrate to room temperature. Once the desired temperature is achieved, the media and organism were placed under a Bio-safety cabinet (Class II) for further processing. The inoculum for microbial culture was prepared by rehydrating the lyophilized pellet containing the organism using the hydration fluid supplied within the unit. Uniquely identified, preconditioned (as mentioned above) plates were inoculated by gently rolling the swab over a third of the plate, while a sterile loop is used to facilitate colony isolation for obtaining the primary culture. The inoculated plates were incubated at 30±2°C, upside down for 24-72 hours (hrs.) for TSA (used for *Staphylococcus aureus*).

4.6. Inoculum Preparation

Primary spore suspension for the organism was prepared by using buffer solution (00267 Lot# 468344 exp. Feb. 27, 2021, Hardy Diagnostics) and bacterial colonies obtained from primary culture. Utilizing aseptic techniques, a sterile cotton swab is rolled 360° over the primary culture obtained from the KWIK-STIK™. This load of organism on the swab was transferred, at room temperature, into a preconditioned, uniquely marked, sterile container containing 90 mL of prefilled buffer by twirling the swab into it. After thoroughly mixing and vortexing the extracted microorganism, its turbidity was verified. The turbidity of the extracted suspension was adjusted to 3.0 McFarland's. This extracted spore suspension aliquot was transferred into 50 mL self-standing test tube (REF 430921 Corning) for experimentation. This microbial cell solution (MCS)/Mother solution was used for the experiment and aerosolization. The following scheme was adapted for preparing various dilutions for the inoculation determination.

Table 2- Inoculum Dilution Scheme

Sl. No.	Dilution ID	Concentration		Inoculating volume
		Inoculum	Buffer	
1	Mother Solution	20-25 Colonies	90 ml	20 mL

4.7. Determination of Inoculum

Microbial viable cell counts in the above prepared suspension was determined by using McFarland Turbidity Standards and Plate Count Method.

McFarland Turbidity Standard: The determination of viable cell counts in the prepared MCS is determined by visually comparing an aliquot obtained after the homogenization in a specimen tube with that of a McFarland Turbidity Standard (Remel, McFarland Equivalence Turbidity Standard; Lot 3102561; REF R20421). The standard used in the experiment is mentioned below.

Table 3- McFarland Scale

McFarland Scale	Viable Cell Counts CFU ($x10^6$/mL)
0.5	<300
1	300
2	600
3	900
4	1200
5	1500

Plate Count Method: The quantitative estimation of viable cell counts in the suspension to be used in this experiment was verified by using serial dilution technique. A 100 µL aliquot of suspension after homogenization of the MCS was transferred into a sterile culture tube (Fisher's scientific Cat. #02911613) containing 900 µL of preconditioned distilled water by using aseptic conditions under laminar air flow. After thoroughly mixing and homogenizing the above prepared cell suspension, 100 µL was transferred into a Petri plate containing the appropriate media, in duplicate, following aseptic techniques under a Bio-safety cabinet. The TSA media was used for cultivating S*taphylococcus aureus* as described in section 4.5. The initial inspection for growth on these plates was performed after 24 hrs. of incubation. The

final observation for the colony growth was made after 72 hrs. in cases of bacterial organism (*S. aureus*). The growth on both plates were counted separately and recorded for all the dilutions. The average of the two counts at the highest dilution were used for determining the viable cell counts in terms of CFU/mL.

4.8. Aerosolization

4.8.1. MCS

Aerosolization was done by an atomizer installed at the PVC pole (Ref. **4.3.2**). Approximately 20 mL of cell suspension was aerosolized for 1 minute (***Figure 1D***). A visual inspection was performed just prior to each aerosolization for the uniformity conformation in the solution. In the event of non-uniformity or clump formation, the aerosolization was aborted. Only after the confirmation of uniformity was verified in the suspension, further steps were taken as mentioned.

4.8.2. Decontamination

The atomizer, located in the southwest (Ref. **4.3.8**) corner, was used for performing the decontamination of the experiment test chamber prior and at the end of every set of trials.

4.9. Sample Collection

4.9.1. Preparation

4.9.1.1. Laboratory supplies required for collecting the environmental samples were removed from the laboratory stock. Wherever needed, all the microbiological media for sample collection were equilibrated to room temperature prior to use. Quality control documents were examined as per the EDLab protocol.

4.9.1.2. The experiment room and monitoring station of the environmental test chamber were thoroughly disinfected and cleaned prior to the experiment with environmentally friendly disinfectants, distilled water and 70% isopropyl alcohol (Supelco 60150 Exp. May 31, 2023) for wall and floor surfaces. Whereas Disinfectant (Halo Mist Disinfect, EPA Beg. No. 84526-1) and an air scrubber were used for air cleaning. A vacuum cleaner equipped with a HEPA was used for dusting and other debris removal.

4.9.1.3. The air machine equipped with the ionizer were placed appropriately and verified for their integrity and functionality.

4.9.1.4. All sensing devices used in the experiment were calibrated and verified as per their specifications.

4.9.1.5. Pumps utilized for collecting the bioaerosol samples were verified for functionality as per their specification. All the pumps used for this purpose were calibrated to draw air at 28.3 lpm.

4.9.1.6. The atomizer was verified to ensure that it is working as per the specification.

4.9.1.7. Pressure and airflow inside the experiment room were monitored for consistency during the entire period of experiment from the monitoring station.

4.9.2. Documentation

4.9.2.1. A chain of custody document was used to record sample details and other desired information. A blank copy of the chain of custody document was made available at the monitoring station for recording throughout the testing.

4.9.2.2. A laboratory notebook was prepared for recording any extraneous notes or other information pertaining to the experiment and/or other details on the project.

4.9.3. Bioaerosols

4.9.3.1. Bioaerosol samples were collected on Tryptic Soy Agar (TSA) Petri plates by using two single stage N-6 Andersen Cascade Impactor (Aerotech 6™ Microbial Sampler, Lot # 2003-651 (I), Lot # 2003-647 (II). That were installed in the monitoring room as mentioned in the experimental design section.

4.9.3.2. Before entering the environmental chamber, each technician dons proper Personal Protective Equipment (PPE), that includes at a minimum an impermeable gown (Tyvek® DUPONT coveralls with boots, elastic hood, and wrists), facemask/goggles, respirators (NIOSH OV/P100, NBR 13696/13697, Honey well part 7581P100L) and hand gloves (Fisher brand Aloe Nitrile gloves 10-041-172).

4.9.3.3. Prior to sample collection, the impactors are disinfected with isopropyl alcohol each time by dismantling the exposure blocks of the impactor along with disinfecting the sampling station by using aseptic techniques.

4.9.3.4. The IAQ Guard Sensor (S/N. 608778), which was placed to record temperature and relative humidity during the experiment, was activated at this stage.

4.9.3.5. A 20 mL cell suspension was aerosolized for (1) minute approximately or until the entire challenge suspension was aerosolized in the experiment chamber before collecting the bioaerosol samples each time. Sample collection started no sooner than after 30 seconds from aerosolization.

4.9.3.6. Air samples were collected at 0, 30, 60, 120, and 180-minute time intervals by drawing 28.3 liters of air for 1 minute for microbials based on the sampling scheme described in the "Scope of Work."

4.9.3.7. Simultaneously, the temperature and relative humidity were recorded using real-time monitoring.

4.9.3.8. Once the samples were collected, the sample containers were removed carefully. The collected specimen was transported aseptically to the laboratory for further processing.

4.9.3.9. All the collected samples were stored in a pre-sterilized tray/container in the monitoring area until they were transported to the laboratory.

4.9.4. Control Samples

4.9.4.1. Positive Controls:

During each set of trials, the viability of the test organism was verified by culturing a 100 µl aliquot directly taken from the MCS used for the experiment. The *S. aureus* was inoculated on TSA plates and incubated at 30±2°C for 24-72 hours for growth verification. (***Table 5)***.

4.9.4.2. Negative Controls:

Reagent water in place of MCS was inoculated on TSA to verify the sterility of the media and water (***Table 5***), in the same manner as mentioned under positive controls (**4.9.4.1**)

4.9.4.3. Blank:

A set of media blanks were run alongside (***Table 5***) the positive and negative controls during this experiment by removing the plate from the packaging, verifying there was no contamination, processing the plates without inoculating them as mentioned under the positive controls section (**4.9.4.1**)

5. TRANSPORTATION

All the collected samples were transported in a sterile container by using aseptic techniques along with their chain of custody (**Appendix 1**). Samples were stored at the sample receiving station of EDLab for their verification and processing.

6. PROCESSING

All the samples received by EDLab were verified for their integrity and appropriate documentation as per the laboratory protocol[5]. The accepted samples were placed in the queue for analysis.

7. ANALYSIS

7.1. Bioaerosols

7.1.1. The collected bioaerosol samples were incubated at 30±2°C in an incubator.

7.1.2. Initially, the incubated plates were inspected for colony growth, if any, after 24 hrs. of incubation; subsequent observations were made after 48 and 72 hrs. of incubation.

7.1.3. Total counts of bacterial colonies were recorded in the laboratory record for each of the samples separately after the cut-off time as mentioned above.

7.1.4. A quantitative estimation of reported colonies was performed by multiplying the observed colonies with a correction (2628) and conversion (35.3) factor and rounding off to the nearest integer.

8. DATA and IMAGES

Results of all the analyzed samples were recorded in the corresponding observation ***Tables 1*** to ***7***. The obtained data was analyzed by using Microsoft Office's EXCEL 365 program. Analytical results were also plotted in ***Graph 1***. Some important stages of the experiments and photographs are presented in ***Figures 1*** to ***13*** in the "PHOTOGRAPHS and FIGURES" section of this report.

9. BIO-WASTE

All bio-waste generated during this experiment was disposed of in compliance per the protocol of the applicable regulation. EDLab vendor Stericycle picks up all the bio-waste as per a fixed schedule.

10. RESULTS

All data, statistical analysis and photographs are presented under the following ***Tables 1*** to **7**, ***Graph 1***, and ***Figures 1*** to ***13***.

Table 4- Microbial Cell Counts for Aerosolization

Organism Description	Viable Cell Counts CFU/mL
Staphylococcus aureus	**5.84×10^7**

The viable cell counts of the solution to be aerosolized were determined by making a series of dilutions up to 100,000X of the stock solution for bacteria using a microbiological growth media TSA for bacteria.

Table 5 - TSA Media Blank and Field Blank Quality Control

Treatment	Temperature (°C)	Growth Media	Growth Pattern		Remarks
			Negative	Positive	
Media Sterility	30±2	Tryptic Soy Agar	✓	X	Pass
Field Blanks			X	X	Pass
Negative Control			X	X	Pass
Positive Control			X	✓	Pass

✓= Yes; X= No

Table 6- Total Quantitative Estimation of Isolated Bioaerosols

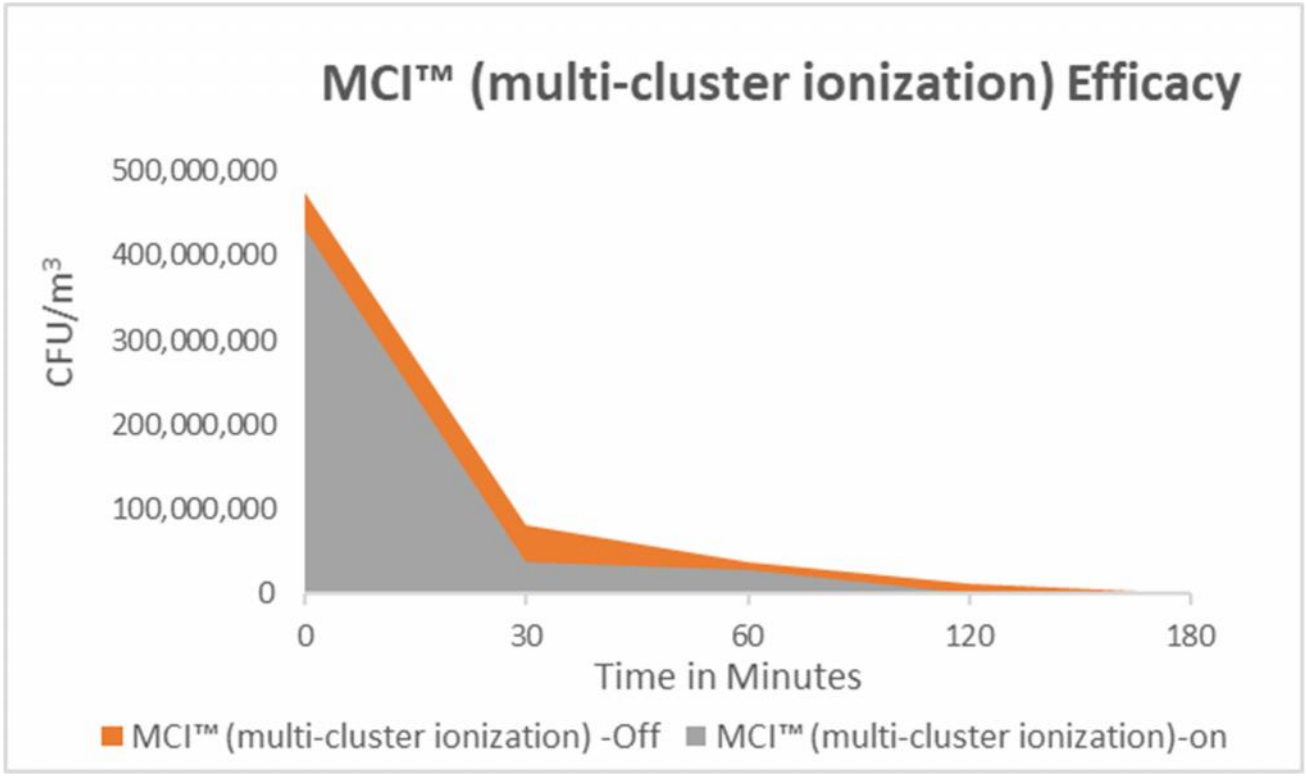
Sample Type	Time Interval (min.)	Set	Total Bacterial Count (CFU/m^3)	Average (CFU/m^3)	Reduction (CFU/m^3)	Reduction Percentage
Baseline	0	I	BDL	BDL	N/A	N/A
		II	BDL			
MCI™ (multi-cluster ionization) Off	0	I	460,131,264	474,974,208	N/A	N/A
		II	489,817,152			
	30	I	92,768,400	81,636,192	393,338,016	82.81%
		II	70,503,984			
	60	I	37,014,592	36,875,439	438,098,769	92.24%
		II	36,736,286			
	120	I	14,935,712	13,126,729	461,847,479	97.24%
		II	11,317,745			
	180	I	556,610	510,226	474,463,982	99.89%
		II	463,842			
MCI™ (multi-cluster ionization) On	0	I	445,288,320	430,445,376	N/A	N/A
		II	415,602,432			
	30	I	37,663,970	37,849,507	392,595,869	91.21%
		II	38,035,044			
	60	I	29,407,583	29,639,504	400,805,872	93.11%
		II	29,871,425			
	120	I	371,074	278,306	430,167,070	99.94%
		II	185,537			
	180	I	BDL	BDL	430.445.376	100.00%
		II	BDL			

BDL = Below Detection Limit; The detection limit = 1 CFU.

Table 7- Environmental Parameters During Bacterial Aerosolization

Sample Type Bacterial	Time Interval (Min.)	Temperature (°F)	Relative Humidity (%)
Baseline	0	69.61	57.10
MCI™ (multi-cluster ionization) Off	0	69.67	57.89
	30	70.31	57.47
	60	70.36	55.44
	120	70.47	53.09
	180	70.53	52.35
MCI™ (multi-cluster ionization) On	0	68.95	61.14
	30	70.23	58.63
	60	70.33	56.89
	120	70.67	54.86
	180	70.63	55.33

Graph 1. Quantitative Estimation Comparison of Isolated Bioaerosols



11. PHOTOGRAPHS and FIGURES

The following section contains photos and figures of some important observations as well as other experimental stages, including graphs based off the experimental findings.

Figure 1- Experimentation Stages

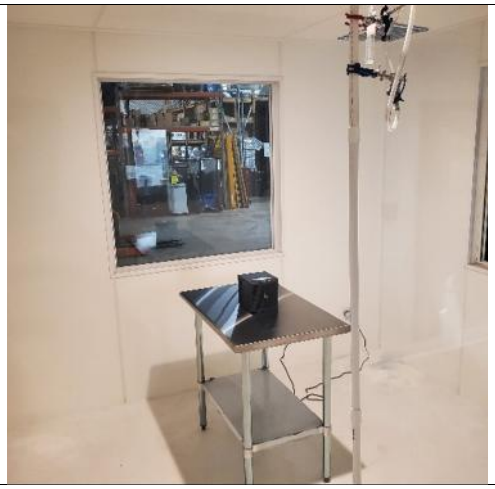
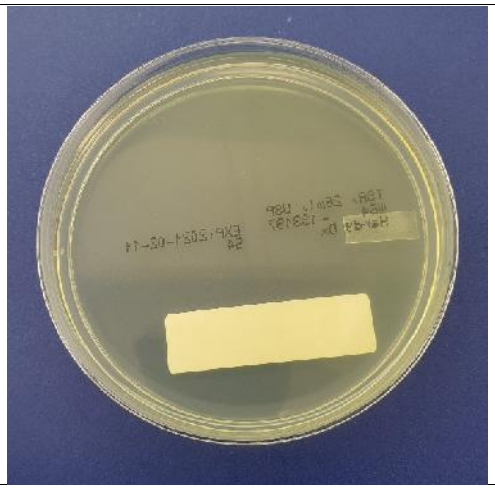
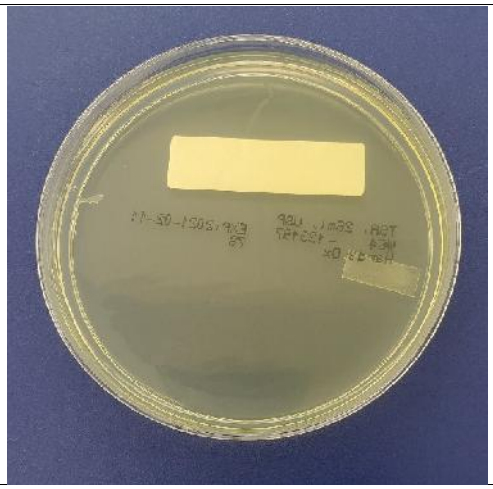
	
A. Experimental Room	**C. MCI™ (multi-cluster ionization)**
	
B. Experimental Station	**D. Aerosolization of Microbial**

Figure 2- Quality Control Samples for TSA

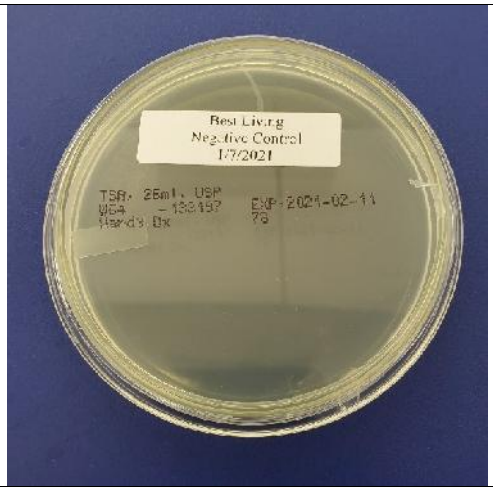


A. TSA Blank Sample (Front)



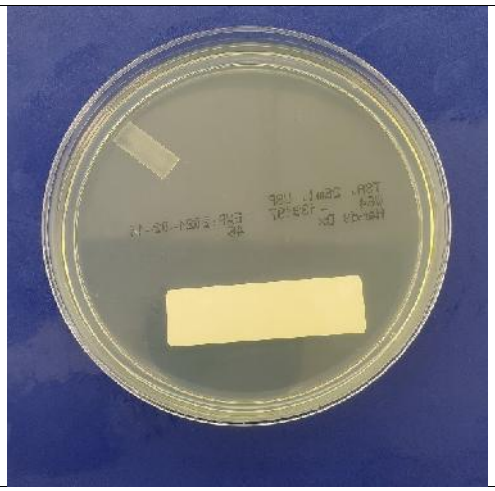
C. TSA Negative Control (Front)

B. TSA Blank Sample (Reverse)

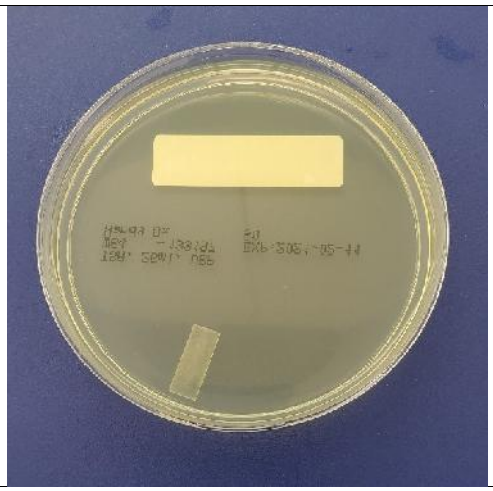


D. TSA Negative Control (Reverse)

Figure 3- Bacterial Bioaerosol Baseline



A. TSA Baseline I (Front)



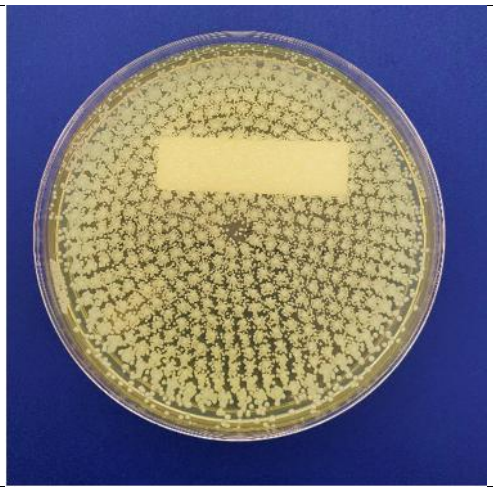
C. TSA Baseline II (Front)

B. TSA Baseline I (Reverse)

D. TSA Baseline II (Reverse)

Figure 4- Bioaerosol Bacterial Samples Pre-Treatment 0 min.

A. Sample Plate I at 0 min. (Front)



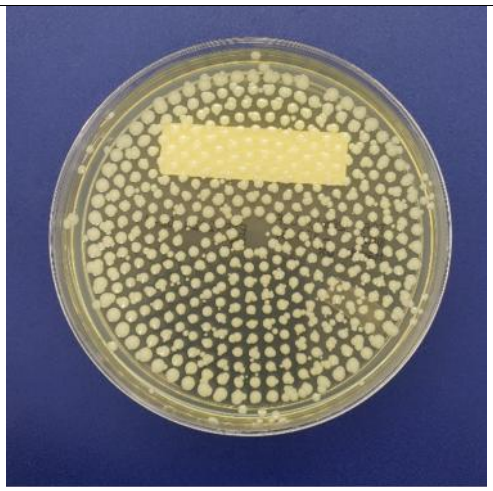
C. Sample Plate II at 0 min. (Front)

B. Sample Plate I at 0 min. (Reverse)

D. Sample Plate II at 0 min. (Reverse)

Figure 5- Bioaerosol Bacterial Samples Pre-Treatment 30 min.

A. Sample Plate I at 30 min. (Front)

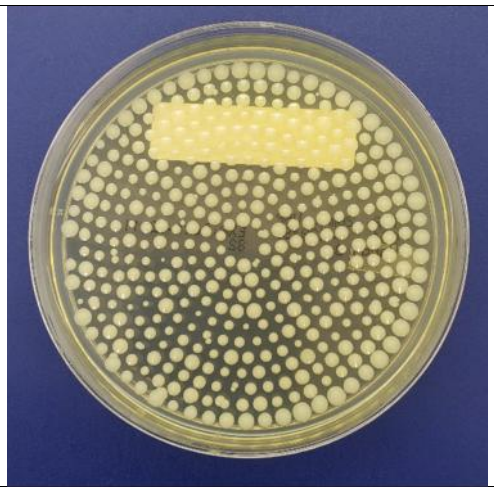


C. Sample Plate II at 30 min. (Front)

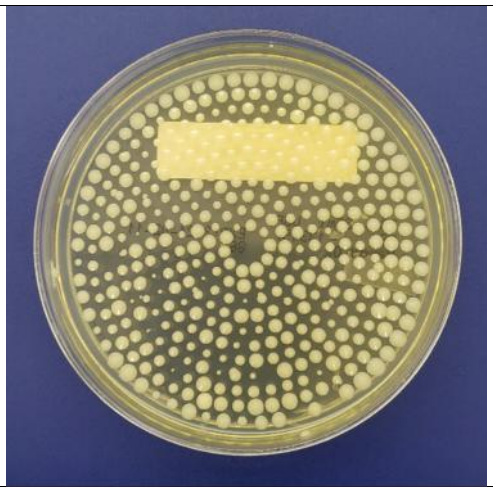
B. Sample Plate I at 30 min. (Reverse)

D. Sample Plate II at 30 min. (Reverse)

Figure 6- Bioaerosol Bacterial Samples Pre-Treatment 60 min.



A. Sample Plate I at 60 min. (Front)



C. Sample Plate II at 60 min. (Front)

B. Sample Plate I at 60 min. (Reverse)

D. Sample Plate II at 60 min. (Reverse)

Figure 7- Bioaerosol Bacterial Samples Pre-Treatment 120 min.

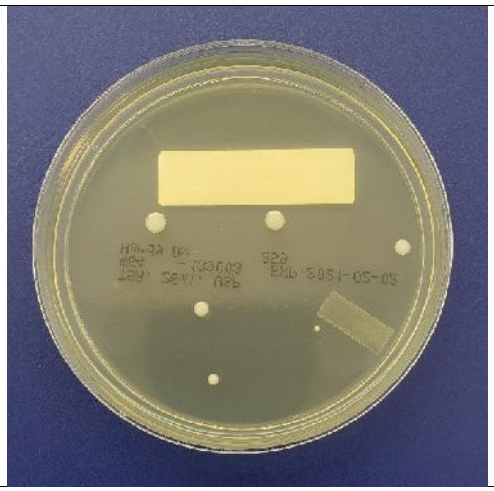
A. Sample Plate I at 120 min. (Front)

C. Sample Plate II at 120 min. (Front)

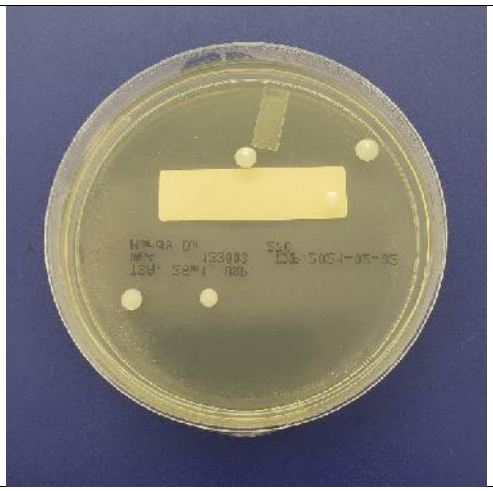
B. Sample Plate I at 120 min. (Reverse)

D. Sample Plate II at 120 min. (Reverse)

Figure 8- Bioaerosol Bacterial Samples Pre-Treatment 180 min.

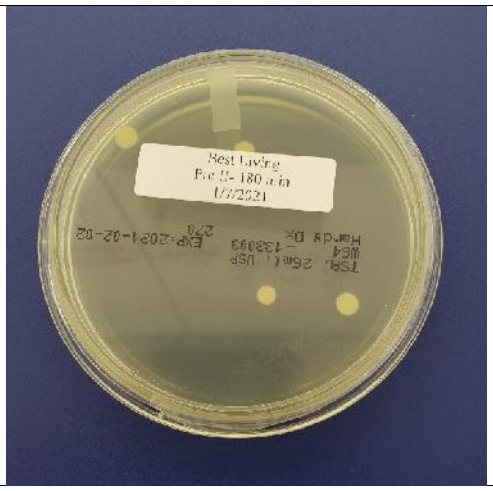


A. Sample Plate I at 180 min. (Front)



C. Sample Plate II at 180 min. (Front)

B. Sample Plate I at 180 min. (Reverse)



D. Sample Plate II at 180 min. (Reverse)

Figure 9- Bioaerosol Bacterial Samples Post-Treatment 0 min.

A. Sample Plate I at 0 min. (Front)

C. Sample Plate II at 0 min. (Front)

B. Sample Plate I at 0 min. (Reverse)

D. Sample Plate II at 0 min. (Reverse)

Figure 10- Bioaerosol Bacterial Samples Post-Treatment 30 min.

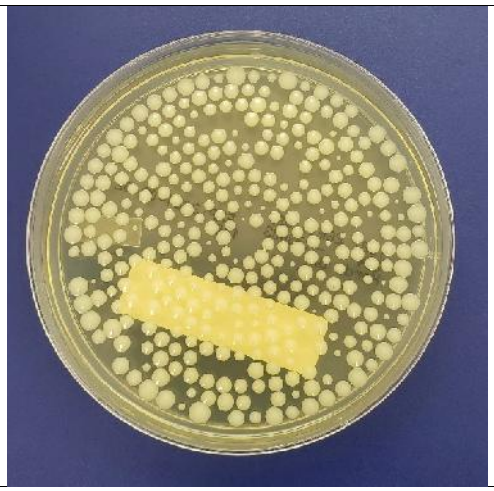
A. Sample Plate I at 30 min. (Front)

C. Sample Plate II at 30 min. (Front)

B. Sample Plate I at 30 min. (Reverse)

D. Sample Plate II at 30 min. (Reverse)

Figure 11- Bioaerosol Bacterial Samples Post-Treatment 60 min.



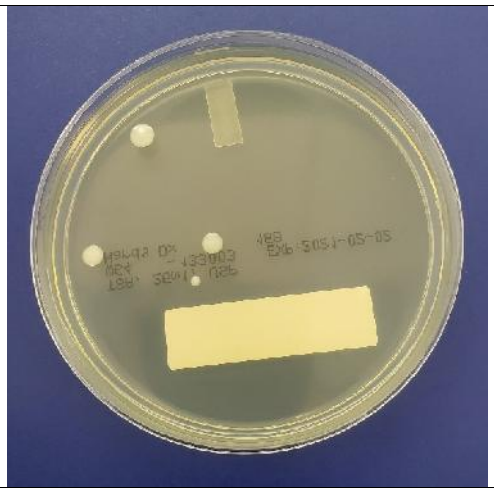
A. Sample Plate I at 60 min. (Front)

C. Sample Plate II at 60 min. (Front)

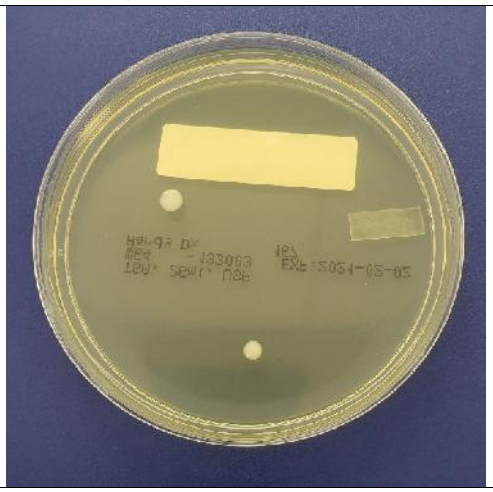
B. Sample Plate I at 60 min. (Reverse)

D. Sample Plate II at 60 min. (Reverse)

Figure 12- Bioaerosol Bacterial Samples Post-Treatment 120 min.



A. Sample Plate I at 120 min. (Front)



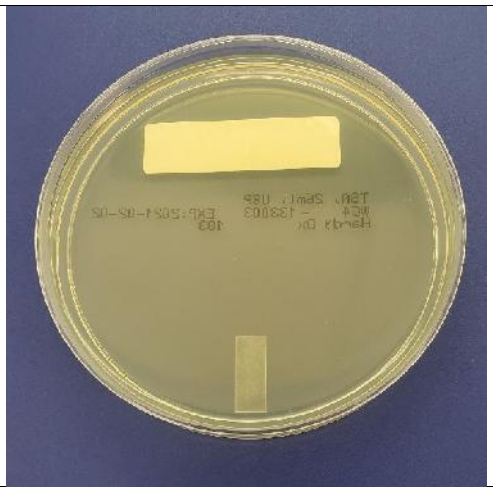
C. Sample Plate II at 120 min. (Front)

B. Sample Plate I at 120 min. (Reverse)

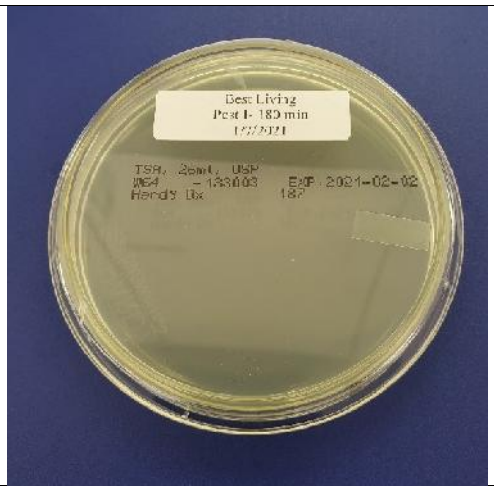
D. Sample Plate II at 120 min. (Reverse)

Figure 13- Bioaerosol Bacterial Samples Post-Treatment 180 min.

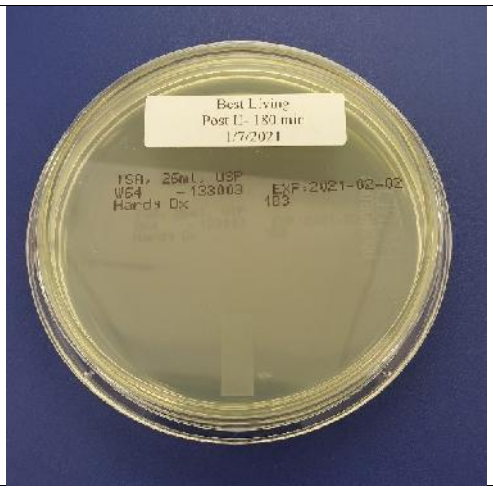
A. Sample Plate I at 180 min. (Front)



C. Sample Plate II at 180 min. (Front)



B. Sample Plate I at 180 min. (Reverse)



D. Sample Plate II at 180 min. (Reverse)

12. CONCLUSION

This study has been performed to understand the efficacy of MCI™ (multi-cluster ionization). The following points summarize the findings:

1. The results indicate antimicrobial efficacy on the organism. Antimicrobial properties for removing airborne bacteria have been established, a trend is noticed that this device acts faster on the microbial inhibition in comparison to the natural decay. (***Tables 6****),* and ***Figures 4-13***).
2. No significant difference was observed in the temperature and humidity during pre- and post-operation of MCI™ (multi-cluster ionization) testing (***Table 7***).
3. An application that inhibits the growth of microorganisms such as bacteria (*Staphylococcus aureus*) may also be helpful in reducing other pathogenic entities such as virus (influenza virus, coronavirus, H1N1 Norovirus and *Legionella*, etc.) due to its antimicrobial properties.
4. A more comprehensive study is required and encouraged for a better understanding of the efficacy of this system in removing specific pathogenic agents/entities.

13. REFERENCES

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END OF REPORT