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Methicillin Resistant S. Aureus Reduction Through Ionization

CLIENT: ACA/IAE

PROJECT: ACA/IAE-ION-MRSA-01

REPORT NUMBER: ACA/IAE-ION-MRSA-01

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: 01/04/2021

CHALLENGE ORGANISIM(S):

- **Methicillin Resistant Staphylococcus Aureus**

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EXPERIMENT SUMMARY

ACA/IAE supplied both a **ACA-RN-0001 and ACA4800GU-1** system for testing purposes to determine efficacy against Methicillin Resistant Staphylococcus Aureus. When operational the units are intended to neutralize bacteria on surfaces and in the air to sanitize enclosed areas.
The ACA-RN-0001 and ACA4800GU-1 are designed to deactivate viral and bacterial pathogens on surfaces and in the air to sanitize enclosed areas. This study was to evaluate the efficacy of one bacterial strain referred to as MRSA 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. Both systems have been used in the following test procedures and the results of each system are identical in performance and ion production.

BACTERIA

BEI Resources Catalog number NR-41886 (CoA attached) Methicillin Resistant S. Aureus 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.

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 MRSA 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.

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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**

EXPERIMENTAL SUMMARY: T0, T10, T20, T30, T60

The test stage can be seen in the design diagram and consisted of a metal and laminate safety test chamber measuring, 20'W x 8'H x 8'D with sealed seams was used for the testing site An equalizing vent was on opened on the side of the test chamber to allow natural air to enter and exit from the chamber. The air temperature fluctuated slightly through the test and ranged from 72.41F to 73.77F. During the control testing and the viral load tests the temperature fluctuation was consistent. The ambient humidity inside the test chamber averaged 53.7%.

- 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 MRSA with a concentration of 33,152 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 ion 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.
- Upon testing completion, samples were provided to lab staff for further review.

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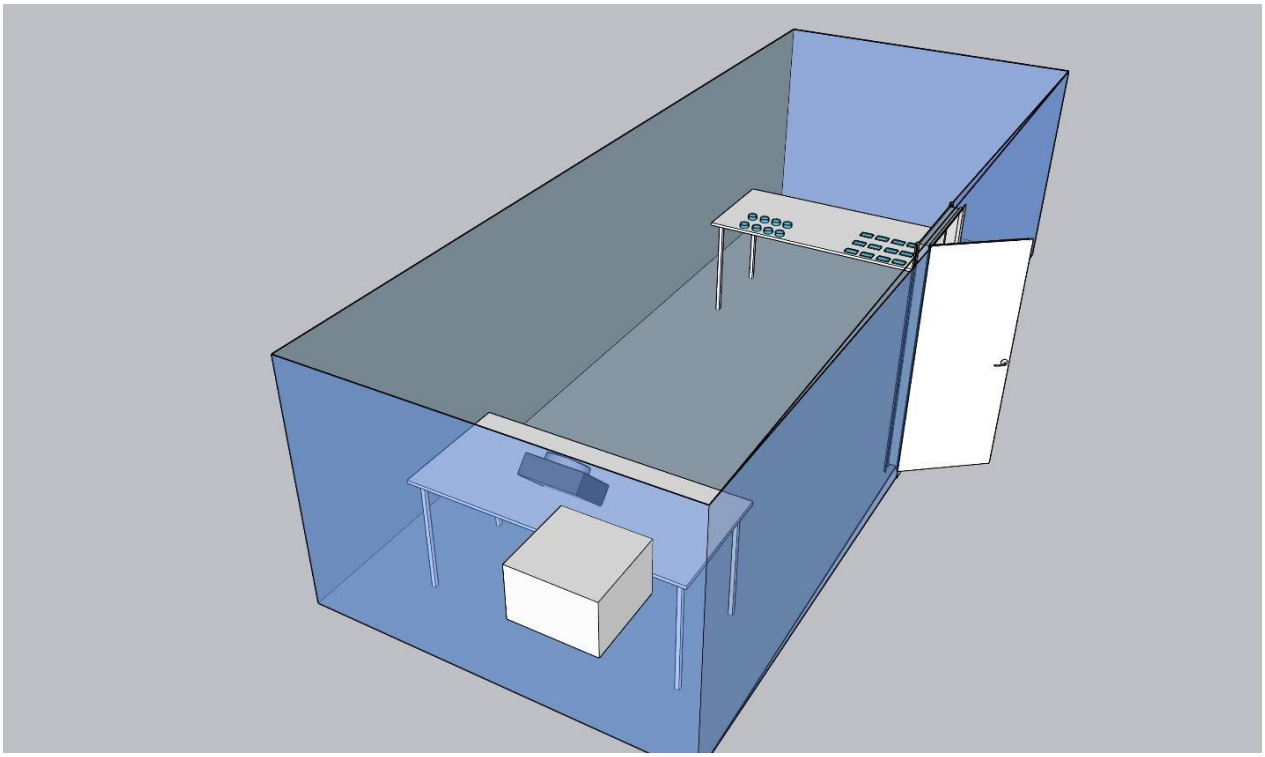
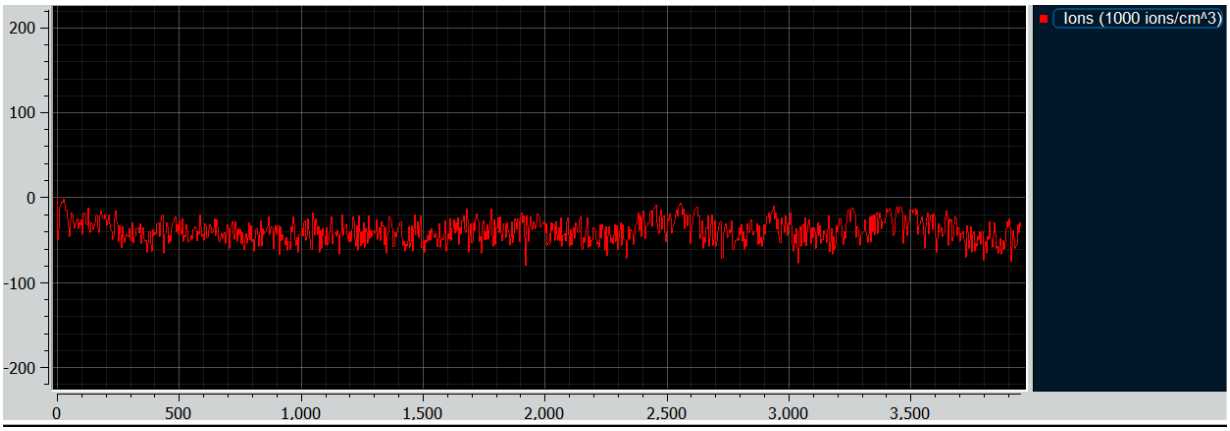
DESIGN LAYOUT:



TEST AVERAGE 27K PER CUBIC CENTIMETER



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RESULTS:

CONTROL RESULTS			EXPERIMENT RESULTS		
Timepoint	CFU/mL	% Reduction	Timepoint	CFU/mL	% Reduction
0 Min	32,212 CFU/mL	1.2	0 Min	31,816 CFU/mL	2.4
10 Min	30,226 CFU/mL	6.1	10 Min	24,358 CFU/mL	23.4
20 Min	29,484 CFU/mL	8.4	20 Min	13,166 CFU/mL	58.6
30 Min	27,842 CFU/mL	13.4	30 Min	5,534 CFU/mL	82.6
60 Min	24,618 CFU/mL	23.6	60 Min	826 CFU/mL	97.4

CONCLUSIONS:

The overall reduction of controls (natural degradation) versus experiment shows the increased degradation of MRSA 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 MRSA in a 60-minute time by 97.4% overall and 73.8% better than natural degradation.

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CERTIFICATE OF ANALYSIS

***Staphylococcus aureus*, Strain M0602 (MRSA)**

Catalog No. NR-41886

Product Description: *Staphylococcus aureus* (*S. aureus*), strain M0602 was isolated in 2004 from a blood sample of an adult male. *S. aureus*, strain M0602 is a methicillin-resistant *S. aureus* (MRSA) strain.

Lot[1]: 62173712 **Manufacturing Date: 14NOV2013**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive cocci	Gram-positive cocci
Colony morphology[2]	Report results	Circular, low convex, entire, smooth and gray (Figure 1)
Motility (wet mount)	Report results	Non-motile
Oxacillin resistance[3]	Resistant	Resistant
Hemolysis[2]	Report results	β-hemolytic
Biochemical characterization:		
Analytical profile index (API Staph®)[4]	Consistent with *S. aureus*	Consistent with *S. aureus*
Catalase	Positive	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 910 base pairs)	Consistent with *S. aureus*	Consistent with *S. aureus*
Riboprinter® Microbial Characterization System	Consistent with *S. aureus*	Consistent with *S. aureus*
Viability (post-freeze)[2]	Growth	Growth

[1]NR-41886 was produced by inoculation of the deposited material into Tryptic Soy broth and grown 24 hours at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 5% defibrinated sheep blood kolles which were grown 24 hours at 37°C in an aerobic atmosphere to produce this lot. Purity of this lot was assessed for 7 days under propagation conditions.
[2]24 hours at 37°C and aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood
[3]24 hours at 30°C and aerobic atmosphere on Mueller Hinton agar containing 6 µg/mL oxacillin and 4% NaCl (Oxacillin Screen Agar BBL™ 221952)
[4]The API Staph® identification includes a test for lysostaphin resistance that was not completed for NR-41886. NR-41886 is identified as *S. aureus* by the API Staph® test regardless of the lysostaphin resistance test results.

Figure 1

Date: 15 FEB 2014 **Signature:**

Title: Technical Manager, BEI Authentication or designee

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