SARS-CoV-2 Neutralization by Needlepoint Bipolar Ionization, Powered by GPS NPBI™ Technology

CLIENT: ACA/IAE

PROJECT: Needlepoint bipolar Ionization “NPBI” applied to COVID19

PRODUCT: ACA-RN-0001 and ACA4800GU-1, Powered by GPS NPBI™ Technology

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STATE ID: CLF 00324630

SAMPLE RECEIVED: 07/10/2020
START DATE: 07/13/2020
REPORT DATE: 08/07/2020
CHALLENGE VIRUS: SARS-CoV-2 USA-WA1/2020
ABSTRACT:

This in vitro study was to characterize the ACA-RN-0001 and ACA4800GU-1, Powered by GPS NPBI™ Technology system and determine efficacy against the SARS COVID-19 virus. The ACA-RN-0001 and ACA4800GU-1 are designed to deactivate viral pathogens on surfaces and in the air to sanitize enclosed areas. This study was to evaluate the efficacy of one viral strain referred to as SARS COVID-19 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.

EXPERIMENTAL SUMMARY:

A custom designed metal container 20’W x 8H’ x 8’D with sealed seams was used for a directed aerosol testing site. The container was tested for leaks using color infused smoke and visual inspection. To prevent accidental exposure, no exhaust was installed on the testing chamber and it maintained a sealed environment throughout testing. The interior was set up to simulate a working environment with rows of chairs and tables to replicate real world environments where there will be air flow obstacles. During the course of the test, 3 air ion counters were placed throughout the container which mapped ion levels for the duration of the test. There were 3 separate testing sites inside the container identified as test site 1 – 3. The air temperature fluctuated slightly through the test and ranged from 77.1F to 77.7F. During the control testing and the viral load tests the temperature fluctuation was consistent. The ambient humidity inside the test chamber was 38.1% and the airflow speed at the time of testing from the ACA4800GU-1 was averaged at 2133 FT/M. During the control testing two fans were placed inside the room to create the same simulated air flow as the ionization unit.

- At each testing site there was an AIC2 Air Ion Counter continually logging the ion count. Each test site contained one 5.5” x 2.5” piece of Kydex-6565, 0.125” thick. One 5.5” x 2.5” piece of Aluminum- 2024 T3, 0.030” thick. One piece of 2.5” x 2.5” Leather- Manufacturer: Aristo P/N King Volaero 1388Q. Test pieces were inoculated with the virus by spraying them with an aerosolized viral solution from 6 inches away to get even coverage of the testing area. 1 sample swab was taken from each test piece at a 5-minute time point, 15-minute time point, and 30-minute time point.
- Each testing surface was sectioned off in the center by 3 separate rows so swabs were not taken from the same point on the surface each time. Swabs were sealed in individual tubular containers and stored in a sealed box for the duration of the test so no further ions could interact with them.
- Upon testing completion, samples were provided to lab staff for further review.
CONTROL SUMMARY:

For the control section two separate AIC2 Air Ion counters were placed on opposite ends of the room. The natural state of ions was counted and little fluctuations were observed until the door to the chamber was opened and equipment was moved at the end of the test. Ion counts were recorded every 0.5 seconds and the average for the duration of the test was 800 ions per cm³ without the needlepoint bipolar ionization units running.

CONTROL ION LEVELS:
TEST SITE 1 – Average ion count 27,846.3

TEST SITE 2 – Average ion count 27,264.4

TEST SITE 3 – Average ion count 26,801.3
Alternate Layout Observations

Two total layouts were reviewed and samples swabs were taken from the primary layout as there was no major difference in ion counts. Test sites 1, 2 and 3 were all placed on the ground under seats. The ionization unit was kept in the same position and the fan was tilted at a 45-degree angle instead of 90 degrees. Based on observations, it can be determined that even with various furnishing changes, ions are able to travel around, over and under obstructions on a consistent basis.
TESTING SUMMARY
### TESTING PROCEDURE:

**VIRAL STOCK:** SARS-CoV-2 USA_WA1/2020 (BEI NR-52281)

<table>
<thead>
<tr>
<th>TEST</th>
<th>SPECIFICATIONS</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification by Infectivity in Vero 6 cells</td>
<td>Cell Rounding and Detachment</td>
<td>Cell Rounding and Detachment</td>
</tr>
<tr>
<td>Sequencing of Species-Specific Region (Approx. 940 Nucleotides)</td>
<td>≥ 98% identity with SARS-CoV 2, isolate USA-WA1/2020 GenBank: MN985325.1</td>
<td>100% identity with SARS-CoV 2, isolate USA-WA1/2020 GenBank: MN985325.1</td>
</tr>
<tr>
<td>(Approx. 940 Nucleotides)</td>
<td>≥ 98% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-WA1/2020 GenBank: MT246667.1</td>
<td>100% identity with SARS-CoV 2, strain FDAARGOS_983 isolate USA-WA1/2020 GenBank: MT246667.1</td>
</tr>
<tr>
<td>Genome Copy Number using BioRad QX200 Droplet Digital PCR.</td>
<td>Report Results</td>
<td>2.07 X 10^9 genome equivalents per mL</td>
</tr>
<tr>
<td>Titer by TCID50 in Vero 6 Cells by Cytopathic effect</td>
<td>Report Results</td>
<td>2.8 X 10^5 TCID50 per mL in 6 days at 37°C and 5% CO2</td>
</tr>
<tr>
<td>Sterility (21-Day Incubation)</td>
<td>No Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td>Harpos HTYE Broth, aerobic</td>
<td>No Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td>Trypticase Soy Broth, aerobic</td>
<td>No Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td>Sabourad Broth, aerobic</td>
<td>No Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td>Sheep Blood Agar, aerobic</td>
<td>No Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td>Sheep Blood Agar, anaerobic</td>
<td>No Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td>Thioglycollate Broth, anaerobic</td>
<td>No Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td>DMEM with 10% FBS</td>
<td>No Growth</td>
<td>No Growth</td>
</tr>
<tr>
<td>Mycoplasma Contamination Agar and Broth Culture</td>
<td>None Detected</td>
<td>None Detected</td>
</tr>
<tr>
<td>DNA Detection by PCR of extracted Test Article nucleic acid.</td>
<td>None Detected</td>
<td>None Detected</td>
</tr>
</tbody>
</table>

### TCID50 PROCEDURE:

**MATERIALS AND EQUIPMENT:**

- Certified Biological Safety Cabinet
- Micropipette and sterile disposable aerosol resistant tips – 20uL, 200 uL, 1000uL
- Inverted Microscope

Innovative Bioanalysis
- Tubes for dilution
- Hemacytometer 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 7 X 10^4 cells 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 48-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 27 samples collected were subject to the same TCID50 assay protocol to determine viral concentration. Each collected swab was vortexed for 1 full minute in 1ml viral preservation media prior to serial dilution.

INNOCULATION OF THE TEST CARRIERS:
Each of the 3 testing sites were simultaneously and equally subjected to a 3ml aerosol or viral media containing a known titer of $2.8 \times 10^5$ TCID50 per mL to ensure saturation of all materials.

**Efficacy Testing:**

Viral media with a known concentration was applied via aerosol to the materials in 3 locations throughout the containment unit and exposed to bipolar ionization for a period of 5, 15, and 30 minutes. Swabs were taken of all material and cultured by the same means as the original viral titration performed on the BEI Resources provided SARS-CoV-2 USA-WA1/2020 viral culture. Preliminary results are as follows:

**Kydex Log10 Reduction from 0 min to 30min**
- Site 1: 3.75, Site 2: 3.7, Site 3: 3.71
- Control: 0.15

**Aluminum Log10 Reduction from 0Min to 30 min**
- Site 1: 3.73, Site 2: 3.65, Site 3: 3.61
- Control: 0.18
Leather Log10 Reduction from 0 min to 30 min: Site 1: 3.72, Site 2: 3.75, Site 3: 3.75 Control: 0.39

Vero6 Cells – Post Infection with deactivated virus

Vero6 Cells and 150μL Control Virus
CONCLUSIONS:

As it pertains to Aluminum-2024 T3.030: the increase in ion concentration had an increased effect on peplomer binding as expected. After 30 minutes, the overall average decrease in active virus was 99.89%. The control sample showed the expected natural degradation given the materials inoculated. Given the specific environment this was tested in, the quality of the materials, and the method in which the virus was dispersed, it is safe to say that the bipolar ionization system used in this experiment has the ability to deactivate SARS-CoV-2 with the given ion counts.

As it pertains to Kydex-6565.125: the increase in ion concentration had an increased effect on peplomer binding as expected. After 30 minutes, the overall average decrease in active virus was 99.99%. The control sample showed the expected natural degradation given the materials inoculated. Given the specific environment this was tested in, the quality of the materials, and the method in which the virus was dispersed, it is safe to say that the bipolar ionization system used in this experiment has the ability to deactivate SARS-CoV-2 with the given ion counts.

As it pertains to Leather: the increase in ion concentration had an increased effect on peplomer binding as expected. After 30 minutes, the overall average decrease in active virus was 99.99%. The natural degradation of the control was expedited leaving the assumption that residual chemicals used to treat the material or the porous surface may have played a larger factor in either the degradation or our inability to collect all of the sample use for inoculation.

- Additional testing was performed in correlation with Innovative Bioanalysis, LLC and can be provided upon request. The data from this experiment showed a negligible difference in total active viral count and can be considered closely comparable.
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Date

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