Surface Time-Kill Study to Evaluate the Antimicrobial Efficacy of the IONaer 7000 Ion Generator against Three Microorganisms

Study Sponsor

IONaer International

Study Personnel

Luisa Ikner, Ph.D.

Charles Gerba, Ph.D.

Report Delivery Date

25 June 2017

THE UNIVERSITY OF ARIZONA®
WEST CENTER
WATER & ENERGY SUSTAINABLE TECHNOLOGY

Study Objective

To evaluate the antimicrobial properties of the IONaer 7000 Ion Generator following 1-hour (for *E. Coli* and *A. baummanii*) or 24-hour (for *S. aureus* and non-enveloped viral surrogate) exposure time against four test organisms inoculated and dried onto hard, nonporous surfaces.

Study Methods

1. Glass slide carriers (1"x 3") were washed in mild soap, double-rinsed in tap water and DI-water, and then autoclaved prior to testing.
2. For *E. coli* and *S. aureus*, bacterial cultures were initiated 22 ± 2 hours prior to testing by inoculating 10 ml of tryptic soy broth (TSB) with one colony of the respective bacteria. On the day of testing, bacterial cells were pelleted by centrifugation (4,000 x g for 10 minutes), and washed twice using 0.01M PBS with successive rounds of centrifugation. For MS2, a pre-titered stock culture (5 x 1011 PFU/ml) was used for the testing.
3. On the day of testing, carrier inoculum cultures were prepared by diluting each of the bacterial cultures and viral stock to achieve target inocula of 7.5 x 105 organisms per 0.020 ml. The carrier inoculum cultures were then amended using fetal bovine serum to achieve a soil load of 2.5%.
4. Clean, dried glass slide carriers were mounted in sterile Petri dishes, and inoculated with 0.02 ml of the test cultures in replicates of two (2) according to the following:
    - Two (2) Time Zero Control Carriers (to be harvested for enumeration immediately upon drying)
    - Two (2) Timed Control Carriers (to be held separately from the exposed test carriers under laminar flow conditions for the study exposure time)
    - Two (2) Test Carriers (to be exposed to the IONaer 7000 Ion Generator for the study exposure time)
5. Inoculated carriers were dried under laminar flow with the Petri dish lids slightly ajar. Drying time for the carriers was approximately 10 minutes.
6. The IONaer 7000 device was placed into a biosafety cabinet chamber, with the laminar flow turned off, for testing. Upon drying, two carriers per organism were placed directly below the device's aluminum enclosure, downstream of the ionizing current, for exposure. The device and fan were powered "on" by plugging in both plugs. The exposure time was initiated when the blue indicator light for ionization glowed steadily (within 30 seconds to 1 minutes of plugging in the device). The biosafety cabinet sash was lowered and closed for the duration of the test.

7. A second set of two dried carriers were held separately in a different biosafety cabinet located in another lab room, and exposed to full laminar flow conditions (i.e. Petri dish lids removed) for the exposure time. These were designated as the Timed Control Carriers.
8. The third set of two dried carriers were harvested immediately into 20 ml of Dey/Engley (D/E) Broth upon drying, and served as the Time Zero controls. Following a 15 second vortex, the detached organisms were diluted 10-fold. Bacterial cultures were plated onto tryptic soy agar (TSA), and MS2 was plated using the double-layer agar overlay technique in combination with an *E. coli* 15597 bacterial host.
9. After the one hour exposure period, the Timed Control and exposed Test Carriers were harvested and plated in the same manner as the Time Zero carriers.
10. All platings were incubated for ~24 hours at 37 °C. Bacterial colony-forming units (CFUs) and viral plaque-forming units (PFUs) were then enumerated, and the reductions calculated.

Study Specifications

Test Organisms (4 total)	Bacteria: *Escheria coli* 25922 *Staphylococcus aureus* 6583 *Acinetobacter baummanii* 19606 Viral Surrogate: MS2 15597
Exposure Time	1 hour (*E. coli*, *A. baummanii*) 24 hours (*S. aureus*, MS2)
Exposure Conditions	22.4°C, 18% R.H.
No. of Replicates	Duplicate

Pathogen	**Total Elimination Percentage**	**Improvement Over Untreated Airflow**
E. coli	99.5%	95.7%
A. baummanii	97.4%	51.4%
S. aureus (staph infection)	99.2%	90.4%
Non-Enveloped Viral Surrogate MS2 (e.g., Norovirus)	99.998%	96%