Virucidal Efficacy Assay

Sponsor	AIONX Antimicrobial Technologies
Sponsor Contact:	Thomas Fuller
Report Date:	June 19, 2020
Viruses Tested:	SARS-CoV-2, USA-WA1/2020
Cell Line:	Vero 76
Contact Time:	10-minutes, 25-minutes, 40 minutes
Experiment #:	SARS2-137

Study Director:

Michelle Mendenhall
Utah State University, Institute for Antiviral Research
5600 Old Main Hill
Logan, UT 84322
michelle.mendenhall@usu.edu

Introduction:
Testing was performed to determine if the test system inactivates SARS-CoV-2 virus when exposed at various time intervals. Virus solutions were placed on the test surface for specified times, then surviving infectious virus was quantified by standard $CCID_{50}$ assays and compared with untreated controls.

Procedures:
Virus, media and cells.
SARS-CoV-2 virus stocks were prepared prior to testing by growing in Vero 76 cells in MEM supplemented with 2% FBS and 50 μg/mL gentamicin (test media).

Test set-up.
The test system was set up as shown in Figure 1, except that it was placed in a biosafety cabinet during testing. A drop of liquid SARS-CoV-2 virus stock was placed on the active grid area of the black coupon. An untreated virus control was placed on the black plastic coupon outside of the active grid area. For toxicity control, a drop containing media only (no virus) was placed on the active grid area of a separate coupon. The test was performed in triplicate and the replicates for each virus were tested at the same time with a separate system set up for each replicate (3 virus test systems and one toxicity test system were running simultaneously).

The system was turned on and the multimeter set to read at 2000 μA and observed to ensure there was a reading to indicate that the circuit was complete. Readings between 160-220 μA were observed throughout the testing period. The samples were covered with a loose lid to prevent drying out of the droplet due to airflow in the biosafety cabinet. The test was performed at three contact times, collecting samples at 10, 25 and 40 minutes. The samples were diluted 1/10 in test media. Samples were stored at -80°C until time of virus quantification.

Virus quantification.
Surviving virus from each sample was quantified by standard $CCID_{50}$ end-point dilution assay. Samples were serially diluted 1/10 in test medium. Then 100 μL of each dilution were plated into quadruplicate wells of 96-well plates containing 80-90% confluent cells. Plates were incubated at 37 ± 2°C with 5% CO_2 for 6 days. Each well was then scored for presence or absence of virus. The $CCID_{50}$ values were calculated using the Reed-Muench (1948) equation.

Controls: Virus controls were tested on the non-grid area of the coupon in parallel and the reduction of virus in test wells compared to virus controls was calculated as the log reduction value (LRV). Toxicity controls were tested with media not containing virus on the active area of

the coupon to see if metal ions or other bi-products generated in the test procedure were toxic to cells. Neutralization controls were tested to ensure that virus inactivation did not continue after the exposure time, and that residual sample in the titer assay plates did not inhibit growth and detection of surviving virus. This was done by adding toxicity samples to titer test plates then spiking each well with a low amount of virus that would produce an observable amount of CPE during the incubation period.

Results:

Table 1 shows results for SARS-CoV-2 virus titers after the three exposure times. Virus control titers were 5.8 log CCID50 per 0.1 mL. After 25- and 40-minute contact times, the test system reduced virus titers by 2.1 and 2.5 log CCID50 per 0.1 mL, respectively (>99%). Virus was not reduced by ≥1 log in the 10-minute time point.

Voltmeter readings varying from 160-220 μA were observed during the incubation. Bubbling was observed and a slight blue tint appeared in the liquid droplet during the longer run times of 25 and 40 minutes.

Toxicity controls showed that the system affected the media leading to a toxic effect in Vero 76 cells at the 1/10 dilution of the sample. Neutralization controls confirmed that virus was detected in the presence of the test sample on cell plates.

Table 1. Virucidal effect of the AIONX test system against SARS-CoV-2 after 10-, 25-, or 40-minute test times.

Contact Time (min)	Test sample Virus Titer[a]	Virus Control Titer[a]	LRV[b]
10	5.3	5.8	0.5
25	3.7	5.8	2.1
40	3.3	5.8	2.5

[a] Log CCID50 per 0.1 mL

[b] LRV (log reduction value) is the $\log_{10}$ reduction of virus compared to the virus control

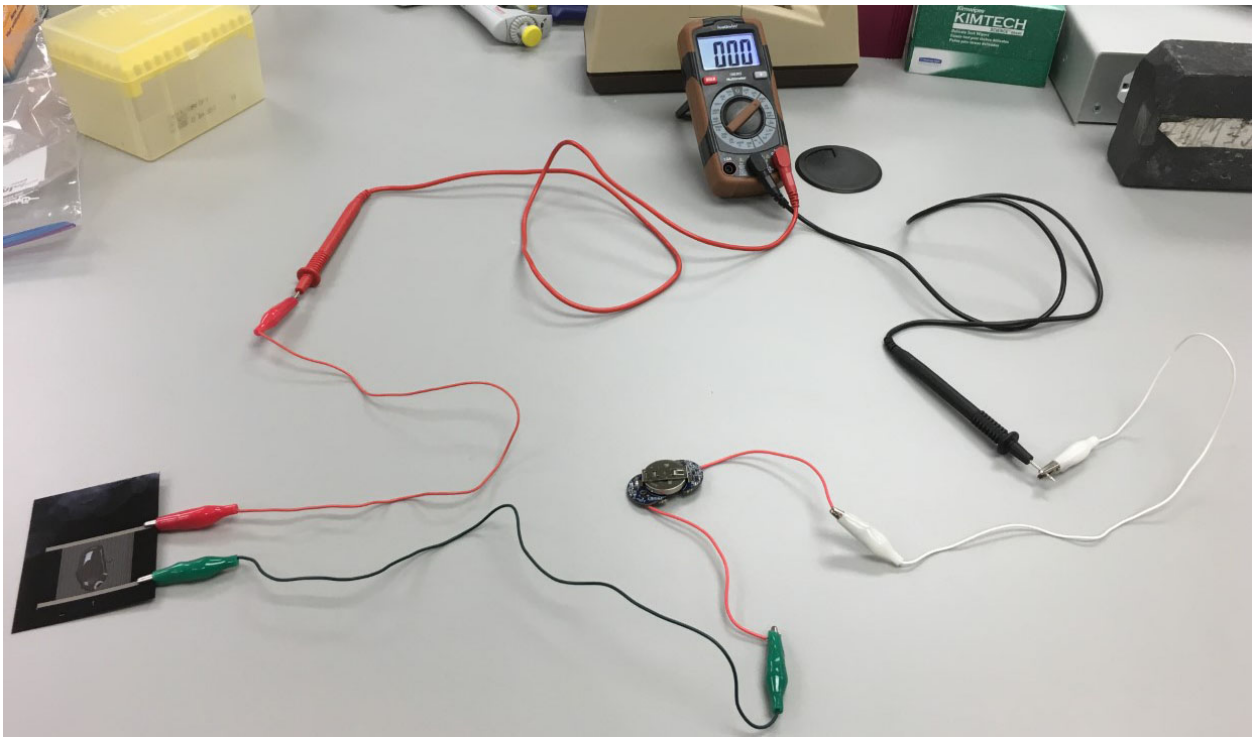

Figure 1. Example of AIONX system set-up. A 3V battery was used to power the printed circuit board (PCB). The multimeter was set to measure μA and was part of the complete circuit; measurements ranged from 160-400 during the test. A droplet of media or water was required on the active grid area of the test coupon (black) to complete the circuit.