March 20, 2020

Enozo SB100 Spray Bottle Inactivates >99.9% of Human Coronavirus

Overview

In-house testing of the Enozo SB100 Ozone Generating Spray Bottle against the human coronavirus surrogate 229E (ATCC VR-740) shows >99.9% inactivation of the virus when treated with aqueous ozone generated by the SB100 bottle. The surrogate virus is the most up-to-date virus commercially available to mimic the SARS-CoV-2 virus responsible for development of the disease COVID-19. The test protocol is based the ASTM E1052 Standard, with considerations for use of ozone outlined below.

Procedures

The ASTM E1052 Standard Test Method for Efficacy of Antimicrobial Agents Against Viruses in Suspension protocol was followed as closely as possible, with special considerations for ozone as the test chemical outlined below. In brief, 1 mL virus was combined with 9 mL aqueous ozone from the Enozo SB100 Ozone Generating Spray Bottle, incubated at room temperature for 30 seconds, and diluted in Eagle's Minimal Essential Medium with 2% Fetal Bovine Serum (EMEM + 2% FBS) to neutralize. Serial dilutions of inactivated ozone + virus were added to 24-hour old cultured human lung fibroblast cells (ATCC CCL-171) and scored for cytotoxic effects at 6 days post-infection. Virus control, cytotoxicity control, and neutralization control were performed in parallel.

Procedural Considerations

- Human Coronavirus was prepared by removing growth medium from 24 hour subcultured human lung fibroblast cells (ATCC CCL-171) in a 75 cm^2 flask, washing 3 times with sterile phosphate buffered saline (PBS), and covering the cell sheet with 3 mL PBS. Cells were frozen at -80 °C for 20 minutes and thawed at 37 °C for 5 minutes for a total of 3 freeze-thaw cycles. Cells were then scraped into the PBS and centrifuged at 2000 rpm for 20 minutes to remove cells. This preparation results in a high titer of active virus and removes virus-inactivating serum from the cell growth medium.
- Virus and test substance were combined by spraying 9 mL undiluted, ozonated water onto 1 mL virus sample rather than by pre-spraying test substance and then adding virus. This consideration reduces the impact of ozone's short half-life by minimizing the time between ozone generation and testing. It also mimics the field use case of spraying directly onto a contaminated area without altering the dynamics of the test that would be performed on a diluted chemical substance.
- The Human Coronavirus 229E is a BL-2 virus that has the same structure, and therefore chemical reactivity, of the SARS-CoV-2 that causes COVID-19. It is the closest surrogate to SARS-CoV-2 that is available for testing, and the US EPA recognizes tests with this surrogate as likely representative of results with SARS-CoV-2.

Results

Activity of virus after spray with either ozonated or unozonated water from the Enozo SB100 spray bottle was tested by adding 2 mL of serial 10-fold dilutions in quadruplicate to a 24-well culture plate that had 24 hours' growth of cultured human fibroblast cells (ATCC CCL-171). Cells were incubated at 35 °C with 5-10% carbon dioxide in air for 6 days and scored for cytotoxic effects. An overview of results is presented below. Wells marked with an "X" showed cytotoxicity. There was no cytotoxicity observed in healthy, untreated cells or in the cytotoxicity controls.

Virus treated with unozonated water (ozone off), sprayed through Enozo SB100 Bottle

10^2	X	X	X	X
10^3	X	X	X	X
10^4	X	X	X	X
10^5	X	X	X	X
10^6	X	X		
10^7				

Median Tissue Culture Infectious Dose ($TCID_{50}$): 5×10^5/mL in 6 days

Virus treated with ozonated water (1 ppm ozone), sprayed through Enozo SB100 Bottle

10^2			X	X
10^3				
10^4				
10^5				
10^6				
10^7				

Median Tissue Culture Infectious Dose ($TCID_{50}$): 5×10^1/mL in 6 days

Viral inactivation with ozone: 50 $TCID_{50}$ / 500000 $TCID_{50}$ = 1×10^{-4}

This corresponds to 99.99% inactivation of virus at 1 ppm ozone.

Reproducibility

The test was repeated to ensure reproducibility, with the following results:

Virus treated with unozonated water (ozone off), sprayed through Enozo SB100 Bottle

10^2	X	X	X	X
10^3	X	X	X	X
10^4	X	X	X	X
10^5	X	X	X	X
10^6	X		X	
10^7	X			

Median Tissue Culture Infectious Dose ($TCID_{50}$): 5 x 10^5/mL in 6 days

Virus treated with ozonated water (1 ppm ozone), sprayed through Enozo SB100 Bottle

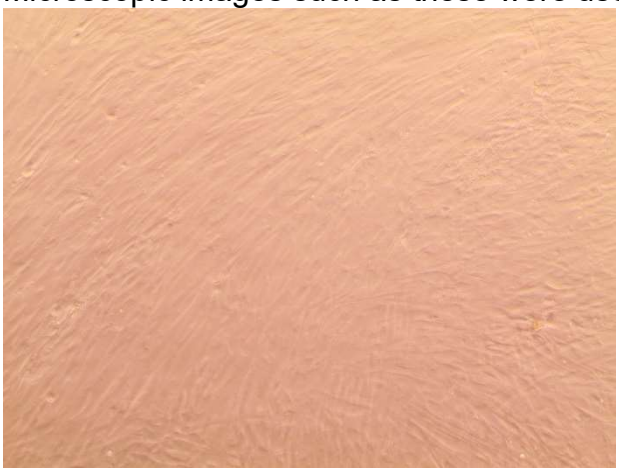
10^2	X	X		X
10^3	X			
10^4				
10^5				
10^6				
10^7				

Median Tissue Culture Infectious Dose ($TCID_{50}$): 1.58 x 10^2/mL in 6 days

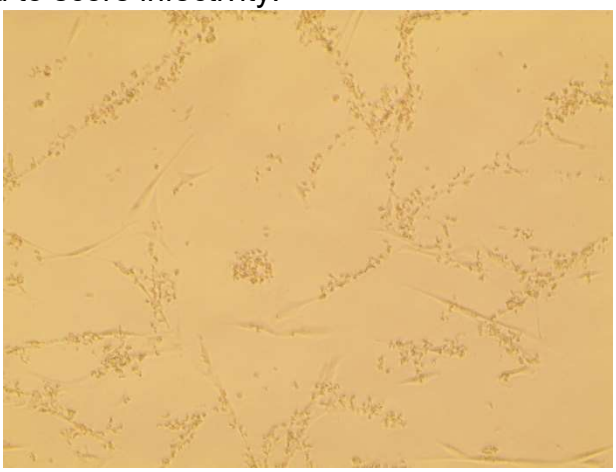
Viral inactivation with ozone: 158 $TCID_{50}$ / 500000 $TCID_{50}$ = 3.16 x 10^{-4}

This corresponds to 99.97% inactivation of virus at 1 ppm ozone.

Representative images (100x magnified) of healthy MRC-5 human lung fibroblast cells (left) and of the same cell type showing cytotoxic effects (right) due to viral infection. Microscopic images such as these were used to score infectivity:



Healthy, elongated human lung fibroblasts after addition of ozone-treated virus (10^3 dilution of virus).



Human lung fibroblasts showing cytotoxic effects after infection with unozonated virus (10^6 dilution of virus).