Flinders UNIVERSITY
College of Science & Engineering

INSPIRING ACHIEVEMENT

TECHNICAL REPORT

Viricidal efficacy of PlasmaShield against Human Coronavirus 229E

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This report was prepared for:
PlasmaShield

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EXECUTIVE SUMMARY

The efficacy of the PlasmaShield device to inactivate aerosolised HCoV229E virus was tested. Purified HCoV229E, at a concentration of 1.93 x 10^8 $TCID_{50}$/mL in PBS, was nebulised for 3 min through 21.5 cm of piping prior to the PlasmaShield and 21.5 cm piping after the PlasmaShield. For the baseline/negative control measurement, the PlasmaShield was replaced with an empty case. Surviving virus aerosols were collected in a liquid impinger sampler. The virus concentration was then determined by infectivity of Huh-7 cells and the $TCID_{50}$/mL calculated. All experiments were conducted in triplicate. The baseline experiment (empty PlasmaShield case) detected 7.85 x 10^6 $TCID_{50}$/mL HCoV229E. This was reduced by approximate a 1.5 x $\log_{10}$ (95% reduction) to 3.9 x 10^5 $TCID_{50}$/mL HCoV229E with the PlasmaShield turned on.

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic is an ongoing global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). To combat the spread of COVID19 it is imperative that we can control SARS-CoV-2 in the environment. This can be achieved using appropriate disinfection strategies, including air purification systems.

The biosafety level of SARS-CoV-2 means that it is not feasible to conduct air quality research involving the aerosolization of the SARS-CoV-2 virus and an appropriate surrogate must be used. The Australian Therapeutic Goods Administration (TGA) recently announced that manufacturers wishing to make label claims of efficacy against COVID-19 for products should use Human coronavirus 229E (HCoV229E) as a surrogate (Therapeutic Goods Administration, 2020).

The aim of this study was to investigate the viricidal efficacy of the PlasmaShield device against HCoV229E as a surrogate organism for SARS-CoV-2.

HCoV229E is an enveloped, positive-sense, single stranded RNA virus. It is a member of family *Coronaviridae*. Members of this family are disseminated via aerosols and fomites and target respiratory tract. HCoV-229E is associated with a range of respiratory diseases. In healthy adults it causes common cold symptoms, with younger children and the elderly considered vulnerable to developing lower respiratory tract infections. In immunocompromised patients these lower respiratory tract infections can be life threatening (Vassilara et al 2018).

METHODOLOGY

HCoV229E is a biosafety level 2 microorganism. Biosafety approval for this work was granted by the Flinders Institutional Biosafety Committee (IBC Ref No. 2021-01).

Propagation of Human coronavirus (HCoV229E)

Huh-7 cells (human male hepatocyte derived cellular carcinoma) were cultured in L-glutamine containing high glucose DMEM (Gibco™, ThermoFisher Scientific) medium supplemented with 10% (V/V) fetal bovine serum (Gibco™, ThermoFisher Scientific). Viral stocks of HCoV229E (ATCC® VR-740™, American Type Culture Collection) were prepared by infecting Huh-7 cell at multiplicity of infection of 0.01 for 5 days, at 37°C and approximately 95% humidity and 5% CO_2, until a significant cytopathic effect was observed. The infected cells were harvested and subjected to three freeze/thaw cycles, and infected-cell lysate was used for the purification of HCoV229E virus.

Purification of Human coronavirus (HCoV229E)

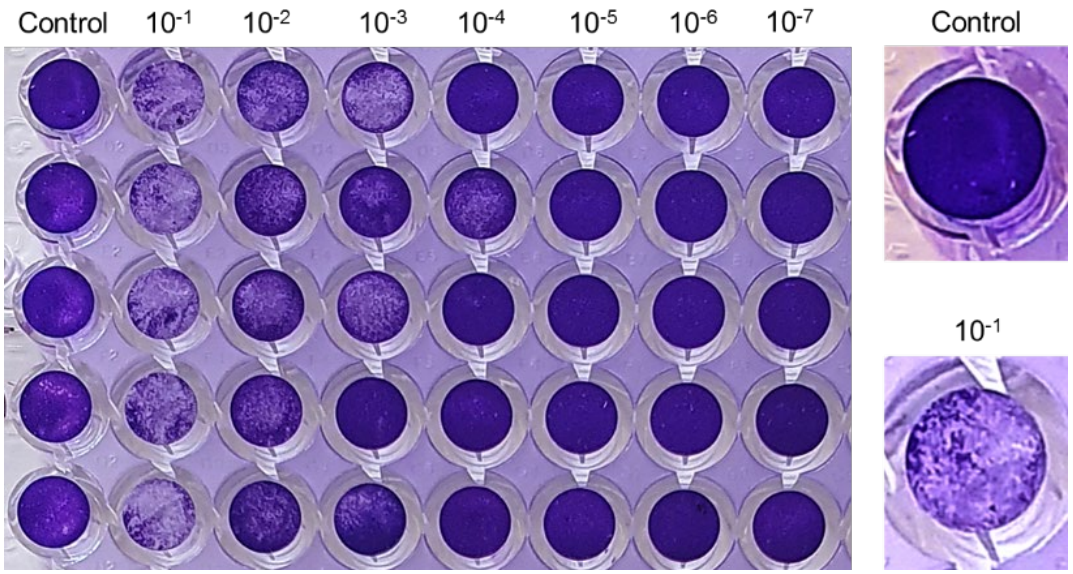
HCoV229E was purified using the High-purity™ Lentivirus Concentration Kit following manufactures instructions. Briefly, 5 mL virus infected cell lysate was filtered using 0.45 µm syringe filter and 4 mL filtrate was obtained. In 4 mL filtrate 1 mL of "5x Lentivirus Concentration Solution" was added and incubated at 4°C overnight (15 to 16 hours). The mixture was centrifuged at 4°C at 3600 RPM for 25 min. After centrifugation the supernatant was discarded, and the remaining pellet was re-centrifuged at 4°C at 3600 RPM for 5 min. This pellet was then resuspended in 1X PBS to provide a concentrated stock solution.

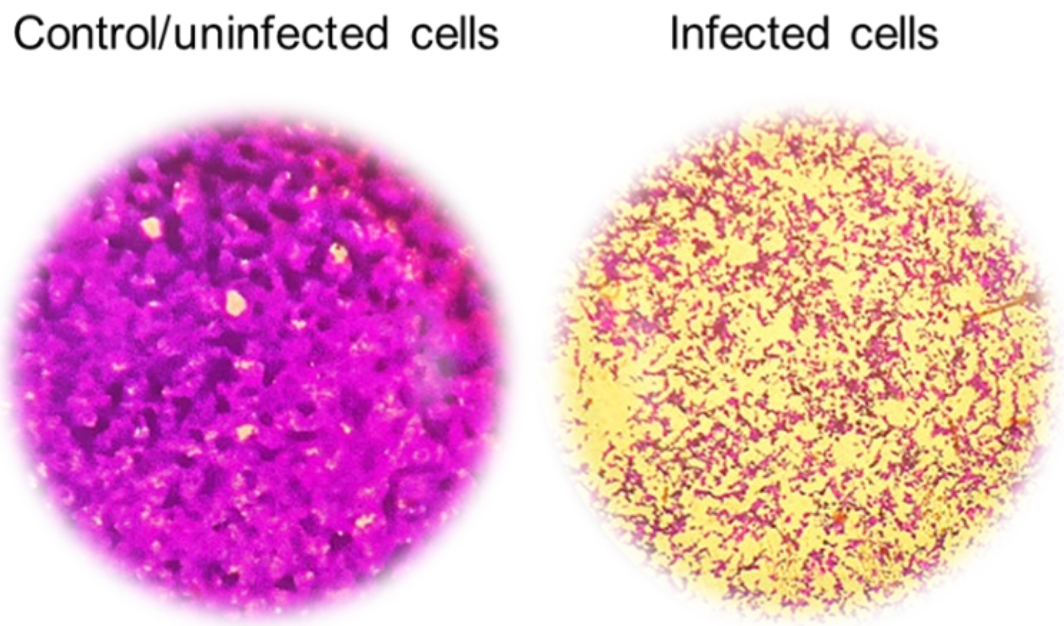
Crystal violet viability assay

Cellular infectivity and survival were estimated using crystal violet assay. Briefly, under standard culture conditions Huh-7 cells were cultured in 96-well flat-bottom microplate to approximately 90% confluency. The virus sample was serially diluted in DMEM medium to various multiplicity of infection. Huh-7 cells were infected with various dilutions (2-fold, 4-fold and 10-fold) of virus and incubated for one hour on a laboratory rocker (ThermoFisher Scientific), followed by incubation under standard culture conditions for 5 days. In the 96-well plate (see Figure 1), column 1 corresponds to control/uninfected cells, column 2 to onwards corresponds to various virus dilutions. After 5 days of incubation cells were fixed with formaldehyde for 12 to 16 hours. The fixed cells were washed with water multiple times and then stained with 0.5% crystal violet (Sigma-Aldrich) for 2 to 3 hours. Finally, stained cells were washed multiple times with water to remove unbound stain. In the crystal violet assay, cellular viability is directly related to crystal violet uptake as shown in Figure 1. The number of wells per virus dilution with and without cytopathic effects were counted and $TCID_{50}$/mL (tissue culture infectious dose, 50%) was calculated (see Appendix for method). Figure 2 presents cytopathic effects of infected and uninfected cells as seen under a microscope.

Survival of HCoV229E in Phosphate Buffered Saline (PBS)

HCoV229E resuspended in 1X PBS was used for nebulisation and tested (positive control) to confirm the concentration of virus used in the experiments.





Experimental set up

The experimental setup is presented in Figure 3 and 4. The purified HCoV229E suspended in 1X PBS (1.93 x 10^8 $TCID_{50}$/mL) was aerosolized using the nebuliser (1-jet Collison nebulizer, CH Technologies, USA) through the removable piping (21.5 cm in length and 25 cm in diameter) and the PlasmaShield device for 3 min. The air flow rate was controlled using the fan and set at 1m/s. This was confirmed using an anemometer (Pocket Pro™

+ Multi 2, HACH Company). The air pressure released from the air cylinder was set to 10 psi. Surviving viruses were then captured in 5 mL DMEM growth medium using a liquid impinger sampler (BioSampler® SKC Inc) and enumerated using the previously described method. The experiments were conducted in triplicate with an empty PlasmaShield case for the baseline/negative control and the PlasmaShield device operating at 100% power.

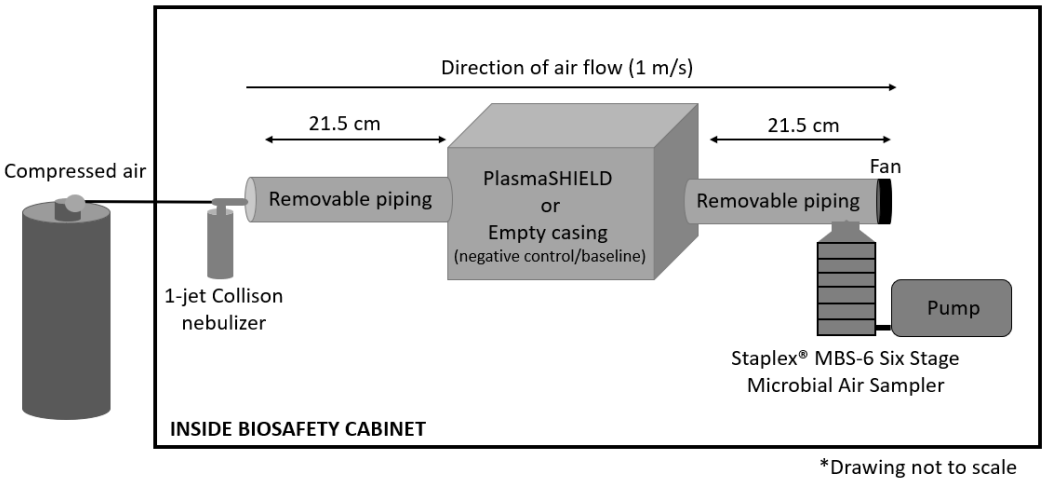


Figure 3:Diagram showing the experimental set up

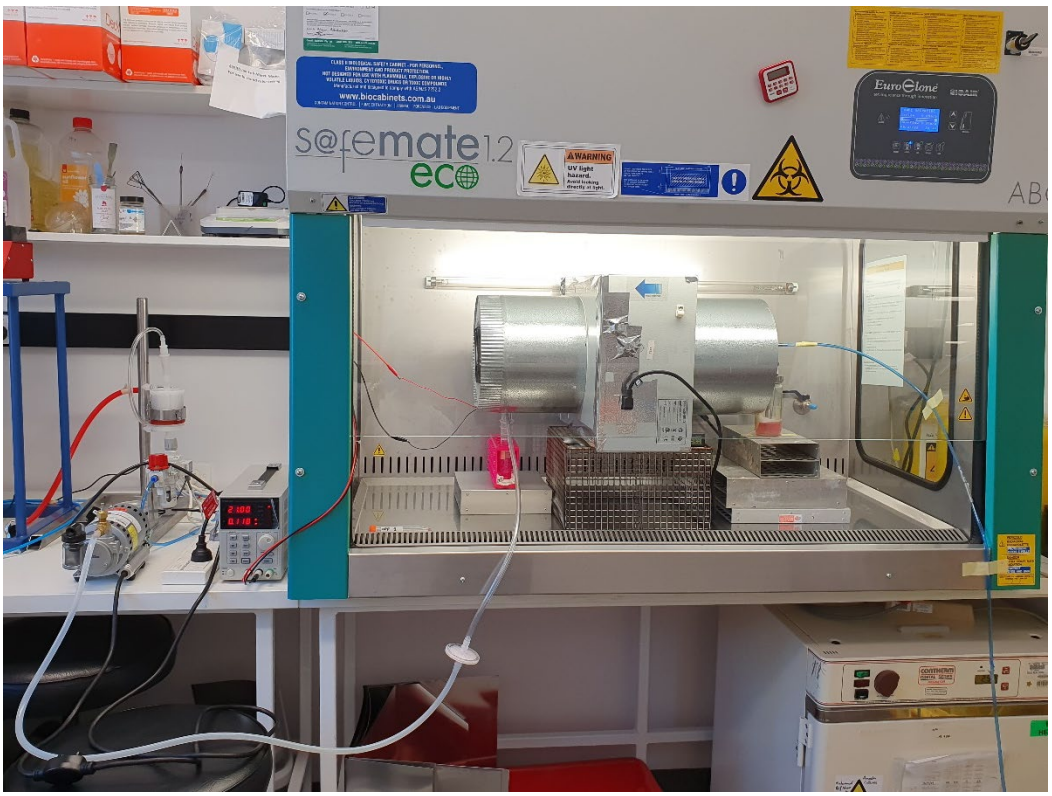


Figure 4:Photo showing the experimental set up

RESULTS

HCoV229E was aerosolised at a concentration of 1.93 x 10^8 $TCID_{50}$/mL in 1X PBS for 3 min. When this passed through the piping and the empty PlasmaShield case, 7.85 x 10^6 $TCID_{50}$/mL HCoV229E was detected. This was reduced by approximate a 1.5 x log_{10} (95% reduction) to 3.9 x 10^5 $TCID_{50}$/mL HCoV229E with the PlasmaShield present and operating at 100% power (See Figures 5 and 6).

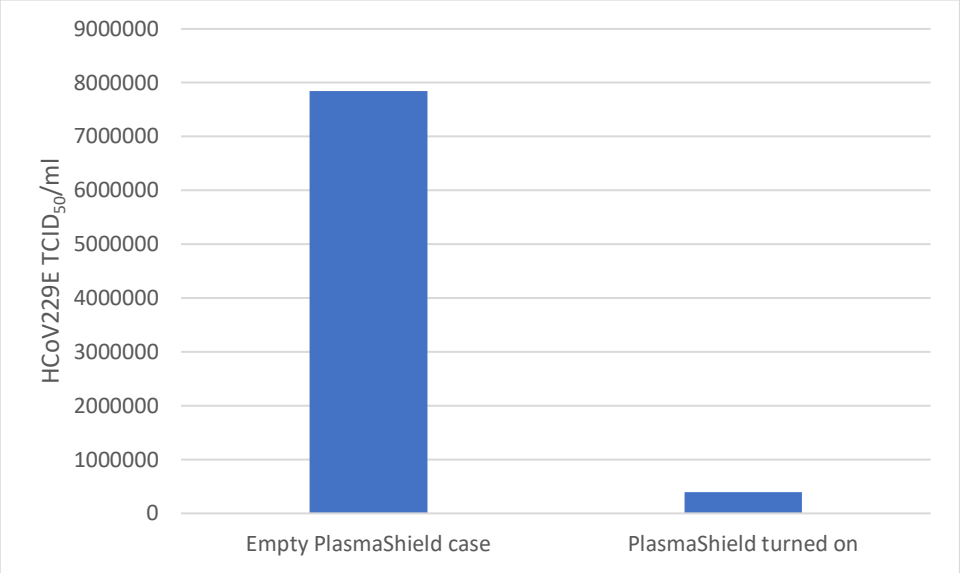


Figure 5: Surviving HCoV229E detected with the PlasmaSHIELD empty case (baseline/negative control) and PlasmaShield operating at 100% power

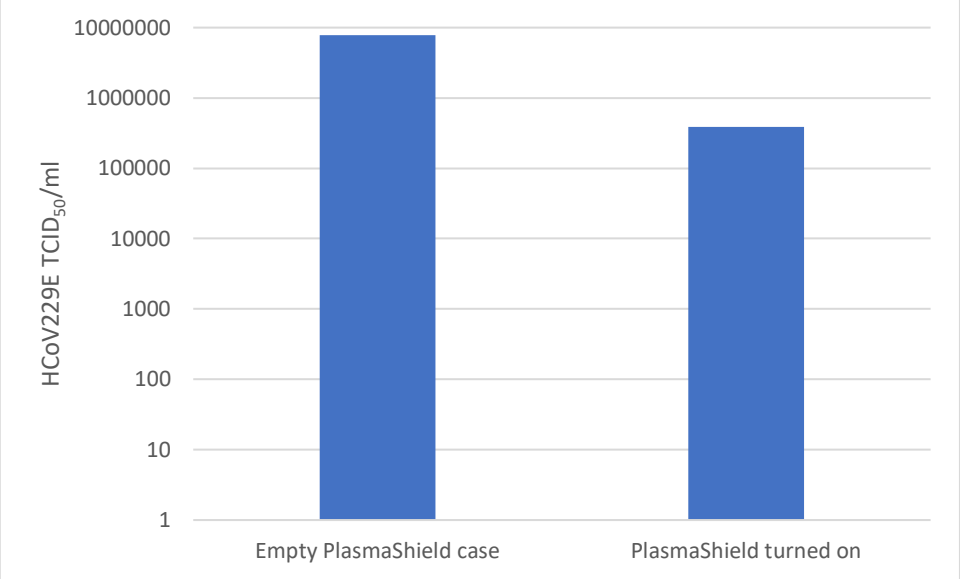


Figure 6: Surviving HCoV229E detected with the PlasmaSHIELD empty case (baseline/negative control) and the PlasmaShield device operating at 100% power, plotted on a log_{10} scale

If you would like to know more about this project, please don't hesitate to contact us.
Kind regards,
Harriet, Kirstin and Peter

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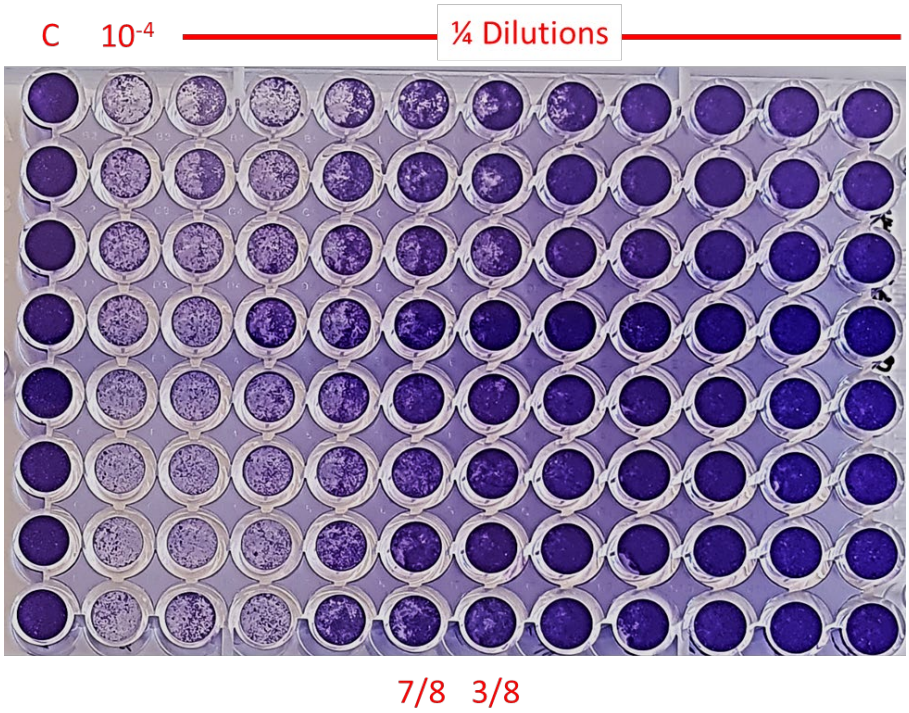
Vassilara, F., Spyridaki, A., Pothitos, G., Deliveliotou, A. and Papadopoulos, A., 2018. A rare case of human coronavirus 229E associated with acute respiratory distress syndrome in a healthy adult. *Case Reports in Infectious Diseases*, 2018, 6796839.

APPENDIX

$TCID_{50}$/mL assay calculations



1. Calculate Proportionate Distance (PD) between the two dilutions in between 50% death:

(% next above 50%) – 50% / (% next above 50%) – (% next below 50%)

PD = 87.5% – 50% / 87.5% – 37.5% = 25/62.5 = 0.75

2. Calculate 50 % end point:

Log lower dilution= dilution in which position is next above 50%

Log lower = 3.906×10^{-7} → = Log (1.563×10^{-6}) = – 6.41

3. Add PD and Log lower dilution:

Add up – 6.41 and 0.75 (it is not mathematical addition or subtraction) → – 7.16

Log TCID50= anti-Log (7.16) = 14454398

4. Calculate TCID 50/ml. Divide by the ml of viral inoculum added to each well:

According to our protocol virus used in each well = 75 µL OR 0.075 ml

TCID 50/ml= 14454398/0.075 = 1.93×10^{8}