APPLICATION OF COLD ATMOSPHERIC PLASMA IN THE TREATMENT OF VIRUSES AND DECONTAMINATION

Vikas Soni[1], Li Lin[1], Sang H. Lee[2], William Kastor[2], Steve Wood[2], Enusha Karunasena[2*], and Michael Keidar[1*]

[1] Department of Mechanical & Aerospace Engineering, MPNL, The George Washington University, Science & Engineering Hall, 800 22nd Street, NW, Washington, DC 20052, USA

[2] Division of Biology, Chemistry, and Materials Science, Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, Building 66, US Food and Drug Administration (FDA), 10903 New Hampshire Avenue, Silver Spring, MD 20993.

*Corresponding Author: enusha.karunasena@fda.hhs.gov; keidar@gwu.edu

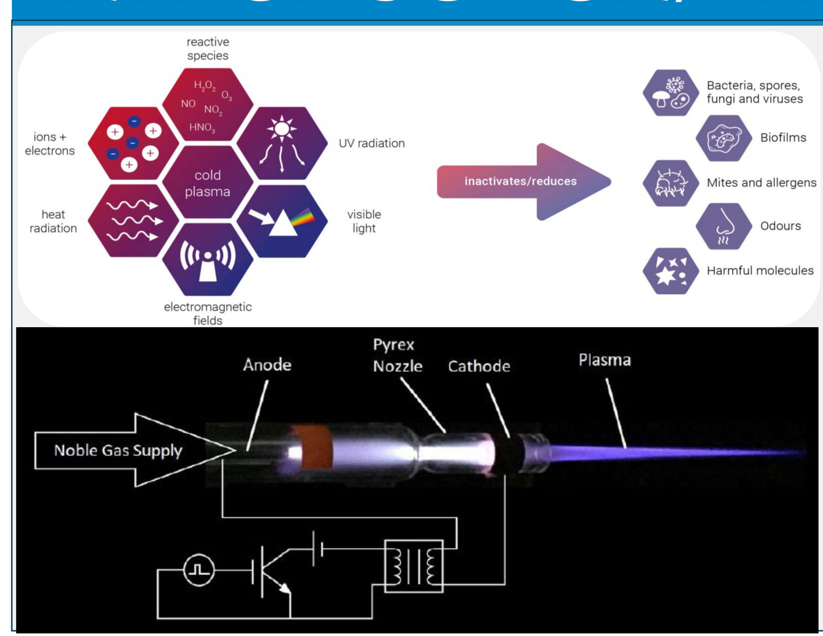
ABSTRACT

During the SARS-CoV-2 pandemic, there were multiple trials reported on testing the efficacy of a few antiviral drugs that were, ultimately, ineffective in curing disease resulting from COVID-19. Consequently, there is an urgent need to develop drugs to treat people and parallel efforts to develop decontamination methods to prevent the spread and inactivate the virus. This work focuses on cold atmospheric plasma (CAP) as a decontamination method [1,2]. Traditional methods to inactivate virus by chemical or physical methods using disinfection/sterilization, include hydrogen peroxide, 70% ethanol, detergents, steam sterilization, ozone and UV exposure. [3]. In the past decade, cold atmospheric plasma (CAP) has earned a lot of interest due to its decontamination/sterilization properties and has been used for the deactivation of many viruses [4,5]. CAP is a non-thermal partly ionized gas formed at atmospheric pressure. It contains a cocktail of reactive oxygen and nitrogen species (OH, O, H_2O_2, O_3, NO, NO_2, etc.), ions, molecules, electromagnetic waves, and UV photons collectively termed reactive agents (RAs) [6,7]. Due to a limited understanding of viral decontamination and a high demand for treatment modality, we tested the effects of CAP against influenza (H1N1) and the SARS-CoV-2 virus. In this study, we tested the application of CAP as a potential method for decontamination [5,6]. CAP exposure treatment of the viral particles was carried out for 30, 60, and 120 seconds. We observed a significant ~2.83 $TCID_{50}$/mL log reduction for 120 seconds of CAP treatment in the case of the influenza virus. Whereas we show preliminary results for SARS-CoV-2 deactivation with a $TCID_{50}$/mL of ~ 0.72 log reduction at 120 secs in PBS. Hence, we propose a new modality of decontamination and preventive interventions through the application of CAP which could be used in decontaminating Personal Protective Equipment (PPE).

OBJECTIVES

During the past three years, COVID-19 resulted in significant loss of life, globally. COVID-19 is a serious life-threatening disease caused by the virus, SARS-CoV-2 and resulted in a global pandemic. Hence alternate modalities were explored. The aim of this study was to develop a potential method of decontamination, rendering viruses inactive. To do this, we developed and tested cold atmospheric plasma (CAP) to inactivate viruses like influenza and SARS-CoV-2.

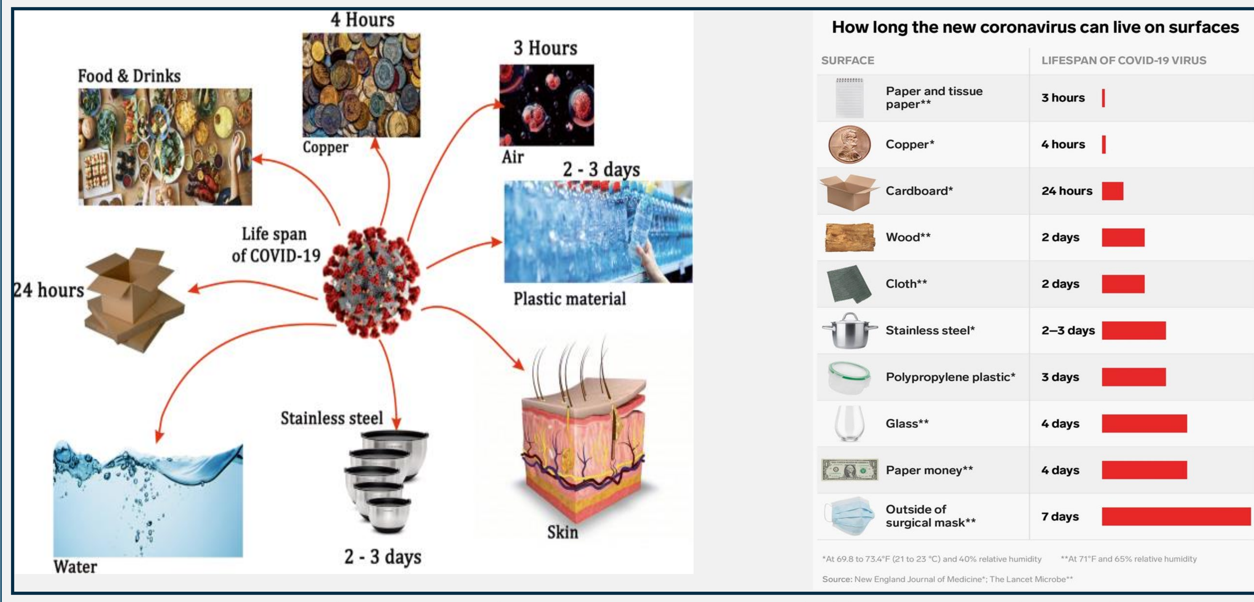
INTRODUCTION/BACKGROUND



Cold-atmospheric plasma (CAP) is a partly ionized gas formed at atmospheric pressure and with quasi-neutral charges composed of reactive species, positive and negative charged ions, charged radicals, neutral atoms, and ultraviolet (UV) photons.

Figure 1,2. Applications and Schematic of CAP jet [6-8]

MATERIALS AND METHODS



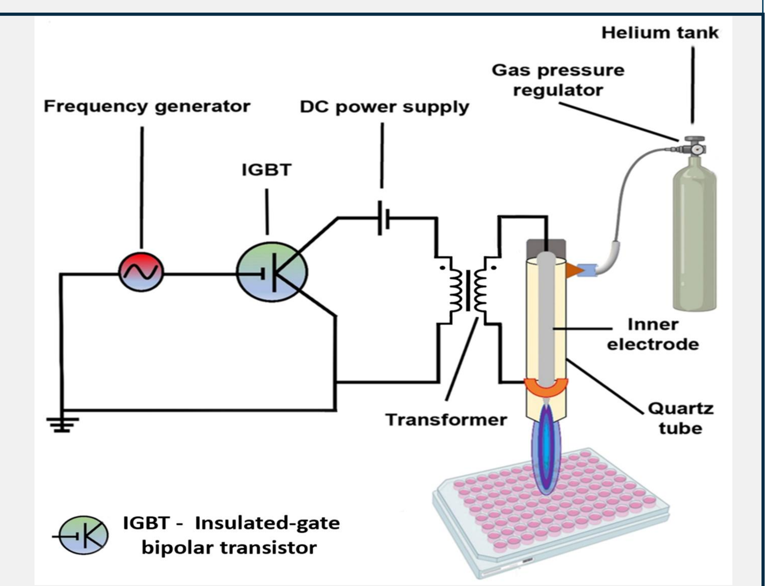
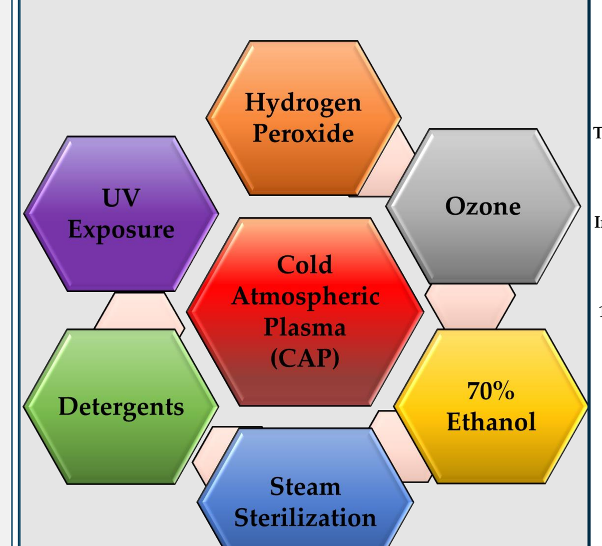
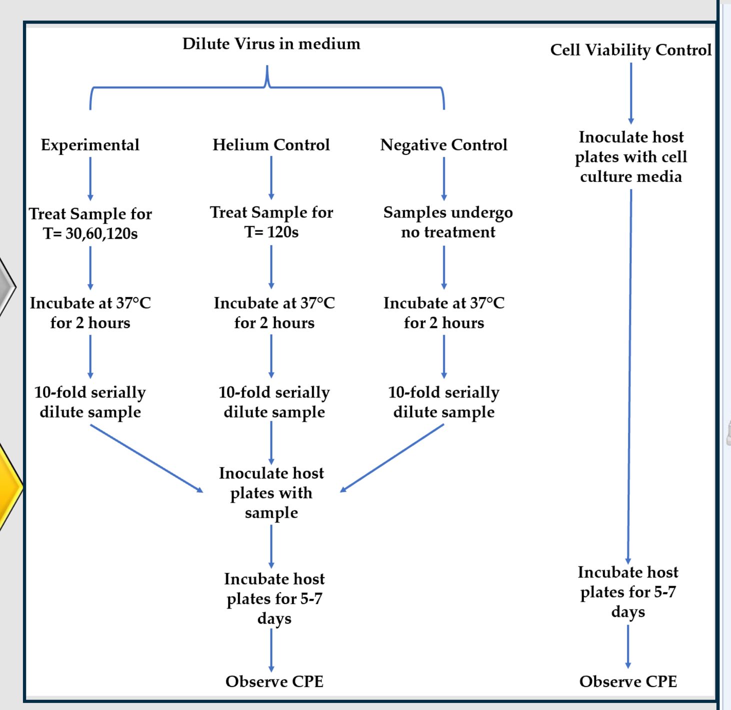


Figure 3 (A,B). Life span of coronavirus on different surfaces [9-11]. Figure 4. Schematic representation of the circuit diagram of CAP jet device used in this study [6].





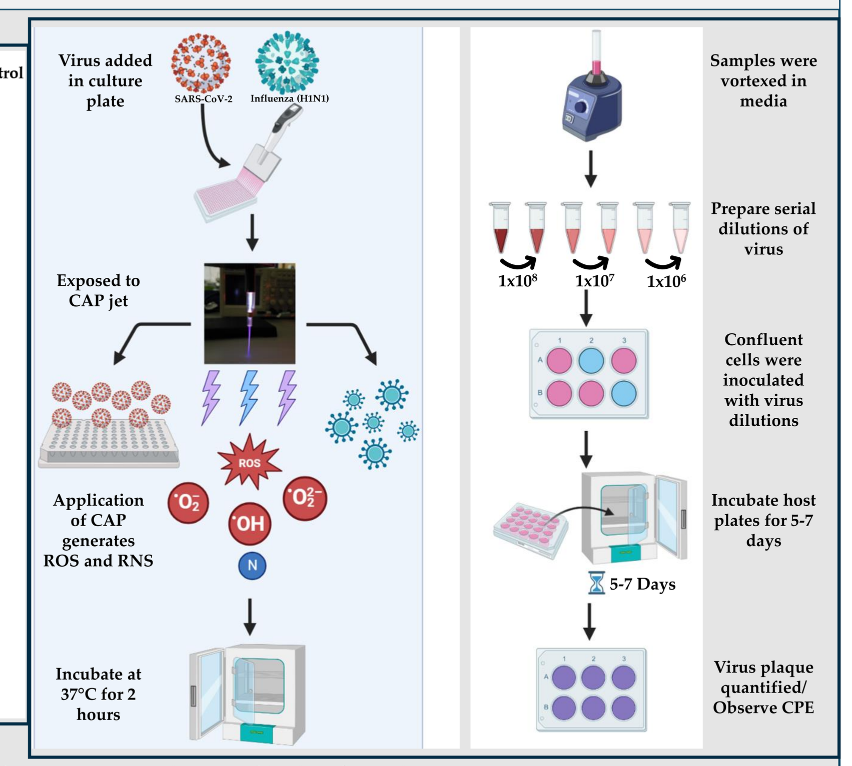


Figure 5(A). Different methods of decontamination on surfaces and PPEs using disinfection and the requirement for a new modality of decontamination method (CAP). Figure 5(B). Flow chart of experimental protocol.
Figure 6. Schematic representation of virus inactivation experiments using $TCID_{50}$ assay [12].

RESULTS AND DISCUSSION

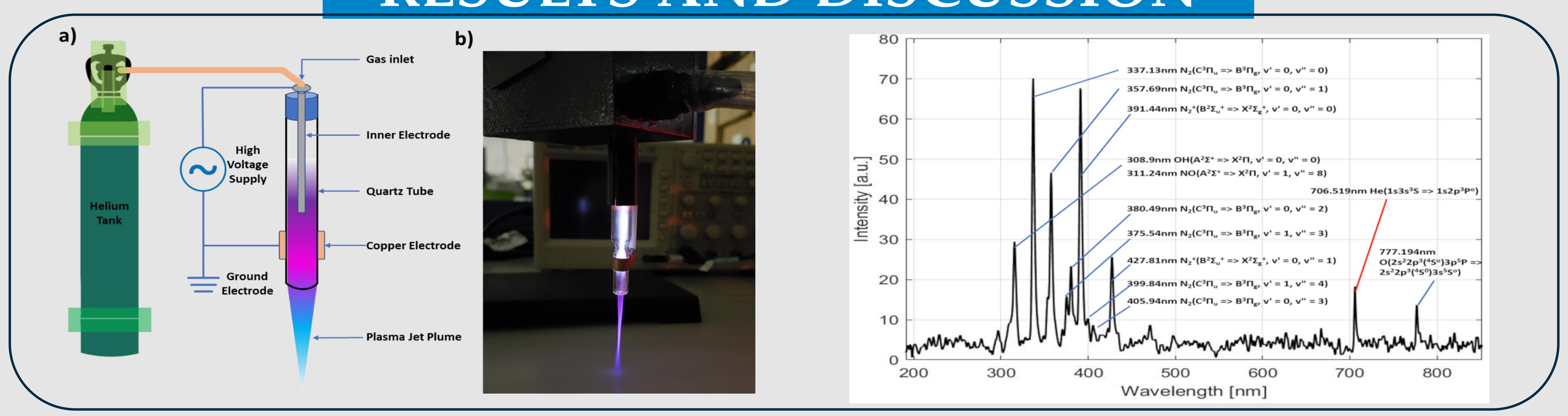
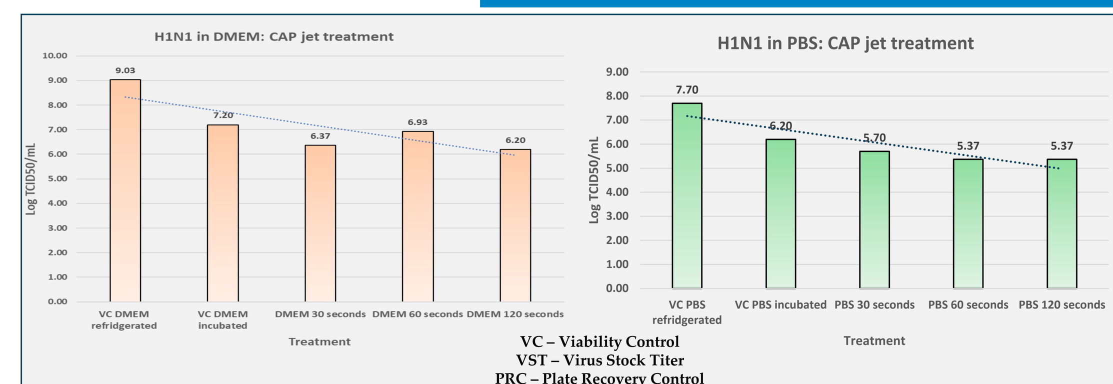


Figure 7(A). Experimental setup of CAP jet used in this study. The applied discharge voltage and frequency of the CAP jet were 10V and 12.5 kHz, respectively. Figure 7(B). Tektronix TDS2004C oscilloscope was used to monitor the current and the discharge voltage was calculated to be ~ 6.5 kV peak-to-peak.

Figure 7(C). Optical Emission Spectroscopy of the CAP jet using SpectraWiz® showing most peaks of nitrogen C3Πu => B3Πg ranging from the spectral region of about 300–490 nm. The CAP jet was characterized using a SpectraWiz® spectroscopy device. Figure illustrates the representative spectra of the CAP jet used. The OES showed a mixture of peaks at various wavelengths at 297 nm oxygen (O_2), 308.9 nm hydroxyl radicals (OH), 311.24 nm nitric oxide (NO), 337.13 nm nitrogen (N_2), 357.69 nm N_2, 375.54 nm N_2, 380.49 nm N_2, 391.44 nm N_2+, 399.84 nm N_2, 405.94 nm N_2, 427.81 nm N_2+, 706.519 nm helium (He) (1s3s3S => 1s2p3Po), and 777.194 nm atomic oxygen (O) [6,7].

RESULTS AND DISCUSSION

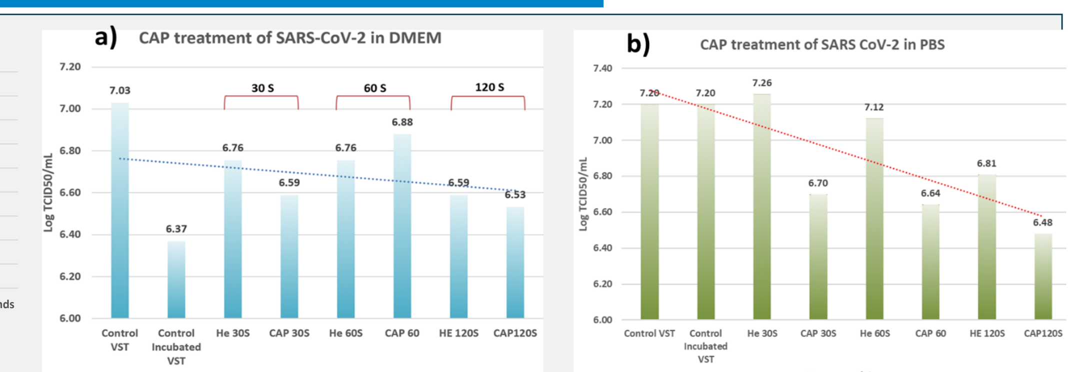


VC – Viability Control
VST – Virus Stock Titer
PRC – Plate Recovery Control

Challenge inoculum	LogTCID50	TCID50/mL	LogTCID50/ml	Log reduction against incubated virus	Log reduction against non-incubated virus
DMEM 30 S	4.67	2.34E+06	6.37	-0.83	-2.66
DMEM 60 S	5.23	8.49E+06	6.93	-0.27	-2.1
DMEM 120 S	4.5	1.58E+06	6.2	-1	-2.83

Challenge inoculum	LogTCID50	TCID50/mL	LogTCID50/ml	Log reduction against incubated virus	Log reduction against non-incubated virus
PBS 30 seconds	4	5.00E+05	5.7	-0.5	-2
PBS 60 seconds	3.67	2.34E+05	5.37	-0.83	-2.33
PBS 120 seconds	3.67	2.34E+05	5.37	-0.83	-2.33

Figure 8. Treatment of H1N1 virus by the application of CAP jet at 30, 60, and 120s in DMEM and PBS as solutions. Log of $TCID_{50}$ was determined in the tables. N = 9.



Condition	Sample Name	LogTCID50	TCID50/mL	LogTCID50/mL	Log reduction against Incubated Virus	Percent reduction
Control VST	VST DMEM	5.33	1.07E+07	7.03	NA	NA
Control Incubated VST	Incubated VST DMEM	4.67	2.34E+06	6.37	NA	NA
He 30S	DMEM 30 sec average	5.057	5.70E+06	6.756	0.387	-143.594
CAP 30S	DMEM 30 sec average	4.89	3.88E+06	6.589	0.22	-65.9587
He 60S	DMEM 1 min average	5.057	5.70E+06	6.756	0.387	-143.594
CAP 60	DMEM 1 min average	5.18	7.57E+06	6.879	0.51	-223.594
HE 120S	DMEM 2 min average	4.89	3.88E+06	6.589	0.22	-65.9587
CAP120S	DMEM 2 min average	4.833	3.41E+06	6.532	0.163	-45.6577

PBS	Sample Name	LogTCID50	TCID50/mL	LogTCID50/ml	Log reduction against Incubated Virus	Percent reduction
Control VST	VST PBS	5.5	1.58E+07	7.2	NA	NA
Control Incubated VST	Incubated VST PBS	5.5	1.58E+07	7.2	NA	NA
He 30S	PBS 30 sec average	5.56	1.80E+07	7.26	0.06	-13.94
CAP 30S	PBS 30 sec average	5	5.00E+06	6.7	-0.5	68.38
He 60S	PBS 1 min average	5.42	1.33E+07	7.12	-0.08	16.18
CAP 60	PBS 1 min average	4.94	4.39E+06	6.64	-0.56	72.25
HE 120S	PBS 2 min average	5.11	6.44E+06	6.81	-0.39	59.26
CAP120S	PBS 2 min average	4.78	3.01E+06	6.48	-0.72	80.95

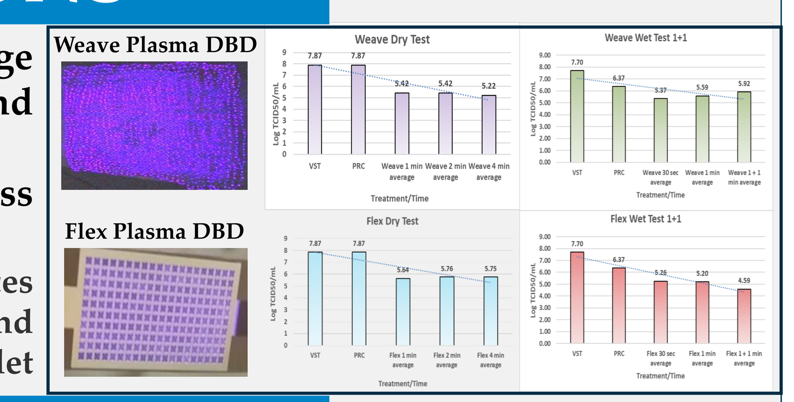
Figure 9. Graphical representation of SARS-CoV-2 virus inactivation experiments by the application of CAP jet using $TCID_{50}$ assay.

CONCLUSIONS

- CAP jet was characterized for the presence of reactive oxygen (ROS) and reactive nitrogen species (RNS).
- Optical Emission Spectroscopy showed various peaks of RONS.
- CAP application was successfully applied to the influenza (H1N1) virus and COVID-19-causing SARS-CoV-2.
- $TCID_{50}$/mL for both the viruses was calculated post-application with CAP and was found to be a significant ~2.83 $TCID_{50}$/mL (DMEM) and ~2.33 $TCID_{50}$/mL (PBS) log reduction for 120 seconds of CAP treatment in the case of the influenza virus and ~ 0.72 log reduction at 120 secs for SARS-CoV-2 in PBS.
- Our preliminary data suggests the application of CAP jet in the inactivation of the virus and potential decontamination of PPEs.

FUTURE DIRECTIONS

- We have tested two new plasma prototypes of dielectric barrier discharge (DBD) developed by Princeton Plasma Physics Lab PPPL named Weave and Flex plasma DBDs.
- The advantages of using these devices are that they are easy to use, take less space, are powered by batteries, and do not require gas supplies.
- We performed a dry and wet viral droplet test for SARS-CoV-2 using these devices and observed a significant ~2.65 and ~ 2.12 $TCID_{50}$/mL log reduction for Weave and Flex DBD in case of dry run and ~0.45 and ~ 1.78 $TCID_{50}$/mL for wet droplet experiments.



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