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PROJECT REPORT **17/01/22**

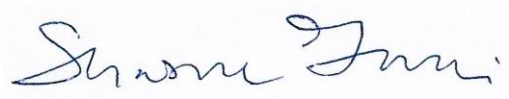
Client: Sharp Business Systems (India) Pvt. Ltd., New Delhi

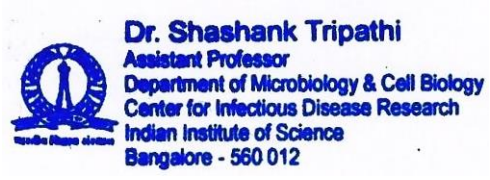
Purpose of study: Testing antiviral coating and disinfectant against SARS-CoV-2

Summary: In this project virucidal efficacy of NaCl electrolyte (HOCl) based disinfectant was tested against SARS-Cov-2 Delta variant in the BSL3 facility at CIDR, IISc. Results indicated reduced infectious virus count by ~99% after 1 minute of treatment with Naturizer solution (HOCl electrolyte).

Principal Investigator:

Dr. Shashank Tripathi





SHARP PROJECT

VIRUCIDAL ACTIVITY OF ELECTROLYSED SALT WATER

MATERIALS AND METHODS

1. Virus

SARS-CoV-2, (Isolate hCoV-19/USA/PHC658/2021, Lineage B.1.617.2, Delta variant) was propagated and titrated by plaque assay in Vero E6 cells.

2. Preparation of Electrolysed salt water (ESW)

The ESW was prepared by directly adding 3 grams of table salt (Make: Tata) followed by 300 mL of drinking water to the sanitizer maker; NATURIZER (SHARP; WJ-S30N-W). The maker was switched on (indicated by blue colour light and formation of bubbles) and allowed for 12 min or until the machine beeps.

3. Measurement of Chloride level

The chloride level of the freshly prepared ESW was tested using the chloride test strips (KYORITSU CHEMICAL-CHECK lab corp.). Here, the tip of the strip was dipped into the ESW solution for a few seconds. The change in strip colour was compared with the given colour chart and a level of chloride was noted.

4. Virucidal activity by plaque assay

The virucidal activity of ESW was measured by plaque assay twice in a BSL3 laboratory. Here, 12-well plates were seeded with $0.2x10^6$ VERO E6 cells/mL/well and incubated at 37ºC with 5% CO_2 for 48h to achieve 100 % confluency. A 100 µL of freshly prepared ESW (1x) or 10 % formaldehyde in PBS (Reference control) was mixed with 100 µL of SARS-CoV-2 virus suspension (2.6×10^7 PFU) in a 1.5 mL Eppendorf vial and incubated at 0, 1, 5 and 15 min, after which they were serially diluted by 10 fold up to 10^{-6} in ice-cold DMEM containing 2 % FBS. A 100 µL of each diluted sample was added to wells and incubated for 1h at 37ºC with intermittent shaking. Then, virus suspension was removed and 1 mL of 0.6 % avicel in DMEM containing 2 % FBS was added and incubated for 48h. After that, avicel was removed completely and fixed with 1mL of 4 % formaldehyde. After 1h of incubation, cells were stained with 1% crystal violet, removed after 5 min and washed with tap water to count the number of plaques obtained and

were represented as PFU/mL using the formula, Number of plaques per well / dilution tested x volume of diluted sample added.

5. Statistical analysis

The data were analyzed using GraphPad Prism v 8.4.3 and represented as mean ± SEM. Statistical variations were determined by one-way ANOVA with Tukey's multiple comparisons test. Values were significant when $*P < 0.05$, $**P < 0.01$ or $***P < 0.001$.

Results:

1. Virucidal assay

The plaque assay data showed the reduction in viral titer after 1 and 5 min post-ESW treatment by 2-3 log_{10} and ~4 log_{10} fold respectively. At 15 min exposure time, ESW reduced the virus below detectable limits, resulting in the reduction of viral titer by >7 log_{10}. However, at 0 min exposure time, virus titer didn't reduce significantly. The 10 % formaldehyde which was used as a reference control also reduced the viral titer by >7 log_{10} after 15 min of exposure time (Fig. 2 and Table 1).

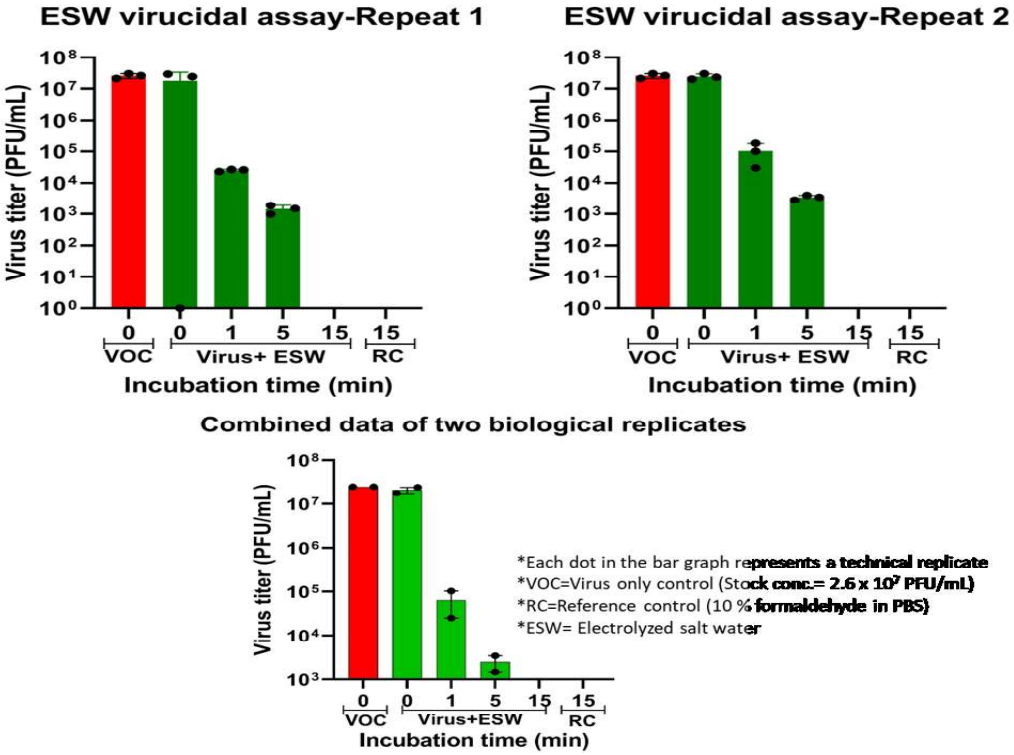


Figure 2: Virucidal activity of ESW by plaque assay. Equal volume of freshly prepared ESW (1x) or 10 % Formaldehyde was mixed with SARS-CoV-2 virus suspension (Delta strain=$2.6x10^7$ PFU/mL) and incubated at 0, 1, 5 and 15 min, after which they were serially diluted (10 fold) in ice-cold DMEM. The 10 % formaldehyde was used as a reference control. The diluted samples were used for plaque assay on VERO-E6 cells.

Table 1: Log and percent reduction of SARS-CoV-2 as observed by plaque assay after exposure to ESW at different time points (0, 1,5, 15 min). The data from experimental repeat-1, repeat-2 and their combined data are shown

REPEAT-1

SL No.	Test organism with exposure time	Average viral titer (PFU/mL)	Log_{10} reduction	Percent reduction (%)
1	SARS-CoV-2 Delta strain (Control)	$2.5x10^7$	-	-
2	SARS-CoV-2 Delta strain (0 min)	$1.76x10^7$	0	**29.60**
3	SARS-CoV-2 Delta strain (1 min)	$2.53x10^4$	~3	**99.89**
4	SARS-CoV-2 Delta strain (5 min)	$1.46x10^3$	~4	**99.99**
5	SARS-CoV-2 Delta strain (15 min)	0	>7	**100**

REPEAT-2

SL No.	Test organism with exposure time	Average viral titer (PFU/mL)	Log_{10} reduction	Percent reduction (%)
1	SARS-CoV-2 Delta strain (Control)	$2.5x10^7$	-	-
2	SARS-CoV-2 Delta strain (0 min)	$2.43x10^7$	0	**2.8**
3	SARS-CoV-2 Delta strain (1 min)	$1.03x10^5$	~3	**99.58**
4	SARS-CoV-2 Delta strain (5 min)	$3.43x10^3$	~4	**99.98**
5	SARS-CoV-2 Delta strain (15 min)	0	>7	**100**

Combined repeats

SL No.	Test organism with exposure time	Average viral titer (PFU/mL)	Log_{10} reduction	Percent reduction (%)
1	SARS-CoV-2 Delta strain (Control)	$2.5x10^7$	-	-
2	SARS-CoV-2 Delta strain (0 min)	$2.09x10^7$	0	**16.4**
3	SARS-CoV-2 Delta strain (1 min)	$6.4x10^4$	~2.5	**99.74**
4	SARS-CoV-2 Delta strain (5 min)	$2.4x10^3$	~4	**99.99**
5	SARS-CoV-2 Delta strain (15 min)	0	>7	**100**