## Virucidal Action of Strongly Acidic Hypochlorous Acid Water (EO-005 Generator) against Novel Coronavirus (COVID-19)

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Test Source: Samples of novel coronavirus (COVID-19) from Diamond Princess cruise ship

Figure 1 Strongly acidic hypochlorous acid water (EO-005 generator) (H) showed virucidal action against the novel coronavirus.

Solution adjusted to pH2.4 with HCl (HCl), and solution of which only ORP was changed by adding sodium thiosulfate to strongly acidic hypochlorous acid water (EO-005 generator) with pH2.4 and ORP 1,150 mv, with hypochlorous acid concentration of 20 ppm (H+T) did not show virucidal action against novel coronavirus, showing that the virucidal action does not depend on the pH at all.

When ammonia is added to strongly acidic hypochlorous acid water (EO-005 generator) (H+A), the pH increases and the ORP decreases, but the concentration of residual chlorine does not decrease. Although the viruses decreased, they were not completely destroyed. It was suggested that this effect is attributed to the residual chlorine.

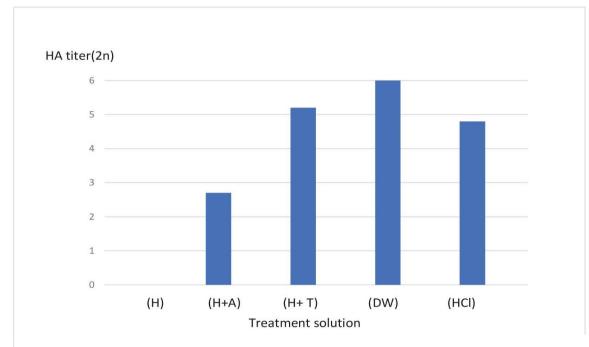


Figure 1 Comparison between strongly acidic hypochlorous acid water and other solutions used to kill the novel coronavirus (COVID-19)

### Figure 1/Table 1

An aqueous solution containing the new coronavirus (COVID-19) strain, which was collected in the Diamond Princess (state room), was mixed with strongly acidic hypochlorous acid water (EO-005 generator) (H), strongly acidic hypochlorous acid water spiked with ammonia (H+A), strongly acidic hypochlorous acid water spiked with sodium thiosulfate (H+T), distilled water (DW), and distilled water adjusted to pH 2.4 (HCl), respectively, at a ratio of 1:9 for 10 seconds each.

The respective solutions containing the virus were then diluted 10-fold with HEM, cultured on plates, and the virus titer in the culture medium was analyzed by the HA method (hazard aggregation analysis method) after 72 hours. The results are shown in Figure 1. The description of the mean value of each solution is shown in Table 1.

Table 1 pH, ORP and residual chlorine concentration in strongly acidic hypochlorous acid water (EO-005 generator) and other solutions

Treatment solution	рН	ORP (mv)	Residual chlorine concentration (ppm)
Strongly acidic hypochlorous acid water	2.40	1150	20.00
Ammonia-spiked strongly acidic water*	8.00	310	19.00
Sodium thiosulfate-spiked strongly acidic water	2.40	345	0.00
Distilled water*	5.60	450	0.00
HCl • Distilled water	2.40	700	0.05

\* Strongly acidic hypochlorous acid water (EO-005 generator) spiked with 1% ammonia at a ratio of 100:1

\* Strongly acidic hypochlorous acid water (EO-005 generator) spiked with 5% sodium thiosulfate at a ratio of 100:1

#### Method

- ① Incubate samples with novel coronavirus (COVID-19) which had been frozen (-80°C) with liquid nitrogen in a culture medium that has been warmed to 37°C for 24 hours
- (2) Add 1,000  $\mu$ L each of tap water and the concerned other solution to 100  $\mu$ L of a novel coronavirus (COVID-19) solution with a concentration  $3.0 \times 10$
- $\bigcirc$  Leave the control solution at room temperature for one minute
- (4) Collect 100  $\mu$ L of the virus solution in (3), and immediately add 900  $\mu$ L of each solution to the MEM medium to culture the solution
- ⑤ Inoculate 200 μL of the solution to novel coronavirus (COVID-19) cells that have been cultivated to confluent on a 24well plate
- 6 Incubate the medium at 37°C for 90 minutes
- 1 Remove 200  $\mu L$  of the inoculated solution
- (8) Collect the supernatant of the culture after 72 hours of incubation at 37°C, and check the virus growth by HA assay (hazard aggregation analytical method)

#### Abstract

It has been found that strongly acidic hypochlorous acid water generated by special electrolysis (EO-005 generator) of saline and tap water has bactericidal and virucidal actions against HIV, hepatitis B, influenza viruses and MRSA. It has also been shown to have a virucidal action against the novel coronavirus (COVID-19) the cause of the pandemic that recently started in China and is spreading worldwide.

This time, the group of Dr. Hiroyuki Yoshida of the International University Organization for Nontraditional Distance Learning Supervision collected samples of novel coronavirus (COVID-19) on the Diamond Princess cruise ship (private rooms) on high alert, and confirmed the virucidal action of each solution. Since the strongly acidic hypochlorous acid water (EO-005 generator) exerts a direct action on part of the viruses/receptors and destroy them by oxidative action, it is expected to destroy all types of virus.

Since the novel coronavirus (COVID-19) is spread through droplet infection in the air (aerosol), it is effective to spray the solution in the air with a sprayer.

# Accordingly, strongly acidic hypochlorous acid water with virucidal action is effective to disinfect the inside of the Diamond Princess cruise ship.

The solution that destroys the novel coronavirus (COVID-19) with strongly acidic hypochlorous acid water (EO-005 generator) has pH of 2.4, oxygen reduction potential (ORP) of 1150 mv and hypochlorous acid concentration of 20 ppm, and it was found to kill the novel coronavirus (COVID-19) at a concentration of 3 million per cc within 10 seconds.

## Diamond Princss Outboard photo









 $\ensuremath{\text{Diamond}}\xspace$  Princss Image at the time of entry



