

## Evaluation of Hypochlorous Acid Water on the Inactivation of SARS-CoV-2

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### INTRODUCTION

It is highly unpredictable to see the end of the COVID-19 pandemic. As of 10 April 2021, about 133.5 million cases with 2.9 million deaths were reported to WHO from 223 countries and territories, with a case fatality rate (CFR) of about 2.2%. In Japan, 496,206 cases have been reported throughout the country with 9,394 deaths among them (CFR; 1.9%). After some improvement, Japan seems to be facing a fourth wave of the infections.

To effectively cope with this pandemic, it is important for each of us to enforce thorough handwashing and disinfection of contaminated materials, both of which are fundamental for personal hygiene. Handwashing is particularly believed to be a very effective self-protective strategy regardless of the availability of a preventive vaccine or efficacious therapies [1]. Therefore, we carried out the experiment to evaluate the potential of hypochlorous acid water (HAW) to inactivate SARS-CoV-2, in light of global shortage of disinfectants such as alcohol- and detergent-based disinfectants.

### METHODS

The strong acid HAW (< pH2.7, effective chlorine concentration = 40-50 ppm) was provided by Enagic International Co. LTD., (Okinawa). The HAW and distilled water samples were mixed with SARS-CoV-2 solution (JPN/TY/WK-521 strain) at 9 (water):1 (virus) volume ratio. The mixtures were incubated for 0.5, 1.0, 5.0, or 10.0 minutes at room temperature (23.5 or 24.0°C). At each time point, chlorine was neutralized by adding 0.012M sodium thiosulfate solution (1/10 volume) and each sample was subsequently supplemented with 10 × MEM (1/10 volume), fetal calf serum (1/50 volume), and appropriate quantities of sodium bicarbonate. Ten-fold serial dilutions (DMEM containing 2% FCS) of each sample were inoculated to TMRSS2-expressing Vero E6 cells on 96-well plates (4 wells for each dilution). A few days later, cytopathic effects were observed and virus titers (TCID<sub>50</sub>/ml) were calculated.

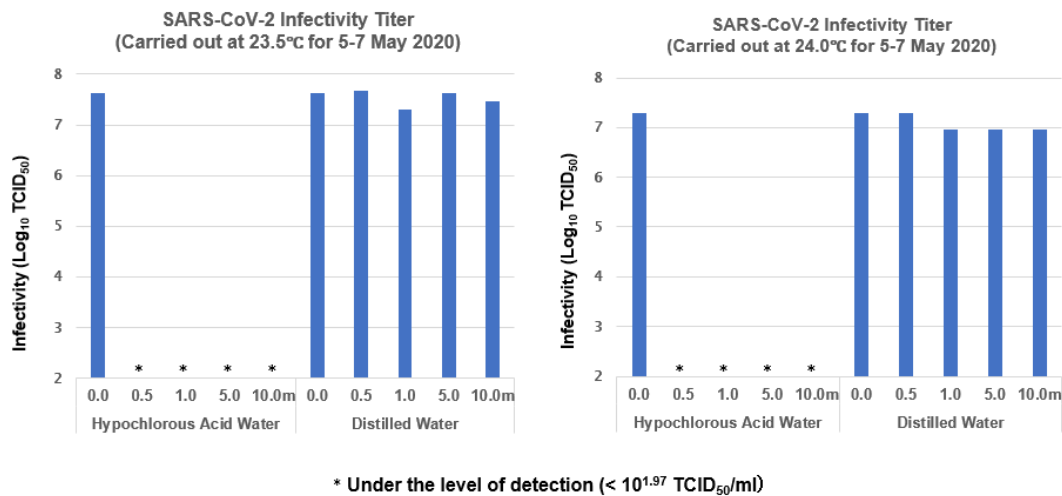
In addition, the weak acid HAW (pH5.5, effective chlorine concentration= 40 ppm) was supplied from the Japan EcoSystems Co., (Tokyo). The HAW and distilled water samples were mixed with SARS-CoV-2 solution at 19 (water):1 (virus) volume ratio. The rest procedures were the same as the above strong acid HAW.

### RESULTS

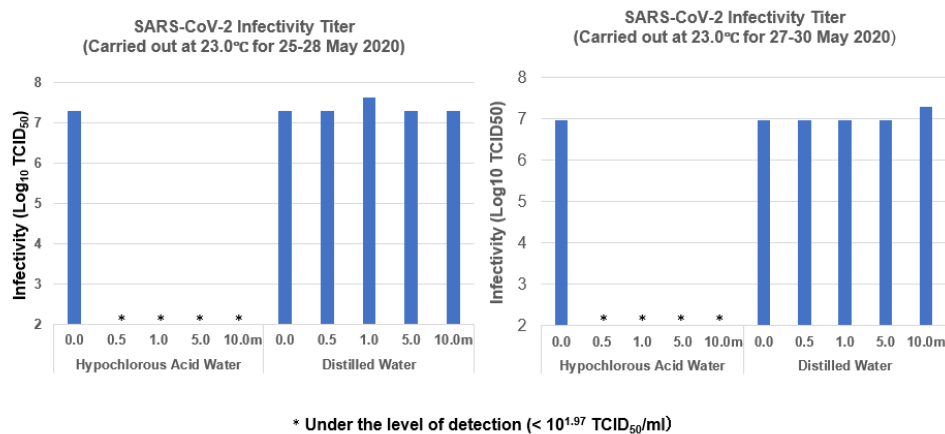
Two independent experiments using the strong acid HAW performed at 23.5<sup>0</sup>C and 24.0<sup>0</sup>C gave similar results at 9:1 volume ratio (Figure 1). The similar results were also obtained from the second experiments using the weak acid HAW at 19.1 volume ratio (Figure 2).

The virus infectious titers of untreated original virus stock solution (i.e., 0 min.) were more than  $10^{7.0}$  TCID<sub>50</sub>/ml in both experiments. As expected, the virus was stable in distilled water and virus titers were constant (more than  $10^{7.0}$  TCID<sub>50</sub>/ml) during the experimental period (-10min.). In contrast, HAW treatment remarkably reduced

infectious virus titers to undetectable levels. It was noteworthy that infectious virus particles were not detected even at 30 seconds of reaction time, indicating that HAW treatment of SARS-CoV-2 instantaneously inactivated the virus under these conditions.



**Figure 1:** Inactivation of SARS-CoV-2 by hypochlorous acid water (HAW, pH<2.7, effective chlorine concentration=40-50 ppm). HAW and distilled water samples were mixed with SARS-CoV-2 at 9 (water): 1 (virus) volume ratio. The mixtures were incubated for 0.5, 1.0, 5.0, or 10.0 minutes at room temperature (23.5 or 24.0°C). Virus titers were calculated as TCID<sub>50</sub>/ml.



**Figure 2:** Inactivation of SARS-CoV-2 by hypochlorous acid water (HAW, pH5.5, effective chlorine concentration=40 ppm). HAW and distilled water samples were mixed with SARS-CoV-2 at 19 (water): 1 (virus) volume ratio. The rest procedures were the same as those in Figure 1.

## CONCLUSIONS

This study has convincingly proved that HAW is highly potent in inactivating SARS-CoV-2 under the conditions of this study. So far, HAW has been approved by the Ministry of Health, Labor and Welfare of the Japanese Government for general food additives and disinfection of door knobs, switches, tables, chairs, floors and walls [2] but its use for hand hygiene has only been suggested. Since HAW is easily accessible, affordable, effective, and safe disinfectant, our data indicate that HAW could be widely used for SARS-CoV-2 inactivation, including handwashing and solid materials as well as potential environmental sprays. It has also been

documented that HAW is very stable for long periods of time provided with light-blocking and sealed conditions [3]. We expect that the effective use of HAW with proper guidelines contributes to the prevention and control of COVID-19 throughout the world.

## References

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