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Virucidal efficacy of hypochlorous acid water for aqueous phase and atomization against SARS-CoV-2

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ABSTRACT

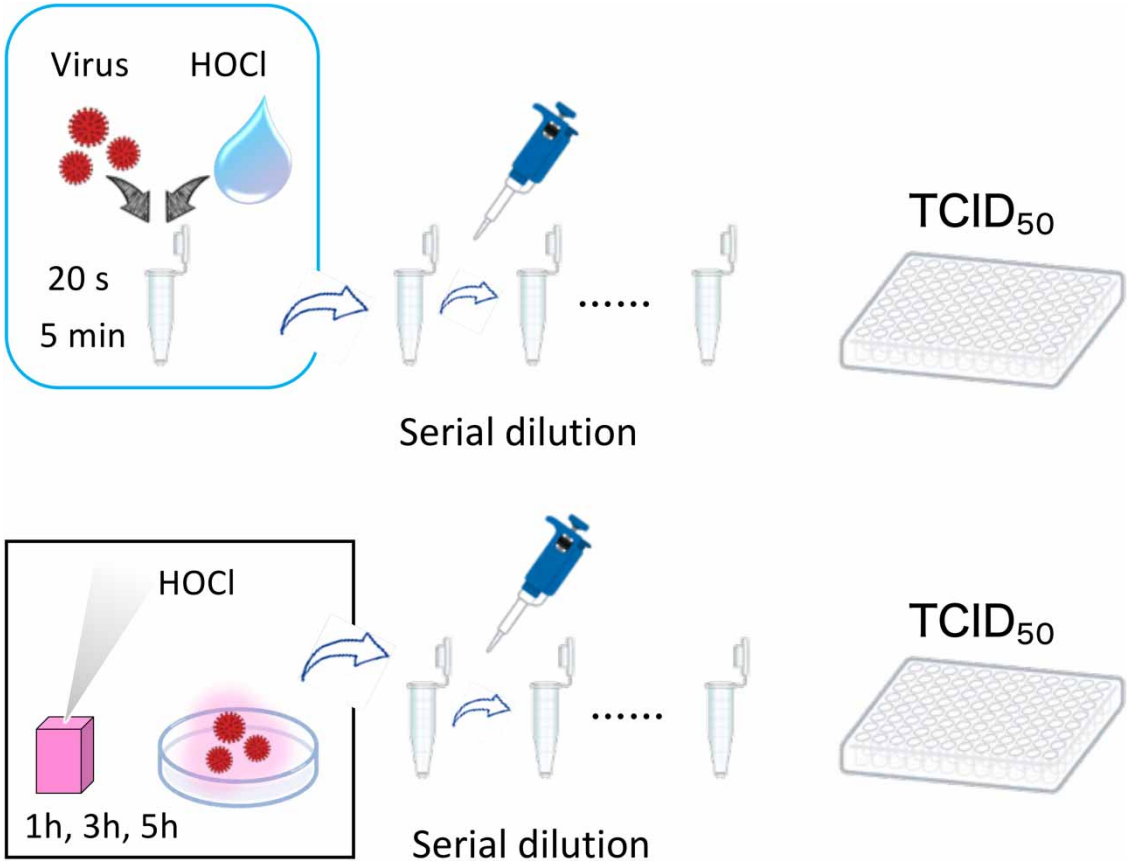
Coronavirus disease 2019 (COVID-19) is an infectious viral disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that emerged at the end of 2019. SARS-CoV-2 can be transmitted through droplets, aerosols, and fomites. Disinfectants such as alcohol, quaternary ammonium salts, and chlorine-releasing agents, including hypochlorous acid, are used to prevent the spread of SARS-CoV-2 infection. In the present study, we investigated the efficacy of ionless hypochlorous acid water (HOCl) in suspension and by spraying to inactivate SARS-CoV-2. The virucidal efficacy of HOCl solution in tests against SARS-CoV-2 was evaluated as 50% tissue culture infectious dose. Although the presence of organic compounds influenced the virucidal efficacy, HOCl treatment for 20 s was significantly effective to inactivate Wuhan and Delta strains in the suspension test. HOCl atomization for several hours significantly reduced the SARS-CoV-2 attached to plastic plates. These results indicate that HOCl solution with elimination containing NaCl and other ions may have high virucidal efficacy against SARS-CoV-2. This study provides important information about the virucidal efficacy and use of HOCl solution.

Key words: atomization, coronavirus disease 2019 (COVID-19), hypochlorous acid water (HOCl), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), suspension test

HIGHLIGHTS

- The virucidal effect of ionless hypochlorous acid water (HOCl) was unaffected by SARS-CoV-2 variants.
- The anti-SARS-CoV-2 effect of HOCl was attenuated by organic compounds in the virus solution.
- The anti-SARS-CoV-2 effect of HOCl was confirmed by HOCl atomization.
- Use of HOCl atomization against anti-SARS-CoV-2 effect can be expected to reduce the burden of cleaning in hospital rooms, daycare centers, schools, etc.

GRAPHICAL ABSTRACT



ABBREVIATIONS

ACC	available chlorine concentration
COVID-19	coronavirus disease 2019
HOCl	hypochlorous acid water
MPO	myeloperoxidase
ROS	reactive oxygen species
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
$TCID_{50}$	50% tissue culture infectious dose

INTRODUCTION

The new pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative virus of coronavirus disease 2019 (COVID-19), which emerged at the end of 2019 and has since spread rapidly worldwide (Zhu *et al.* 2020). The clinical symptoms of COVID-19 vary widely from asymptomatic to severe, and it can be fatal (Chen *et al.* 2020; Guan *et al.* 2020). As of 28 August 2023, the World Health Organization (WHO) confirmed 770 million cases and >6.9 million deaths (WHO 2023).

SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus, which can spread through asymptomatic and symptomatic individuals through ejected respiratory droplets and aerosols when breathing, coughing, sneezing, and speaking under insufficient ventilation (Hu *et al.* 2021; Leung 2021). The virus can survive from a few hours to several days on various material surfaces, such as glass, plastic, natural rubber, and metal (Riddell *et al.* 2020; van Doremalen *et al.* 2020; Hirose *et al.* 2021; Wiktorczyk-Kapischke *et al.* 2021). Therefore, the droplet infection and fomite transmission are feasible routes of infection. Consequently, to prevent human-to-human viral transmission, it is important to inactivate SARS-CoV-2 in the form of

released droplets or on the surface of fomites. In addition, handwashing and hand disinfection are also important to prevent SARS-CoV-2 infection.

For this purpose, the use of chemical disinfectants and sanitizers can reduce the high risk of pathogen transmission. We have explored or developed various disinfectants and sanitizers to prevent infectious diseases. The main components used in disinfectants and sanitizers contain alcohol, quaternary ammonium salts, and amphoteric surfactants (McDonnell & Russell 1999; Tischer *et al.* 2012; Gerba 2015; Tsujimura *et al.* 2015; Falk 2019; Xiao *et al.* 2022). At the beginning of the COVID-19 pandemic, the demand for alcohol disinfectants increased. Therefore, alternative disinfectants were evaluated in Japan. Ionless hypochlorous acid water (HOCl) is one of the several reactive oxygen species produced by phagocytes, such as macrophages and neutrophils, playing a key role in disinfection of bacteria, fungi, protozoa, and viruses (Aratani 2018). HOCl is a strong oxidizing agent with antipathogen efficacy. HOCl has rapid and broad-spectrum antimicrobial efficacy against clinically relevant microorganisms *in vitro* and *in vivo* (Wang *et al.* 2007). HOCl was reportedly superior to hypochlorite or hydrogen peroxide in terms of therapeutic index, which is an indicator of the safety margin of a disinfectant (Wang *et al.* 2007). HOCl can inactivate Gram-negative and positive bacteria, fungi, and viruses (WHO 2021). HOCl exhibits high virucidal efficacy by interacting with viral structural proteins (capsid or surface compound), lipid envelope, and DNA/RNA (Block & Rowan 2020; Hawkins & Davies 2021; Qiao *et al.* 2022). HOCl can react better with thiol-containing compounds, such as cysteine, methionine, and glutathione, than other cellular components and therefore induces protein unfolding and aggregation (Gray *et al.* 2013; da Cruz Nizer *et al.* 2020). Hawkins & Davies (2002) reported that HOCl-induced DNA damage. HOCl reacts with unsaturated fatty acids and cholesterol and induces a change in membrane properties (Spickett *et al.* 2000; da Cruz Nizer *et al.* 2020). Previous studies have reported the viricidal efficacy of HOCl solution against influenza virus, herpes simplex virus, human immunodeficiency virus 1, and coronavirus in the aqueous phase (Tachikawa *et al.* 1999; Miyaoka *et al.* 2021). According to Hatanaka *et al.* (2022) and Takeda *et al.* (2021), HOCl solution containing 28 and 51–56 ppm of chlorine concentration is efficient against SARS-CoV-2. A study reported that >35 ppm of HOCl is efficient against SARS-CoV-2 infection (NITE: National Institute of Technology and Evaluation 2022). In the study, each experimental condition by five facilities was different, for example, the producing method of HOCl solution (electrolyzed or not electrolyzed), the concentration of fetal bovine serum (FBS; 1–5%) in the virus solution, and reaction rates (1:9 or 1:19) with HOCl and virus solution.

Atomization with HOCl reduces bacteria on surfaces *in vitro* and in the hospital room (Hakim *et al.* 2016; Miyazaki *et al.* 2022). The virucidal efficacy of HOCl atomization was reported against norovirus, avian influenza virus, and SARS-CoV-2 on surfaces (Park *et al.* 2007; Hakim *et al.* 2015; Urushidani *et al.* 2022). Details of the appropriate use of HOCl against SARS-CoV-2 in the aqueous phase and under atomization are unclear.

In the present study, we investigated whether HOCl treatment would influence the infectivity of SARS-CoV-2 to susceptible cells in the presence of the different concentrations of organic compounds and variants. We also explored whether HOCl atomization would provide effective disinfection to prevent SARS-CoV-2 infection.

MATERIALS AND METHODS

Cell culture

VeroE6 cells expressing the transmembrane serine protease TMPRSS2 (VeroE6/TMPRSS2: JCRB1819) were purchased from the National Institutes of Biomedical Innovation, Health and Nutrition (JCRB Cell Bank, Osaka, Japan). VeroE6/TMPRSS2 cells were cultured in Dulbecco's modified Eagle's medium (D-MEM) GlutaMAX (Thermo Fisher Scientific, Tokyo, Japan) containing 5% heat-inactivated FBS (Vitromex, Vilshofen, Germany), 100 units/mL penicillin, and 100 mg/mL streptomycin (Nacalai Tesque, Inc., Kyoto, Japan), and 1 mg/mL G-418 solution (Roche Diagnostics GmbH, Mannheim, Germany) under biosafety level 2.

Virus

The Pango lineage A virus strain that was prevalent in the early stages of the COVID-19 pandemic (JPN/TY/WK-521) was provided by the National Institute of Infectious Diseases. To compare more pathogenic and transmissible variants of concern with Pango lineage A virus, the Pango lineage B.1.617.2 virus strain (KUH355) was isolated clinically and provided by the Kitasato University Hospital. Both viruses were used to infect VeroE6/TMPRSS2 cells for virus expansion, and virus-infected cells were cultured in D-MEM containing 2% FBS. After 48 h of incubation, each virus-infected cell culture supernatant was collected and centrifuged at 3,000 rpm for 10 min at room temperature (23 °C) to eliminate cells and debris. Culture

supernatants were divided and stocked at −80 °C until use. All viruses were treated in the biological safety cabinet class II type A2 (LAL-1500XPA2 + : Oriental Giken Inc., Tokyo, Japan) of biosafety level 3 (BSL3).

Reagents

HOCl was provided by NIPRO Corporation (Osaka, Japan). HOCl was produced by the electrolysis of the saturated sodium chloride (NaCl) solution. The products were purified by reverse osmosis filtration to remove ions, such as Na^{+}. The conductivity and pH of 500 ppm HOCl used in this study were 280.1 mS/cm and 6.24, respectively.

Suspension test

The JPN/TY/WK-521 (2×10^7 pfu/mL) and KUH355 (1.5×10^7 pfu/mL) strains were mixed with 1–200 ppm of HOCl or water for injection (Otuska Pharmaceutical Co., Ltd, Tokyo, Japan) at a ratio of 1:9 and 1:99 for 20 s and 5 min, respectively. Three viral solutions were treated with the same HOCl concentration or water for injection. After treatment, the reaction was stopped by adding 10% sodium thiosulfate (FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan) to the virus solution and HOCl mixture. Ten-fold serial dilutions of these solutions were then prepared using D-MEM. Each diluted mixture was then added to VeroE6/TMPRSS2 cells. After 3 days of incubation, culture supernatants were eliminated. Then, VeroE6/TMPRSS2 cells were fixed with the cold methanol (Nacalai Tesque) and stained with the 0.5% methylene blue (FUJIFILM Wako Pure Chemical Corporation) solution for calculation of the tissue culture infectious dose 50 ($TCID_{50}$) using the Behrens–Karber method (Behrens & Karber 1953). Live cells were stained blue and macroscopically observed, whereas dead cells from SARS-CoV-2 infection were not stained:

$$TCID_{50} = (\text{lowest dilution at which cytopathic effect was observed}) \times 10^{\sum - 0.5}$$

$$\sum = (\text{number of wells with observed cytopathic effect})/(\text{number of specimens})$$

The average value of $TCID_{50}$ was used as the viral infectivity. All experiments were performed in the biological safety cabinet of BSL3.

Spray test

The test chamber used for the spray tests was placed into the biological safety cabinet in BSL3. KUH355 (1.5×10^7 pfu/mL) virus solutions diluted 10- and 100-fold with double distilled water (ddw) were placed in plastic plates and placed on a wet towel in the test chamber (Figure 3). 30–500 ppm and 10–300 ppm of HOCl or distilled water were sprayed for 5 h using two ultrasonic atomizers. Each of the three plastic plates was treated with HOCl or distilled water at the same time. Plastic plates were collected at 1, 3, and 5 h, and 0.95 mL of recovery liquid (D-MEM) was added to the plastic plates, and the virus was suspended. Ten-fold serial dilutions of the virus solutions were prepared using D-MEM. Each diluted mixture was added into VeroE6/TMPRSS2 cells. After 3 days of incubation, the culture supernatants were eliminated. Then, VeroE6/TMPRSS2 cells were fixed with cold methanol and stained with 0.5% of methylene blue solution for the calculation of $TCID_{50}$ by the Behrens–Karber method. All experiments were performed in the biological safety cabinet of BSL3.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 8.2 (GraphPad Software, La Jolla, CA). Statistical significance was analyzed using one-way analysis of variance (ANOVA) with the Bonferroni post hoc test. Differences were considered statistically significant at $P < 0.05$.

Ethical approval statement

All infectious experiments were reviewed and approved by the Biosafety Committee of Kitasato University (approval numbers: A06-116 and 199) and the Kitasato University Medical Ethics Organization (approval number: COVID-19-CT001).

RESULTS

Evaluation of HOCl virucidal efficacy against SARS-CoV-2 by suspension test

Virucidal efficacies of HOCl against SARS-CoV-2 are shown in Figure 1. Virus stocks containing 2% FBS were mixed with 10, 30, 50, 100, and 200 ppm HOCl solution for 20 s and 5 min at room temperature at a ratio of 1: 9. In the presence of 0.2%

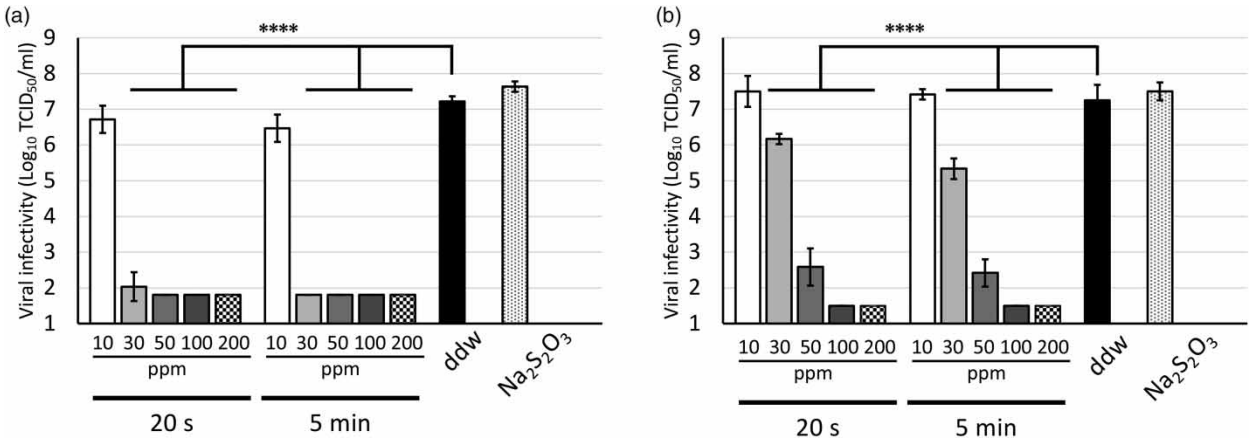


Figure 1 | Evaluation of HOCl virucidal efficacy against SARS-CoV-2. SARS-CoV-2 virus solution was treated with various concentrations of ionless hypochlorous acid water, as described in Materials and Methods. (a) Pango lineage A virus (JPN/TY/WK-521) strain treated with 10 (white bar), 30 (light gray bar), 50 (gray bar), 100 (dark gray bar), and 200 (black dot bar) ppm of HOCl solution. (b) Pango lineage B.1.617.2 virus (KUH355) strain treated with 10 (white bar), 30 (light gray bar), 50 (gray bar), 100 (dark gray bar), and 200 (black dot bar) ppm of HOCl solution. The ddw (black bar) indicates that the virus solution was treated with water for injection. $Na_2S_2O_3$ (gray dot bar) indicates that 200 ppm HOCl solution pretreated with 10% $Na_2S_2O_3$ solution was mixed with the SARS-CoV-2 virus solution. Data were analyzed using one-way ANOVA with the Bonferroni post hoc test. **** $p < 0.0001$.

FBS, the HOCl solution with the concentration not less than 30 ppm was significantly effective against the Pango lineage A virus strain (JPN/TY/WK-521), resulting in a 5.2–5.4 $\log_{10}$ decrease. In contrast, the 10 ppm HOCl solution had no anti-SARS-CoV-2 effect (Figure 1(a)). Using the Pango lineage B.1.617.2 virus strain (KUH355), the HOCl solution with the concentration not less than 30 ppm was significantly effective against SARS-CoV-2, resulting in a 1.3–5.8 $\log_{10}$ decrease. In contrast, the HOCl solution at 10 ppm did not exert any SARS-CoV-2 virucidal efficacy (Figure 1(b)). The cytopathic effects of 200 ppm HOCl and $Na_2S_2O_3$ stop solution on VeroE6/TMPRSS2 cells were not observed (Supplementary Figure 1).

Virucidal efficacies of HOCl against SARS-CoV-2 in the presence of 0.02% FBS are shown in Figure 2. In the presence of a 0.02% FBS, the HOCl solution with concentration not less than 10 ppm was significantly effective against both the JPN/TY/WK-521 and KUH355 strains of SARS-CoV-2, resulting in a 5.3 and 6.7 $\log_{10}$ decrease, respectively. In contrast, 1 ppm HOCl

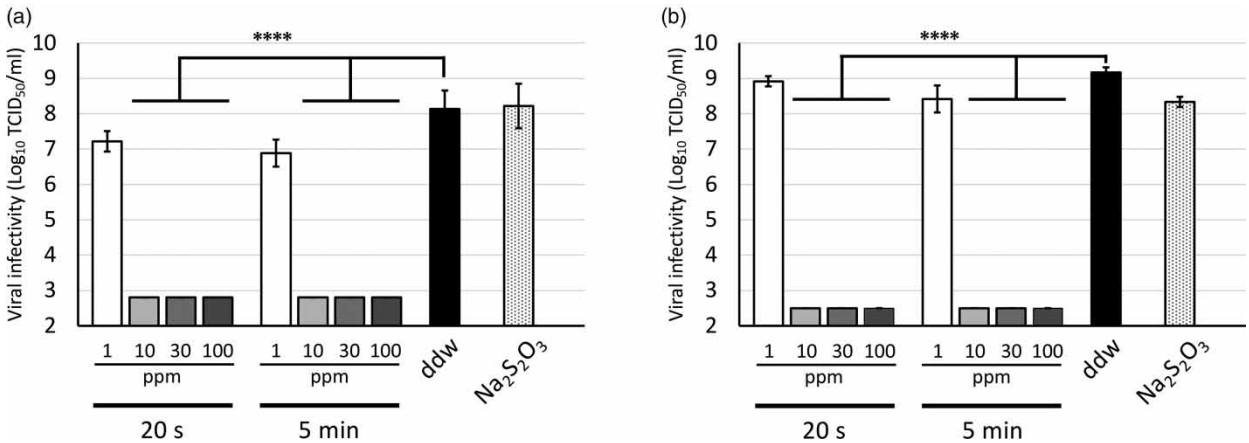


Figure 2 | Influence of organic compounds on the SARS-CoV-2 virucidal efficacy of HOCl. SARS-CoV-2 virus solution was treated with various concentration of ionless hypochlorous acid water, as described in Materials and Methods. (a) JPN/TY/WK-521 strain treated with 1 (white bar), 30 (light gray bar), 50 (gray bar), and 100 (dark gray bar) ppm of HOCl solution. (b) KUH355 strain treated with 1 (white bar), 30 (light gray bar), 50 (gray bar), and 100 (dark gray bar) ppm of HOCl solution. The ddw (black bar) indicates that the viral solution was treated with water for injection. $Na_2S_2O_3$ (gray dot bar) indicates that 200 ppm HOCl solution pretreated by 10% $Na_2S_2O_3$ solution was pretreated with SARS-CoV-2 virus solution. Data were analyzed using one-way ANOVA with the Bonferroni post hoc test. **** $p < 0.0001$.

solution had no virucidal effect on SARS-CoV-2 (Figure 2). The virucidal efficacy of HOCl in the presence of 0.02% FBS was 10 times greater than that in the presence of 0.2% FBS; 200 ppm HOCl pretreated with 10% $Na_2S_2O_3$ did not observe the virucidal efficacy against SARS-CoV-2 in the presence of 0.2 and 0.02% FBS (Figures 1 and 2).

Evaluation of virucidal efficacy of HOCl atomization against SARS-CoV-2

Although the virucidal efficacy of HOCl was dependent on the presence of organic compounds, the suspension test indicated sufficient SARS-CoV-2 virucidal efficacy. To investigate the SARS-CoV-2 virucidal efficacy of atomized HOCl, we conducted the spray test in the test chamber (Figure 3). When sprayed with 500 ppm HOCl, no clear change was observed in the virus titer of the KUH355 strain solution containing 0.2% FBS after spraying for 1 h, but it was significantly suppressed after spraying for 3 and 5 h, by 2.3 $\log_{10}$ and 5.7 $\log_{10}$, respectively (Figure 4(a)). Next, the KUH355 strain solution containing 0.02% FBS was atomized by 10, 30, and 300 ppm HOCl solution for 1, 3, and 5 h. When sprayed with 300 ppm HOCl, the virus titer of KUH355 strain solution containing 0.02% FBS was significantly suppressed after spraying for 1, 3, and 5 h, by 5.1 $\log_{10}$, 4.2 $\log_{10}$, and 4.1 $\log_{10}$, respectively (Figure 4(b)). In the presence of 0.02% FBS, the atomized HOCl solution at 30 ppm was significantly effective for 3 and 5 h, resulting in a 2.8 and 4.1 $\log_{10}$ decrease, respectively (Figure 4(b)), suggesting that HOCl atomization showed dose- and time-dependent effectiveness despite the presence of organic compounds. The virus titer of the control group showed a gradual decrease in a time-dependent manner for KUH355 strain (Figure 4(a) and 4(b)). We also continuously monitored the change in relative humidity and atmospheric available chlorine concentration (ACC) in the test chamber. When 10, 30, 300, and 500 ppm HOCl was atomized in the same way as for the spray test, ACC remained constant as relative humidity of about 90% was maintained in the chamber during the test. ACC at 5 h after spraying with 10, 30, 300, and 500 ppm HOCl showed 0.01 ± 0.008, 0.051 ± 0.012, 0.175 ± 0.050, and 0.311 ± 0.029 ppm, respectively (Supplementary Figure 2).

DISCUSSION

This study was performed to determine whether HOCl, produced by electrolysis of saturated chloride solution and filtrated through a reverse osmosis membrane to remove ions, had antiseptic efficacy against SARS-CoV-2 infectivity in the presence of organic compounds and under conditions of atomization. In the suspension test, we demonstrated a reduction of SARS-CoV-2 infectivity by contact with the HOCl solution for 20 s and 5 min. We found that the presence of organic compounds affected SARS-CoV-2 infectivity depending on the level of the ACC in the HOCl solution. We also observed that HOCl atomization significantly reduced SARS-CoV-2 infectivity in the spray test. The presence of organic compounds, including FBS, greatly affected the virucidal efficacy against SARS-CoV-2 in the suspension test and atomization test.

HOCl, a reactive chlorine species similar to ClO^- and Cl_2, is a high-potency oxidizing agent (Deborde & von Gunten 2008). In the innate immune system, HOCl is generated from hydrogen peroxide and chloride ions by myeloperoxidase (MPO) in phagocytes (Pattison *et al.* 2012; Winterbourn & Kettle 2013). Then, neutrophils and monocytes utilize HOCl to eliminate invading microorganisms (Gray *et al.* 2013; Da Cruz Nizer *et al.* 2020). MPO-knockout mice physiologically demonstrate increased susceptibility to infection with *Candida albicans* and *Klebsiella pneumoniae* in comparison with

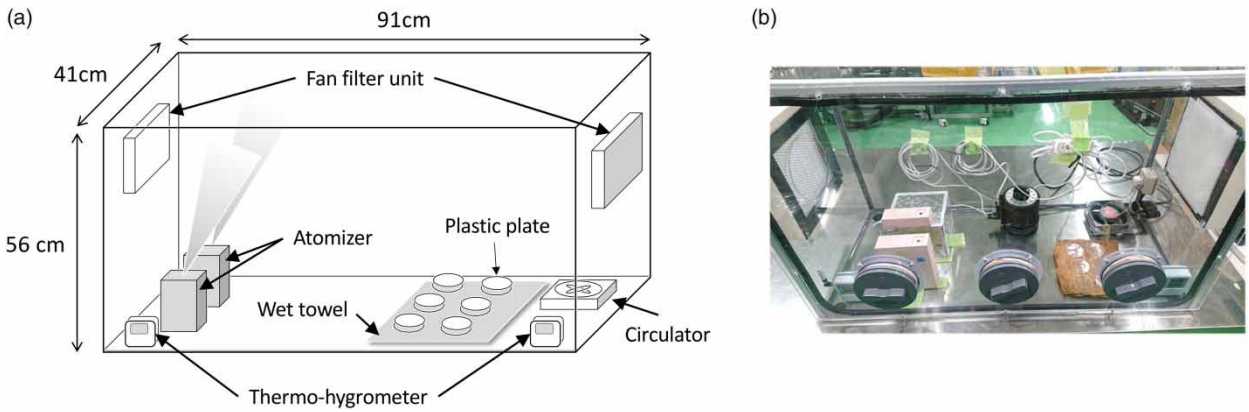


Figure 3 | Diagrammatic representation of the chamber. (a) Schema of the chamber used for the spray test. (b) Photo graph of the chamber.

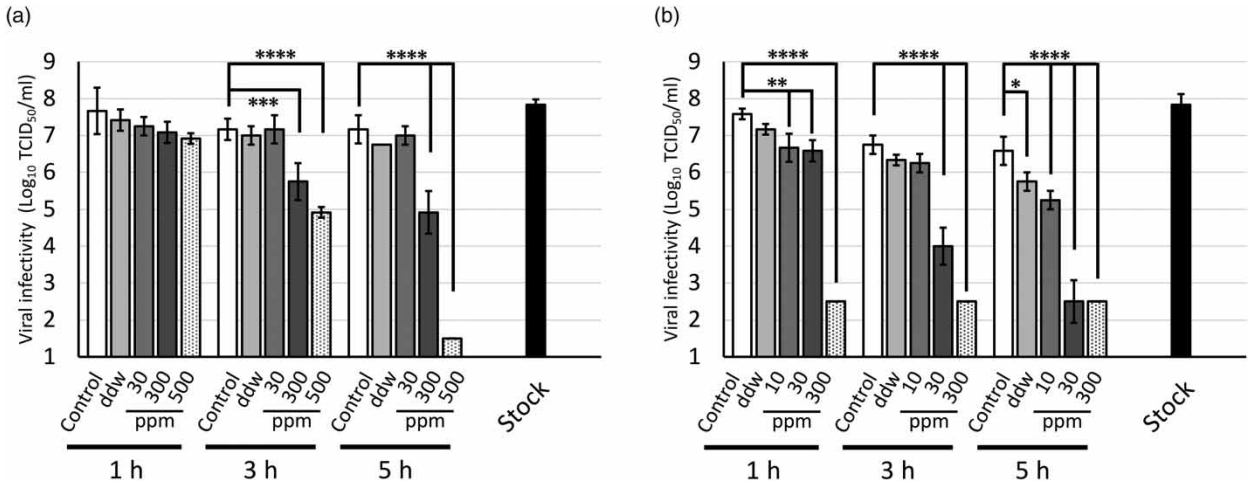


Figure 4 | Evaluation of SARS-CoV-2 virucidal efficacy of atomized HOCl. SARS-CoV-2 virus solution was treated with various concentrations of ionless hypochlorous acid water, as described in Materials and Methods. (a) KUH355 virus strain in the presence of 0.2% FBS condition atomized with 30 (gray bar), 300 (dark gray bar), and 500 (dot bar) ppm of HOCl solution. (b) KUH355 strain in the presence of 0.02% FBS condition atomized with 10 (gray bar), 30 (dark gray bar), and 300 (dot bar) ppm of HOCl solution. The control (white bar) indicates the sequential reduction of the viral titer during the spray test. The stock (black bar) indicates the viral titer of a virus stock solution. The ddw (light gray bar) indicates that the virus solution was atomized with double distilled water. Data were analyzed using one-way ANOVA with the Bonferroni post hoc test. * $p < 0.05$. ** $p < 0.005$. **** $p < 0.0001$.

wild-type mice (Hirche *et al.* 2005; Homme *et al.* 2013). The HOCl solution shows powerful antibacterial efficacy against Gram-positive bacteria (*Bacillus* spp., *Clostridium* spp., *Staphylococcus* spp., *Streptococcus* spp.) and Gram-negative bacteria (*Bordetella* spp., *Campylobacter* spp., *Vibrio* spp., *Pseudomonas* spp.); Actinomycetaceae (*Corynebacterium* spp.); and Enterobacteriaceae (*Escherichia* spp., *Shigella* spp., *Yersinia* spp.), as well as virucidal efficacy against DNA (Adenoviridae, Herpesviridae) and RNA (Orthomyxoviridae, Coronaviridae) viruses (Tachikawa *et al.* 1999; Tagawa *et al.* 2000; Huang *et al.* 2008; Taharaguchi *et al.* 2014; Tamaki *et al.* 2014; Takeda *et al.* 2020; Miyaoka *et al.* 2021).

Previous studies using the suspension test have reported that treatment with HOCl solution can inactivate SARS-CoV-2 in cultured cells, such as VeroE6/TMPRSS2 cells. Hatanaka *et al.* (2022) have reported that 28.1 ppm HOCl solution achieved a 4-log reduction of SARS-CoV-2 infectivity within 10 s. In addition, 29.7 and 59.4 ppm HOCl solution achieved a 3.7–3.9- and 5-log reduction of SARS-CoV-2 infectivity within 10 s to 3 min in the presence of 0.1% FBS. Takeda *et al.* (2021) have reported that a residual chlorine concentration of 51–56 ppm in HOCl solution achieved >4-log reduction of SARS-CoV-2 infectivity within 20 s in the presence of 0.05% FBS. NITE summarized the results of the virucidal efficacy against SARS-CoV-2 at five facilities. They showed that a 19–200 ppm HOCl solution achieved a 3- and 4-log reduction of SARS-CoV-2 infectivity within 20 s to 5 min in the presence of 0.05–0.25% FBS. These studies have reported that viral solutions were prepared in the viral growth medium containing 1–5% FBS. The reaction rates of the virus solution and HOCl were 1:9 or 1:19. Then, they evaluated virucidal efficacy at a final concentration of 0.05–0.25% FBS. Moreover, previous studies have reported that the amount of protein in saliva, which is the cause of the droplet infection, is usually 100 times lower than that in plasma. Saliva contains 2% of proteins and 98% of water (Nunes *et al.* 2011; Kang & Kho 2018). Therefore, we chose the concentration of 0.2% and 0.02% FBS. In the present study, we found that 30 ppm HOCl solution achieved >5-log reduction of JPN/TY/WK-521 strain infectivity within 20 s in the presence of 0.2% FBS. In contrast, we demonstrated that 30 ppm HOCl solution achieved a 1.1-log reduction in KUH355 strain infectivity within 20 s in the presence of 0.2% FBS. This difference in the virucidal efficacy might affect the Delta variant, which is more transmissible than the original variant (Mistry *et al.* 2022). We also indicated that 10 ppm HOCl solution achieved sufficient reduction (>5 log) of JPN/TY/WK-521 and KUH355 virus strains infectivity within 20 s in the presence of 0.02% FBS. Thus, 30 ppm HOCl solution for 20 s may be sufficient to prevent the infection of the original SARS-CoV-2 strain. In contrast, the more transmissible mutant SARS-CoV-2 strain may require 50 ppm HOCl solution for > 20 s.

To reduce the risk of airborne transmission of pathogens, the bactericidal and virucidal efficacies of HOCl solution have been previously evaluated using atomization. Hakim *et al.* (2016) have reported that 50 and 100 ppm HOCl solution achieved an approximately 4-log reduction of *Escherichia coli* and *Salmonella infantis* in dry conditions within 5 min of atomization.

Benedusi *et al.* (2022) have also reported that 300 ppm HOCl solution showed 69–99.9% reduction of *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* with 10 min of atomization, although bactericidal activities were affected by the differences in material surfaces, such as semi-porous, flat, and porous. They also showed that 300 ppm HOCl solution achieved >95% reduction of human coronavirus 229E and adenovirus with 5–10 min of atomization. Urushidani *et al.* (2022) intermittently sprayed 250 or 8,700 ppm HOCl solutions for 2.5–5 s (each 4 min) in the test chamber and reported that 250 ppm HOCl solution did not neutralize air-dried SARS-CoV-2 infectivity after 16 min HOCl exposure. However, 8,700 ppm HOCl solution reduced air-dried SARS-CoV-2 infectivity to decrease the limit of detection within 12 min for 16 min HOCl exposure. In the present study, we found that atomized HOCl at 300 and 500 ppm for 3 and 5 h achieved a 1.4–2.3 and 2.3–5.7 log reduction of KUH355 virus solution infectivity in the presence of 0.2% FBS. We also observed that 30 ppm HOCl atomization for 3 and 5 h achieved 2.8–4.1 log reduction of KUH355 viral solution infectivity in the presence of 0.02% FBS. Moreover, SARS-CoV-2 infectivity in the presence of 0.02% FBS was reduced by >4-log by 300 ppm of HOCl atomization for 1, 3, and 5 h. Our results suggest that the HOCl solution may have effective virucidal efficacy against SARS-CoV-2, although a longer atomization time compared with a previous report may be needed. Our data also showed that atmospheric ACC is constantly maintained at <0.40 ppm in 200-L test chamber after atomization with 500 ppm HOCl. Our test chamber was almost twofold in volume as that reported in another study, in which the chlorine concentration was approximately 10 ppm with 8,700 ppm atomization. Therefore, differences in chlorine concentration in the experimental chamber may have influenced the virucidal efficacies against SARS-CoV-2 we observed in this study.

The WHO does not recommend the disinfection of COVID-19 by fogging environmental surfaces with disinfectant, as it may increase the risk of adverse effects to the eyes, respiratory tract, and skin. Because HOCl reportedly did not cause these adverse effects, the adverse effects of HOCl are probably related to the chlorine concentration and pH of the exposed HOCl. In addition, no adverse events were observed by drinking 20–60 ppm HOCl water in animal studies, washing wounds with 200 ppm HOCl solution, and mouth washing with 80 and 200 ppm HOCl solution in clinical studies (Morita *et al.* 2011; Kubota *et al.* 2015; Block & Rowan 2020; Alzahrani *et al.* 2022; Sevinç Güll *et al.* 2022). Although the effect of the high purity of HOCl on adverse events is unclear, HOCl should be atomized under controlled conditions at the appropriate concentration. Our results, which prove the relationship between the virucidal efficacy and the ACC of HOCl, suggest that HOCl atomization for disinfection may be useful for cleaning unmanned hospital rooms, schools, daycare centers, and various facilities at night.

CONCLUSIONS

We have clarified that both aqueous and atomized HOCl solutions are effective in neutralizing SARS-CoV-2 infectivity. The virucidal efficacy of HOCl was not influenced by the difference in SARS-CoV-2 variants (Pango lineage A and B virus strains). In addition, we showed the virucidal efficacy of HOCl atomization against SARS-CoV-2. HOCl atomization may reduce the burden of cleaning and disinfection work in various facilities such as hospital rooms, daycare centers, and schools.

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FINANCIAL INTERESTS

Author M.K. received research support from NIPRO Corporation. All authors except M.K. have no financial interests.

CONTRIBUTIONS

M.K., R.E., H.F., N.S., and N.K. performed the study concept and design. M.K. and R.E. performed writing the original draft. M.K., R.E., S.M., H.F., N.S., N.K., H.K., and H.H. performed the development of methodology and writing, review, and revision of the manuscript. M.K., R.E., H.F., and N.S. provided acquisition, analysis and interpretation of data, and statistical

analysis. H.F., N.S., and N.K. provided reagents, materials, test chamber, and instrumentations. N.K., H.K., and H.H. supervised and provided the financial support for this project. All authors have read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST STATEMENT

M.K. received research support from NIPRO Corporation. All authors except M.K. have no financial interests.

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