Effectiveness of different disinfectants and combinations against SARS-CoV-2 nucleic acid in COVID-19 quarantine wards

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Abstract

Background

To detect the contamination of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the surroundings of coronavirus disease 2019 (COVID-19) patients and to evaluate the effectiveness of regular disinfectants and combinations against SARS-CoV-2 RNA.

Methods

We sampled the patients’ high contact surfaces in COVID-19 pediatric quarantine wards from April to June 2022. After conducting cleaning procedures using disinfectants, including trichloroisocyanuric acid (TCCA; 500, 1000, and 2000 mg/L), 5% hydrogen peroxide (H$_2$O$_2$), 0.5% povidone-iodine (PI), 75% ethanol (EA), 0.2% chlorhexidine gluconate (CHG), 0.2% quaternary ammonia compound (QAC), and five combinations, environmental samples in bathroom were collected at 0, 30 s, 10, 30, and 60 min. All samples were delivered to the medical laboratory for SARS-CoV-2 nucleic acid (ORF1ab and N) detection using real-time PCR.

Results

SARS-CoV-2 RNA was largely detected on surfaces in the COVID-19 quarantine ward and was highest in the floor, bathroom, and bed sheet. The ORF1ab and N genes remained detectable after 60 min of treatment with QAC, PI, EA, and CHG. H$_2$O$_2$ and TCCA2000 completely degraded SARS-CoV-2 RNA in 30 s, which was faster than TCCA1000 (10 min). Clearance of ORF1ab and N by TCCA500 required 10 and 60 min, respectively, whereas combination of TCCA500 with EA or PI destroyed ORF1ab and N faster at 30 s and 30 min, respectively.

Conclusion

The surroundings of patients with COVID-19 are contaminated by SARS-CoV-2 RNA. Effectiveness of disinfectants and combinations varies, N gene persists longer time than ORF1ab after some disinfection.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel human coronavirus that was first identified in late 2019 and caused the coronavirus disease 2019 (COVID-19), which continues to threaten global public health and cause worldwide economic collapse(Desimmie et al.,2021). SARS-CoV-2 can survive for a long time on the human skin or inanimate surfaces, such as metal, glass, or plastic;
therefore, proper disinfection procedures are essential in preventing the contact transmission of SARS-CoV-2 (Hirose et al., 2021; Kampf et al., 2020a).

As an enveloped RNA virus with a fragile outer lipid layer, SARS-CoV-2 is more sensitive to disinfectants than non-enveloped viruses, and any disinfectant that interferes with the integrity of the SARS-CoV-2 envelope could effectively degrade the virus and prevent its infectivity. Chemical disinfectants vary in their mechanism of action; the majority of disinfectants target the outer lipid layer of SARS-CoV-2 and inactivate viral particles (Choi et al., 2021). There are many regular disinfectants against SARS-CoV-2 environmental carriage: high-level disinfectants include hydrogen peroxide ($\text{H}_2\text{O}_2$) and chlorine disinfectants such as trichloroisocyanuric acid (TCCA); intermediate-level disinfectants include ethanol (EA), quaternary ammonia compound (QAC), and povidone-iodine (PI); and low-level disinfectants include chlorhexidine gluconate (CHG) and benzalkonium chloride (BC) (Elzein et al., 2021; Ghafoor et al., 2021). Compared with other disinfectants, low-level disinfectant CHG is significantly inferior in terms of effectiveness against SARS-CoV-2 (Hirose et al., 2021).

Disinfectants may affect the lipid membrane, cytoplasmic membrane, energy metabolism, cytoplasm, nucleus, enzymes, or proteins (Maris, 1995). TCCA is a chlorine disinfectant that is widely used against SARS-CoV-2 because of its function as a chlorinating agent and oxidant. It reacts with a wide variety of biological molecules such as proteins, amino acids, peptides, lipids, and DNA or RNA (Fukuzaki, 2006). $\text{H}_2\text{O}_2$ is a peroxide-based disinfectant that interferes with different viral components, including lipid membranes, proteins, and nucleic acids. A concentration of 1–3% $\text{H}_2\text{O}_2$ inactivates SARS-CoV-2 within 1 min (Ghafoor et al., 2021). Most intermediate- or low-level disinfectants, including EA, QAC, CHG, and PI, disinfect the virus by reacting with proteins or lipids to destabilize the cell wall and interfere with osmosis, but fail to degrade nucleic acids (Hirose et al., 2021; Kampf et al., 2020b; van Doremalen et al., 2020; Wu et al., 2022; Xiling et al., 2021).

Although there are previous studies regarding environmental SARS-CoV-2 contamination and the effect of disinfectant usage (Santarpia et al., 2020; S. Zhang et al., 2020), the effects of different disinfectants and their combinations in hospital settings, especially COVID-19 quarantine wards, are very limitedly studied, and some conclusions remain unclear. Considering that RNA detection is simple and widely used worldwide to detect SARS-CoV-2, we detected SARS-CoV-2 nucleic acid gene including the open reading frame 1ab ($\text{ORF1ab}$) and nucleoprotein ($\text{N}$ genes) on different environmental surfaces within COVID-19 quarantine wards, and conducted regular disinfection procedures using a single or combined use of eight disinfectants, including TCCA (500, 1000, and 2000 mg/L), 5% $\text{H}_2\text{O}_2$, 0.5% PI, 75% EA, 0.2% CHG, and 0.2% QAC. The effectiveness of the disinfectants was analyzed based on SARS-CoV-2 nucleic acid ($\text{ORF1ab}$ and $\text{N}$ genes) detection.

In this study, we systemically evaluated the presence and contamination of SARS-CoV-2 nucleic acids on environmental surfaces in the pediatric COVID-19 quarantine ward and the elimination effectiveness of different kinds of disinfectants.
Materials And Methods

Setting and environmental sample collection

Environmental sample collection was performed in the COVID-19 pediatric quarantine wards from April to June 2022 in a designated hospital, Renji Hospital (South branch), School of Medicine, Shanghai Jiao Tong University. All admitted children and their adult caregivers in pediatric wards in the hospital from April to June 2022 had mild or no disease symptoms. The regular cleaning procedure in the COVID-19 quarantine wards was conducted four times daily at 8 AM, 12 PM, 4 PM, and 8 PM. High-touch surfaces were cleaned with disinfectant wipes to ensure that the surface remained wet for the required contact time. The floor was cleaned using disinfectant mop.

We collected samples from nine sites involved seven patient’s surrounding samplings including the bedrails, bed sheets, floor, doorknobs, chairs, windows, and bathrooms, and hands samples from patient and their adult caregiver in each ward. Nasopharyngeal swab was used for sampling collection, and the collections were conducted at least one hour before the regular 8 AM cleaning (from 6:30–8 AM).

Five different wards were chosen for five experiments in this step, a total of 45 samples were collected from each of nine patient’s high contact surfaces in five different wards (5 wards × 9 sites × 1 sample per site). The procedure and details of sampling collection in this study were shown in Table S1.

Disinfectants And Combinations Used In This Study

The eight disinfectants used in this study were as follows: four high-level disinfectants, TCCA500 (available chlorine: 500 mg/L), TCCA1000 (1000 mg/L), TCCA2000 (2000 mg/L), and 5% H$_2$O$_2$; three intermediate-level disinfectants, 0.5% PI, 75% EA, and 0.2% CHG; and one low-level disinfectant, 0.2% QAC. Double-distilled water (ddH$_2$O) treatment was used as the control.

Five disinfectant combinations (defined as combinations 1–5) were used in this study. Combination 1 represented a mixture of three intermediate- and one low-level disinfectant (0.2% QAC, 0.5% PI, 75% EA, and 0.2% CHG), while combinations 2–5 represented TCCA500 mixing with other intermediate- or low-level disinfectants, including 75% EA (combination 2), 0.2% QAC (combination 3), 0.5% PI (combination 4), and 0.5% CHG (combination 5). The details of the disinfectants and their combinations used in this study were shown in Table S2.

Disinfecting Procedure And Samplings In This Study

The bathroom was selected as the sampling site because of its occlusive environment and the potential fecal–oral transmission of SARS-CoV-2. The disinfection procedure was conducted at least 1 h before 8 AM. The removal of all visible blood and inorganic and organic matter in the bathroom was as critical as the germicidal activity of the disinfecting agent. A surface that could not be cleaned adequately was
protected with barriers. The bathroom surface, including the door knob, sink, mirror, shower stall, bedside toilet, and entire toilet, was sprayed with different disinfectants and combinations.

There were fourteen treatments including ddH2O (n = 1), eight disinfectants (n = 8), and five combinations (n = 5) in this study. Meanwhile, we set five different time points (0 s, 30 s, 10, 30, and 60 min) for samples collection. Fourteen different bathrooms were selected and exposed by fourteen treatments respectively. After treatment, we collected the mixed bathroom sample (n = 1) including the doorknob, floor, hand sink, and toilet flush button in each of 14 bathrooms at five different time points. We obtain 70 samples in this step (14 bathrooms × 5 time points × 1 mixed sample per bathroom).

Two more replicated experiments were conducted, and those 14 previously-used bathrooms were selected again for 14 different treatments in each experiment. Identical areas at different bathroom sites were sampled for disinfection.

A total of 210 samples (3 experiments × 70 samples) were collected in this procedure. The interval between each replicate experiment was > 48 h. The procedure and details of sampling collection in this study were shown in Table S1.

**RT-PCR detection for SARS-CoV-2 nucleic acid**

All the environmental samples were delivered to the medical laboratory for SARS-CoV-2 nucleic acid detection. Viral RNA was extracted from environmental samples using kits (BioGerm, Shanghai, China) and real-time PCR (RT-PCR) was performed (ABI QuantStudio Dx, Thermo Fisher Scientific, Waltham, USA) to detect the presence and approximate amount of viral RNA in a sample. The nucleic acids of these samples were extracted and amplified by RT-PCR for the open reading frame 1ab (ORF1ab) and nucleoprotein (N) genes of SARS-CoV-2.

The CT value was used to determine the number of original RNA copies in the samples. No quantitative real-time PCR test kit has been approved by the National Medical Products Administration of China. Therefore, we used the CT value to reflect RNA copies in the samples. In the SARS-CoV-2 RNA detection assay, CT ≥ 40 was considered negative; therefore, we defined negative CT values as 40.

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, California, USA). Data were analyzed using the rank-sum test, χ2 test, or Fisher’s exact test, as appropriate. Statistical significance was set at P< 0.05.

**Results**

SARS-CoV-2 nucleic acid on different environmental surfaces
The ORF1ab and N genes from SARS-CoV-2 nucleic acids were widely detectable on environmental surfaces in pediatric COVID-19 quarantine wards. Both ORF1ab and N were 100% positive in samples from the bed sheets (5/5), floor (5/5), and bathrooms (5/5). The ORF1ab gene was 40% (2/5) positive in bedrails, doorknobs, and children's hands, and 20% (1/5) were positive in chairs and windows, but undetectable in adults' hands (0/5). The N gene was 100% (5/5) positive in bedrails, 80% (4/5) positive in doorknobs and chairs, 60% (3/5) positive in children's hands, 40% (2/5) positive in windows, and 20% (1/5) positive in adults' hands samples (Table 1).

<table>
<thead>
<tr>
<th>Sites</th>
<th>Number of samples (percent %)</th>
<th>ORF1ab positive</th>
<th>N positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bedrail</td>
<td>5</td>
<td>2 (40%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Bed sheet</td>
<td>5</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Floor</td>
<td>5</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Doorknob</td>
<td>5</td>
<td>2 (40%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Chair</td>
<td>5</td>
<td>1 (20%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Window</td>
<td>5</td>
<td>1 (20%)</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>Children's hands</td>
<td>5</td>
<td>2 (40%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Adults' hands</td>
<td>5</td>
<td>0 (0%)</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Bathroom</td>
<td>5</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
</tbody>
</table>

The CT values of the ORF1ab and N genes also varied in different environmental samples. As shown in Fig. 1A, the ORF1ab gene was significantly detected in the bed sheets, floor, and bathroom samples, with CT values of 35.8 ± 1.0, 31.5 ± 1.6, and 33.8 ± 0.8, respectively. The N gene persisted for a longer time and was significantly detected in the bedrails, bed sheets, floor, doorknobs, chairs, children's hands, and bathroom samples with CT values of 36.1 ± 0.8, 34.4 ± 0.7, 31.3 ± 0.7, 36.7 ± 0.9, 36.9 ± 1.0, 37.4 ± 1.1, and 33.2 ± 0.7, respectively (shown in Fig. 1B). Notably, the CT values of ORF1ab and N were the lowest in the floor, bathroom, and bed sheets samples, indicating a high viral load in these samples.

**Effectiveness of eight disinfectants against SARS-CoV-2**

The effectiveness of disinfectants in the bathrooms was further evaluated. Figure 2 depicts the CT values of ORF1ab (Fig. 2A) and N (Fig. 2B) after 1 h of treatment with 0.5% PI, 75% EA, 0.2% CHG, 5% H₂O₂, TCCA500, TCCA1000, and TCCA2000.
As shown in Fig. 2, the low- or intermediate-effect disinfectants, including QAC, PI, EA, and CHG, could not completely degrade the RNA of SARS-CoV-2 (ORF1ab and N genes) throughout the experiment. After 60 min of disinfectant treatment, the CT values of ORF1ab and N genes in samples treated with 0.2% QAC, 0.5% PI, 75% EA, and 0.2% CHG were 33.5 ± 0.9 and 34.3 ± 0.4, 33.5 ± 0.4 and 33.2 ± 0.6, 32.5 ± 0.5 and 32.8 ± 0.5, and 36.0 ± 1.1 and 35.4 ± 0.6, respectively.

In the high-effect disinfectant treatment groups, H2O2 and TCCA2000 completely degraded the RNA of SARS-CoV-2 (CT ≥ 40) at 30 s, faster than TCCA1000, which required 10 min. However, TCCA500 showed a weaker effect on the RNA degradation of SARS-CoV-2, and the clearance of ORF1ab and N genes (CT value ≥ 40) required 10 and 60 min, respectively.

Effectiveness of five disinfectant combinations against SARS-CoV-2

As shown in Fig. 3, the combined use of 0.2% QAC, 0.5% PI, 75% EA, and 0.2% CHG (combination 1) showed no difference to the single use, and the CT values of ORF1ab and N were 34.8 ± 0.9 and 35.4 ± 0.7, respectively, after 60 min of treatment.

The N gene persisted for a longer time (60 min) than ORF1ab (10 min) after TCCA500 disinfection. Notably, the combination of TCCA500 with 75% EA (combination 2) or 0.5% PI (combination 4) degraded ORF1ab faster at 30 s and 10 min and destroyed the N gene at 10 and 30 min, respectively. After combining with 0.2% QAC (combination 3), TCCA500 destroyed ORF1ab faster at 30 s but showed no difference in N gene degradation. Moreover, the combination of TCCA500 and 0.2% CHG (combination 5) showed no difference in the degradation of either the ORF1ab or N gene, as compared to the single use of TCCA500.

Discussion

Previous studies have shown that various contaminated surfaces by SARS-CoV-2 can still be infectious for some time (Tharayil et al., 2022). The incubation times of human-to-human transmissions have been described between 2–10 days, facilitating the spread of SARS-CoV-2 via droplets, contaminated hands or surfaces (Kampf et al., 2020a). In this study, SARS-CoV-2 RNA is largely detected on different surfaces in the COVID-19 pediatric quarantine wards, and the environmental carriage of ORF1ab and N genes was highest in the samples from the floor, bedsheets, and bathroom. Our findings show that mild and asymptomatic COVID-19 patients can contaminate their surroundings, indicating that the isolation of asymptomatic patients at home poses a risk to their family members.

Chemical disinfectants are widely used in hospitals, and increased cleaning and disinfection of healthcare surfaces is essential for effective prevention and control of SARS-CoV-2 (Choi et al., 2021). SARS-CoV-2 viral RNA has been found on environmental surfaces in hospital rooms, quarantine rooms, and other hospital settings, suggesting its potential transmission via surfaces (Aytogan et al., 2020; Kotwa et al., 2022; Santarpia et al., 2020; Wei et al., 2020; Zhou et al., 2021). Environmental surfaces may be contaminated by respiratory droplets and aerosols, as they settle from the air or through contact transfer.
from an infected person. Some studies have attempted to isolate viable SARS-CoV-2 from environmental surfaces using cell culture but were either unsuccessful or found weak evidence of a viable virus (Colaneri et al., 2020; Santarpia et al., 2020; Zhou et al., 2021).

It has been found that viable SARS-CoV-2 can survive on various surfaces under experimental conditions ranging from 3 h to up to more than 7 d (Chin et al., 2020; van Doremalen et al., 2020). Environmental disinfection is necessary to prevent the transmission and infection waves of COVID-19. There are many disinfectants that can effectively inactivate SARS-CoV-2 (Xiao et al., 2022). Lipid solvents, including EA (> 75%), formaldehyde (> 0.7%), isopropanol (> 70%), PI (> 0.23%), and sodium hypochlorite (> 0.21%), can be used to inactivate SARS-CoV-2 (Dhama et al., 2021; Talic et al., 2021). The effects of disinfectants differ according to the destruction of the viral genetic structure.

To date, various types of biocidal agents are used worldwide for disinfection against SARS-CoV2 in healthcare settings (Viana et al., 2022). \( \text{H}_2\text{O}_2 \) exhibits virucidal activity at 0.5% or 1% concentration and inactivates SARS-CoV-2 virus (Fantozzi et al., 2022; Kampf et al., 2020b). EA, PI, and QAC are effective disinfectants for the surface disinfection of SARS-CoV-2 (Baker et al., 2020; Chin et al., 2020; O’Donnell et al., 2020). EA can easily inactivate SARS-CoV-2 by penetrating the cell and causing leakage of intracellular components, leading to cell death (Lee et al., 2022; O’Donnell et al., 2020). In this study, we evaluate the effects of different disinfectants to SARS-CoV-2 RNA instead of the virus. Meanwhile, the combinations of different disinfectants are involved to further clarify the combing effects of disinfectants.

SARS-CoV-2 RNA was recently detected in feces, predicting its potential transmission via the fecal–oral route (Jones et al., 2020). A study published by Yeo et al. confirmed the possible transmission of SARS-CoV-2 via the fecal–oral route (Yeo et al., 2020). SARS-CoV-2 was firstly isolated from a stool specimen in China (Y. Zhang et al., 2020). In this study, we found that SARS-CoV-2 RNA was 100% present in the bathroom, and the CT values of bathroom samples were very low. Considering the high viral load and potential fecal–oral transmission of SARS-CoV2 in bathroom, we chose the bathroom for further study to evaluate the effectiveness of different disinfectants and combinations.

The elimination effectiveness against SARS-CoV-2 RNA varied with different types of disinfectants and their combinations. We found that low- or intermediate-effect disinfectants, including QAC, PI, EA, and CHG, could not completely degrade \( \text{ORF1ab} \) and \( \text{N} \) genes throughout the 60 min treatment. However, high-level disinfectants that bind to and react with proteins, amino acids, peptides, lipids, and nucleic acids show better clearance of SARS-CoV-2 RNA, among which \( \text{H}_2\text{O}_2 \), TCCA2000, and TCCA1000 destroy \( \text{ORF1ab} \) and \( \text{N} \) genes within 30 s, 30 s, and 10 min, respectively. TCCA500 presented a weaker disinfection effect on the \( \text{ORF1ab} \) and \( \text{N} \) genes, but the combination of TCCA500 with 75% EA or 0.5% PI could destroy the \( \text{ORF1ab} \) and \( \text{N} \) genes faster at 30 s and 30 min, respectively. We hypothesized that the powerful effectiveness of the combination is attributed to the synergistic effects of membrane damage and cell lysis of SARS-CoV-2 by 75% EA or 0.5% PI, and the degradation of the nucleic acid by TCCA500.
Without the exposure to disinfectants, SARS-CoV-2 RNA can be detected on the surface of some materials for a long time: One study showed that infectious virus was detectable within 48 hours in the environments, and SARS-CoV-2 RNA remained detectable for 7 days (Matson et al., 2020). Our study demonstrates that SARS-CoV-2 nucleic acid can persist for a long time in the surroundings of COVID-19 patients even if regular disinfection has been conducted. The disinfectants such as H₂O₂ and TCCA which directly react with nucleic acids can destroy SARS-CoV-2 RNA much faster than other disinfectants. It is noticeable that \textit{N} gene can persist longer time than \textit{ORF1ab} gene after disinfection using TCCA500 and combinations. Studies have demonstrated that nucleoprotein which is coded by \textit{N} gene is very critical for optimal CoV genomic replication and has the ability to interfere with the host cell-cycle cellular machinery. Seemingly, the persistence of \textit{N} gene in environment after some disinfection is confusing and deserve further study.

**Conclusions**

Our finding indicates that the surroundings of COVID-19 patients with mild or no symptoms are extensively contaminated with SARS-CoV-2 nucleic acids. The effectiveness of disinfectants and combinations against SARS-CoV-2 RNA differs with respect to the destruction of the viral genetic structure. High-level disinfectants H₂O₂, HCCA1000, and HCCA2000 show perfect degradation of SARS-CoV-2 RNA. Intermediate-level disinfectants, 75% EA or 0.5% PI, destroyed SARS-CoV-2 RNA faster when combined with HCCA500.

**Declarations**

**Funding**

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**Competing interests**

The authors declare that they have no conflicts of interest, financial or otherwise, related to the publication of this paper.

**Authors' contributions**

Wenhao Zhou, Pan Fu and Chuanqing Wang designed the experiments and wrote the manuscript. Ying Zhang and Jianguo Zhou performed experiments, data analysis, and data collection. Guoping Lu analyzed the data and revised the manuscript. Haitao Zhu, Chunmei Lu, Lan Ye, and Lingfeng Chunyu participated in the experiments and data analyses. Thanks professor Haiqun Ban and his team members in Renji Hospital to provide the guidance of this experiment.
Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Children’s Hospital of Fudan University (ethical approval number:2022-88). Informed consent was obtained from all children's legal guardians. All experiments were performed in accordance with relevant guidelines and regulations. All experimental protocols were approved by the Ethics Committee of the Children's Hospital of Fudan University.

Consent for publication

Not applicable. The experiment protocol for involving humans was in accordance to the institutional guidelines.

References


Figure 1

CT values for (A) ORF1ab and (B) N genes of SARS-CoV-2 on different environmental surfaces in hospital setting. CT-values ≥ 40 in control group was defined as CT = 40. The CT-values of ORF1ab gene from bed sheets, floor, and bathroom were 35.8 ± 1.0, 31.5 ± 1.6, and 33.8 ± 0.8, respectively. The CT-values of N gene from bedrails, bed sheets, floor, doorknobs, chairs, children’s hands, and bathroom were 36.1 ± 0.8, 34.4 ± 0.7, 31.3 ± 0.7, 36.7 ± 0.9, 36.9 ± 1.0, 37.4 ± 1.1, and 33.2 ± 0.7, respectively. (*p < 0.05, **p < 0.01, and ***p < 0.001)

Figure 2

CT values for (A) ORF1ab and (B) N gene of eight disinfectants on SARS-CoV-2 inactivation at different time point. Plots showed the means and standard error across three replicates. After 60 min of disinfectants treatment by 0.2% QAC, 0.5% PI, 75% EA, and 0.2% CHG, the CT values for ORF1ab and N genes from samples showed no significant differences. H₂O₂ and TCCA2000 could completely degrade
the RNA of SARS-CoV-2 (CT≥40) in 30 s, however, TCCA1000 need 10 min. Clearance of ORF1ab and N genes (CT≥40) treated with TCCA500 required 10 and 60 min, respectively.

![Figure 3](image)

**Figure 3**

CT values for (A) ORF1ab and (B) N genes of five disinfectant combinations on SARS-CoV-2 inactivation at different time point. Combination 1 represents 0.2% QAC, 0.5% PI, 75% EA, and 0.2% CHG; Combination 2 represents TCCA500 and 75% EA; Combination 3 represents TCCA500 and 0.2% QAC; Combination 4 represents TCCA500 and 0.5% PI; and Combination 5 represents TCCA500 and 0.2% CHG. Combination 1 cannot destroy both ORF1ab and N gene through 60 min treatment. Combinations 2, 3, 4, and 5 degraded ORF1ab at 30 s, 30 s, 10 min, and 10 min, and degraded N gene at 10, 60, 30, and 60 min respectively.

**Supplementary Files**

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