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Research Article

High concentrations of hypochlorous acid-based disinfectant in the environment reduced the load of SARS-CoV-2 in nucleic acid amplification testing

During the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) pandemic, chlorine-containing disinfectants have been widely used in nucleic acid amplification testing laboratories. Whether the use of disinfectants affect the results of viral nucleic acid amplification is unknown. We examined the impact of different hypochlorous acid (HOCl) concentrations on the quantitative results of SARS-CoV-2 by real-time reverse-transcription polymerase chain reaction (RT-PCR). We also explored the mechanisms and models of action of chlorine-containing disinfectants that affected the detection of SARS-CoV-2. The results showed that different HOCl concentrations and different action times had an impact on the SARS-CoV-2 results. High concentrations of ambient HOCl have a greater impact than low concentrations, and this effect will increase with the extension of the action time and with the increase in ambient humidity. Compared with the enzymes or the extracted RNA required for RT-PCR, the impact of HOCl on the SARS-CoV-2 detection is more likely to be caused by damage to primers and probes in the PCR system. The false negative result still existed after changing the ambient disinfectant to ethanol but not peracetic acid. The use of HOCl in the environment will have an unpredictable impact on the nucleic acid test results of SARS-CoV-2. In order to reduce the possibility of false negative of SARS-CoV-2 nucleic acid test and prevent the spread of epidemic disease, environmental disinfectants should be used at the beginning and end of the experiment rather than during the experimental operation.

Keywords:
Disinfectant / Hypochlorous acid / Nucleic acid amplification testing / SARS-CoV-2
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1 Introduction

The spread of the new pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused an expanding pandemic of coronavirus disease 2019 (COVID-19) [1]. As of March 14, 2021, 186 countries, territories and regions have reported a total of 119 million cases and there were fewer than 3.3 million new cases and more than 60 000 new deaths at last week [2]. Laboratory viral nucleic acid amplification testing (NAAT), such as real-time reverse-transcription polymerase chain reaction (RT-PCR), has been recommended by the World Health Organization (WHO) as a standard confirmation protocol for SARS-CoV-2 detection owing to their high sensitivity, specificity, and efficiency [3–5], but the reliability of this method has been questioned [6], as false-negative results have been reported by multiple researchers [7–9]. The false-negative results of RT-PCR are affected by the sample type, collection method, and specific test performed [10] and threaten healthy people who do not take protective measures [11]. Misdiagnosis of SARS-CoV-2 can be extremely detrimental for patients' medical treatment and public health. Generally, an accurate viral nucleic acid detection result requires rigorous quality control in sampling, sample transportation, nucleic acid extraction, and rRT-PCR. Any processing failure at any step can lead to unexpected detection bias. Therefore, there is an urgent need to identify and eliminate such preanalytical or analytical bias to improve the accuracy of infected patient identification.

We have previously reported that thermal inactivation at 56°C before PCR could lead to a false-negative result in

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Abbreviations: **COVID-19**, Coronavirus Disease 2019; ***Ct***, cycling threshold; **HOCl**, hypochlorous acid; **NAAT**, nucleic acid amplification testing; **PCR-MixR**, PCR-Mastermix and RNA template; **RH**, relative humidity; **RT-PCR**, real-time reverse-transcription polymerase chain reaction; **SARS-CoV-2**, Severe Acute Respiratory Syndrome Coronavirus Type 2; **WHO**, World Health Organization

Color online: See article online to view Figs. 1 and 2 in color.

patients with low viral loads, mainly by disrupting viral RNA integrity [12]. Similar results have also been reported by Yang Pan and coworkers; up to 46.7% of weak-positive samples were identified as negative using rRT-PCR after thermal inactivation [13]. Nevertheless, such an impact only accounts for a small part of false-negative results. Indeed, latency time, sample type, and sample pretreatment will also reduce the detection rate within infected patients [10]. Hypochlorite (HOCl) displays a broad spectrum of antimicrobial activity and is effective against several common pathogens at various concentrations [14]. Under the current pandemic of the COVID-19, HOCl disinfectant is widely used in medical places and testing institutions. A 0.1% HOCl solution (1000 mg/L) was recommended by the WHO for waste management and ambient cleaning in clinical laboratories for SARS-CoV-2 detection [15]. A total of 0.5% (5000 mg/L) HOCl solution was recommended for blood and body fluids large spills (i.e. more than about 10 mL) [16]. Hypochlorite-based products include liquid (sodium hypochlorite), solid, or powdered (calcium hypochlorite) formulations. These formulations dissolve in water to create a dilute aqueous chlorine solution in which undissociated HOCl is active as the antimicrobial compound [14]. Although it is known that oxidants like HOCl can affect DNA and inhibit PCR, no studies have been conducted on the inhibition of PCR by environmental HOCL aerosols. We sought to explore whether the use of high concentrations of HOCl to disinfect the laboratory surface objects may also affect the detection of SARS-CoV-2 by RT-PCR.

2 Materials and Methods

2.1 Reagents, instruments, and consumables

Synthetic SARS-CoV-2 pseudovirus: The synthetic SARS-CoV-2 virus S1 (1.94E+04 copies/mL) were provided by Guangzhou BDS Biological Technology Co., Ltd. Nucleic acid extraction and purification reagent: Shanghai Fosun (LOT: 20 200 614, Fosun, China) and NX-48 Viral RNA Kit (lot: CVN111-200720, Genolution, Korea). Fluorescence quantitative PCR amplification reagent: Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing, Sansure Biotech Inc. (lot: 2 020 051), and DaAn Inc. (lot:2 020 089), China). Disinfectant: (1) Hypochlorous acid solution (HOCL): KONVIDA® Sodium dichloroisocyanurate disinfectant tablets (effective chlorine content: 500+50 mg/tablet (circular flake), LOT:20 190 120, Xizi Sanitary disinfection medicine apparatus Co. Ltd., China); configurational approach: 1000 mg/L = 2 tablet:1 liter H_2O; 5000 mg/L = 10 tablet:1 liter H_2O, dissolve the tablet completely in water for use immediately. (2) Disinfectiant Alcohol: OYEAH® Disinfectiant Alcohol (alcohol content:75% ± 5% (v/v), China). Commercialized reagent for use directly; (3) Peracetic Acid disinfectant ($C_2H_4O_3 \geq$ 0.5%, Ounuokang, China), commercialized reagent for use directly.

Automatic nucleic acid extractor: NX-48 (SN:1 712 245, Genolution, Korea); TANBead (E13200202, TANBEAD, Taiwan of China). Fluorescence quantitative PCR instrument: Roche Cobas Z480 (SN:54 339, Roche, Germany). Medical Thermohygrometer: Anymetre Thermohygrometer (Product model:TH-20, China). PCR consumables:0.1 mL PCR reaction tube (Applied Biosystems, Life Technologies, China);centrifuge tube (1.5 mL DNase-free and RNase-free centrifuge tubes, AXYGEN, China);tip: (10, 200, and 1000 μL tips with filters, AXYGEN, China) ; pipette gun (Eppendorf, Germany); refrigerated centrifuge (Legend Micro 21R, Thermo Fisher, China).

2.2 Preparation of experimental conditions

Simulation of aerosol formation used by different disinfectants dosage and time in the laboratory, that information is listed in Table 1. The same volume of HOCl (5000 and 1000 mg/L), ethanol (75%), and peracetic acid (≥0.5%) solution were put into uncovered bottles (capacity 50 mL, radius 8 cm), and then put them in same size closed simulated lab containers ($30 \times 25 \times 30$ cm^3). In this situation, a certain experimental environment can be created after the HOCl or other disinfectants evaporated within the confined space. The relative humidity (RH) of the environment is determined by the size of the simulated laboratory space and the volatile surface area of the disinfectant and the time of the coincubation, which is monitored in the simulated laboratory using the Anymetre thermometer and hygrometer (product model: TH-20, China). The total volatile superficial area of the disinfectant is equal to the numbers of bottles without caps multiplied by the bottle mouth area. Those experiments were completed in another laboratory, which did not use HOCl disinfectant during the experiment, so HOCl aerosol did not exist in the working laboratory, and the ambient base humidity in our laboratory is 55–60%^{RH} at 23.5 ± 1°C. See Table 1 for details.

The operation process as follows: (1) Configured different disinfectants as required; (2) placed the disinfectants in the simulated laboratory for different periods to form initial humidity according to Table 1; (3) loaded PCR reagents of different components into PCR reaction tubes according to Table 2; (4) placed PCR reaction tubes to the corresponding simulation laboratory; (5) incubated disinfectants and PCR components in the simulation laboratory together; (6) after different times, took out different groups of PCR reaction tubes; (7) add the corresponding remaining PCR components into PCR reaction tubes according to Table 2, covered the PCR reaction tube caps; (8) finally, moved them to the real-time PCR machine for PCR amplification. (see Tables 1 and 2 for details).

2.3 RNA extraction and quantitative RT-PCR assay

The 200 μL of sample was used for nucleic acid extraction and purification using a 48 Viral RNA Kit (Genolution, Korea). The extracted RNA was added to the reaction tubes to react

Table 1. The formation of different initial relative humidity in a simulated laboratory box

Types of disinfectant		The numbers of filled with 50 mL disinfectants' lidless bottle	The evaporated time of disinfectant before experiment (min)	Initial RH
HOCl	1000 mg/L	1	60	I: 65–70%^{RH}
		2	60	II: 70–75%^{RH}
		3	60	III: 75–80%^{RH}
	5000 mg/L	1	30	I: 65–70%^{RH}
		2	30	II: 70–75%^{RH}
		3	30	III: 75–80%^{RH}
Ethanol (75%)		1	30	III: 75–80%^{RH}
Peracetic acid ($C_2H_4O_3 \geq 0.5\%$)		1	30	III: 75–80%^{RH}

Table 2. The types of disinfectant and the co-incubated PCR components used in each experiment

Experimental No.	Disinfectants	RH	Coincubated PCR components	Other components added before amplifying
1	1000 and 5000 mg/L HOCl	I: 65–70% ^{RH}	PCR-Mastermix	RNA Template
2	1000 mg/L HOCl	I: 65–70%^{RH} II: 70–75%^{RH} III: 75–80%^{RH}	PCR-Mastermix	RNA Template
3	1000 mg/L HOCl	III: 75–80%^{RH}	a: PCR dNTPs mix, b: PCR enzyme, c: PCR-Mastermix, d: RNA Template	a: PCR enzyme +RNA, b: PCR dNTPs mix++RNA, c: RNA, d: PCR-Mastermix
4	1000 and 5000 mg/L HOCl, 75% ethanol, peracetic acid ($\geq$0.5%)	III: 75–80%^{RH}	PCR-Mastermix	RNA Template

with the pretreated the PCR-Mastermix samples. The RNA of S1 pseudovirus was extracted by RNA extraction and purification reagent and used as RNA template for subsequent experiments. A standard curve was generated using different gradient concentrations of standard samples, which were prepared by diluting standard S1 (1.94E+04 copies/mL, Guangzhou BDS) with RNA-/DNase-free deionized water. The final concentrations of S2, S3, S4, and S5 were 1/3, 1/9, 1/27, and 1/81 of S1, respectively. The cycling thresholds (*Ct*) were calculated by a Roche fluorescence quantitative PCR instrument, and the S1 concentration after the experiment was calculated by the standard curve constructed based on the standard product. All tests were repeated three times. After the PCR reaction reagent interacted with the environment formed by the disinfectant evaporated for a certain period and then added the remaining PCR components, the RT-PCR was performed at last (see Table 2 for details). Our laboratory was accredited by China National Accreditation Service for Conformity Assessment and certificated by College of American Pathologists in 2012. Almost at the same time, our laboratory also obtained the certificate of technical acceptance of clinical gene amplification laboratory issued by Zhejiang Provincial Clinical Laboratory Center in 2013. The laboratory molecular detection system has been operating stably for many years. All medical personnel participating in PCR tests are authorized and qualified professionals with official permission to perform these tests in China.

2.4 Statistical analysis

Data are presented as the mean ± standard deviation. The Shapiro–Wilk test was used to determine whether a set of data fit a normal distribution. The Kruskal–Wallis H test was used to analyze the differences between samples from more than two groups that underwent the same treatment or to analyze the differences between more than two incubation time periods of one certain sample. One-way ANOVA was used to analyze the differences between different incubation time periods of one certain sample while the data fit a normal distribution. The Mann–Whitney U test was used to analyze the differences between samples in two groups that underwent the same treatment or to analyze the differences between two incubation time periods of one certain sample. SPSS 23.0 (IBM) and Prism 8 (GraphPad, La Jolla, USA) were used for all statistical determinations. *p*-values less than 0.05 were considered significant.

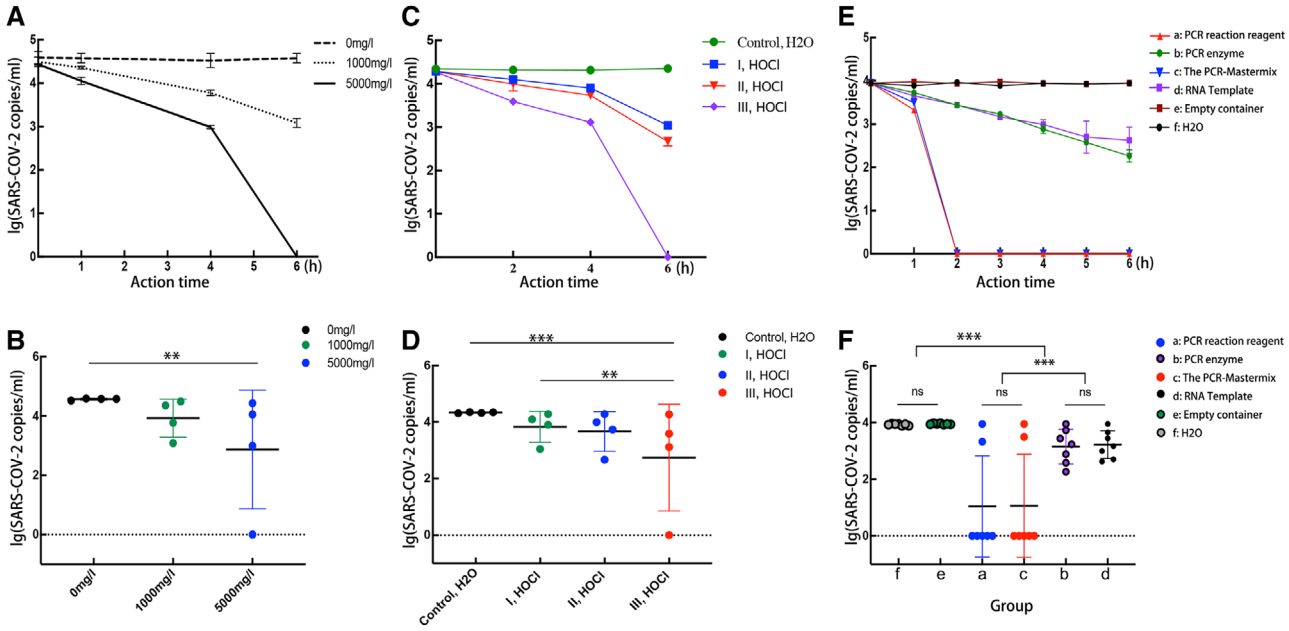

Figure 1. Exploration of the influence of HOCl concentration and environmental humidity on the detection amount of SARS-CoV-2 nucleic acid at different periods and its influencing factors.

3 Results

3.1 The number of SARS-CoV-2 copies decreased with increasing HOCl concentration in the environment and with the extension of exposure time

The PCR-Mastermix was subjected to HOCl concentrations of 5000, 1000, and 0 mg/L for different time periods (6, 5, 4, 3, 2, 1 h) in simulated laboratory containers of 65–70%^{RH} at 23.5 ± 1°C, and the changes in the SARS-CoV-2 viral load were detected by NAAT. We examined the number of copies of SARS-CoV-2 when the PCR reaction tubes containing PCR-Mastermix were exposed to different HOCl disinfectants with concentrations of 5000, 1000, and 0 mg/L. We found that the SARS-CoV-2 copy numbers in the three groups with the same exposure time (*chi-square value* = 19.682, $p < 0.001$) differed greatly. When the HOCl concentration was 0 mg/L in the environment, the amplification capacity of the PCR-Mastermix and RNA template (MixR) for SARS-CoV-2 did not change with incubation time ($F = 0.152$, $p > 0.05$). When the HOCl concentration was 5000 and 1000 mg/L, the amplification capacity of the SARS-CoV-2 PCR-MixR was significantly decreased compared with 0 mg/L HOCl (5000 mg/L and 0 mg/L HOCl: $Z = -3.710$, $p < 0.001$; 1000 mg/L and 0 mg/L HOCl: $Z = -3.802$, $p < 0.001$). HOCl (5000 mg/L) showed a greater influence on the detection ability of SARS-CoV-2 than HOCl (1000 mg/L) after the incubation time reached 4 h ($Z = -2.580$, $p < 0.01$) and 5000 mg/L HOCl completely lost the detection ability of SARS-CoV-2 after 6 h. See Figure 1A and B for details. The number of SARS-CoV-2 copies significantly decreased with increasing HOCl concentration.

A and B represent the influence of different HOCl concentrations on SARS-CoV-2 detection. The SARS-CoV-2 amplification system was subjected to the ambient HOCL concentration of 5000, 1000, and 0 mg/L for different hours respectively (humidity 65–70%, temperature 23.5 ± 1°C), and the changes of SARS-CoV-2 virus concentration were observed by NAAT. (A-F) The *Y*-axis shows the logarithm of 10 for the concentration of SARS-CoV-2 and the *X*-axis represents the interaction time between the PCR-Mastermix and the environment. C and D represents the effect of different environmental humidity caused by HOCl on the detection of SARS-CoV-2. The SARS-CoV-2 PCR-Mastermix acted with 65–70%^{RH}, 70–75%^{RH} , and 75–80%^{RH} environmental humidity for different hours respectively (1000 mg/L HOCl, temperature 23.5 ± 1°C), and the concentration of SARS-CoV-2 was observed by NAAT method. A–C and E: The *X*-axis shows the action hours of different experimental environment and SARS-CoV-2 PCR-Mastermix. E and F represents the influence of different amplifying substances on the NAAT of SARS-CoV-2. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3.2 The number of SARS-CoV-2 copies decreased with increasing ambient humidity caused by HOCl

We exposed the PCR reaction tubes containing SARS-CoV-2 PCR-MixR to the control conditions (65–70%^{RH}, 0 mg/L HOCl) and the different ambient humidity groups (I: 65–70%^{RH}, II: 70–75%^{RH}, and III: 75–80%^{RH}, 1000 mg/L HOCl, Table 1) in the above simulation laboratory for different time

Table 3. PCR components coincubated with disinfectants and added components after incubation

Group	Group No.	Abbreviation	Main Ingredient
Experimental group	**a**	PCR dNTPs mix (SARS-CoV-2 reaction reagent mix)	dNTPs, MgCl2, Primers (ORF1ab gene, E gene and N gene of SARS-CoV-2), Probes, Rnasin, PCR buffer
	b	PCR enzyme	Taq DNA polymerase,Reverse Transcriptase, UNG enzyme
	c	The PCR-Mastermix	The group a and b mixture no RNA template
	d	RNA Template	The extracted and purified RNA Template
Control group	**e**	Empty container	Empty PCR reaction tubes
	f	H_2O	PCR reaction tubes filled with water

at 23.5±1°C. When the concentration of HOCl in the environment was 0 mg/L, that is, using deionized water as a control, the viral load did not decrease with the incubation time extension (*chi-square value* = 1.093, $p > 0.05$). However, ambient humidity had a negative influence on SARS-CoV-2 loads (*chi-square value* = 22.116, $p < 0.001$). Ambient humidity of 75–80%^{RH} (III) with 1000 mg/L.

HOCl showed greater influence compared with I and II after the incubation time reached 2 h (III and I: $Z = -2.036$, $p < 0.05$; III and II: $Z = -1.992$, $p < 0.05$). The influence of ambient humidity in groups I and II on the detection of SARS-CoV-2 nucleic acid was still not statistically significant, even after incubation for 6 h ($Z = -1.964$, $p > 0.05$). See Figure 1C and D.

3.3 Exploration of the mechanism of HOCl on the inhibition of PCR amplification

The PCR amplification system was divided into four experimental groups and two control groups. Except for group e, the other groups are added respectively according to the volume required for PCR amplification to PCR reaction tubes, see Table 3 for details. They were placed in 1000 mg/L HOCl in the above simulation laboratory for different time periods at 75-80%^{RH} and 23.5 ± 1°C; then, other components required for NAAT of SARS-CoV-2 were added to these groups. With prolonged exposure time, the SARS-CoV-2 viral concentration of the four experimental groups decreased gradually, but the two control groups remained unchanged (Figure 1E). Viral concentrations were completely undetectable after 2 h of exposure in groups a and c (including the dNTPs, primers, and probes), and approximately one-tenth of the viral concentration was detected after 6 hof exposure in groups b and d (including the PCR enzyme and RNA template) (see Figure 1E). There were significant differences between the experimental groups and the control groups in detected SARS-CoV-2 copies ($Z = -8.162$, $p < 0.001$). Ambient HOCl caused more serious damage to groups a and c than to groups b and d ($Z = -4.451$, $p < 0.001$). There was no statistical difference in the effect of environmental HOCl on SARS-CoV-2 NAAT by PCR enzyme or RNA template ($Z = -0.201$, $p > 0.05$). Compared with group a, despite the addition of PCR enzyme in group c, there was also no difference in the effect of environmental HOCl on SARS-CoV-2 detection between this two groups ($Z = -0.142$, $p > 0.05$). There was also no difference between the eight empty tubes and eight tubes filled with water ($Z = -1.523$, $p > 0.05$) (see Figure 1F). The influence of environmental HOCl on the PCR amplification reaction system of SARS-CoV-2 NAAT detection was more likely to be caused by the destruction of dNTPs, primers and probes, rather than the PCR enzyme or RNA template.

3.4 The effect of different disinfectants in the environment on detection of SARS-CoV-2

To assess the effect on SARS-CoV-2 detection by different disinfectants, we set one control group (0 mg/L HOCl, deionized water) and four experimental groups (75% ethanol disinfectant, peracetic acid ($C_2H_4O_3 \geq 0.5\%$), 1000 mg/L HOCl, and 5000 mg/L HOCl). The SARS-CoV-2 PCR-MixR were loaded in PCR reaction tubes and then placed respectively in the above simulation laboratory with 75–80%^{RH} at 23.5 ± 1°C. After exposure for 6, 5, 4, 3, 2, 1, and 0 h, we observed that there was a significant difference between the above five groups in number of detected SARS-CoV-2 copies (*chi-square value* = 61.380, $p < 0.001$). Furthermore, both 1000 and 5000 mg/L HOCl suppressed the detection of SARS-CoV-2 compared with deionized water (5000 mg/L and 0 mg/L HOCl: $Z = -5.124$, $p < 0.001$; 1000 mg/L and 0 mg/L HOCl: $Z = -5.117$, $p < 0.001$), and 5000 mg/L HOCl more suppressed the detection of SARS-CoV-2 compared with 1000 mg/L HOCl ($Z = -2.747$, $p < 0.01$), see Figure 2A,B,E,F. When 1000 mg/L HOCl was used as disinfectant in the simulation laboratory, both the concentration of SARS-CoV-2 and the fluorescence value of platform stage of the amplification curve decreased gradually with the extension of incubation time with the PCR-MixR, see Figure 2A. When the disinfectant was replaced with 5000 mg/L HOCl, SARS-CoV-2 concentration and the fluorescence value of platform stage decreased more obviously, see Figure 2B. When ethanol and PCR-MixR were incubated together, the concentration of SARS-CoV-2 had a decreasing trend after 2 h, but the fluorescence value during the amplification platform stage of the amplification curve did not decrease significantly; but when incubated together for 3 h,

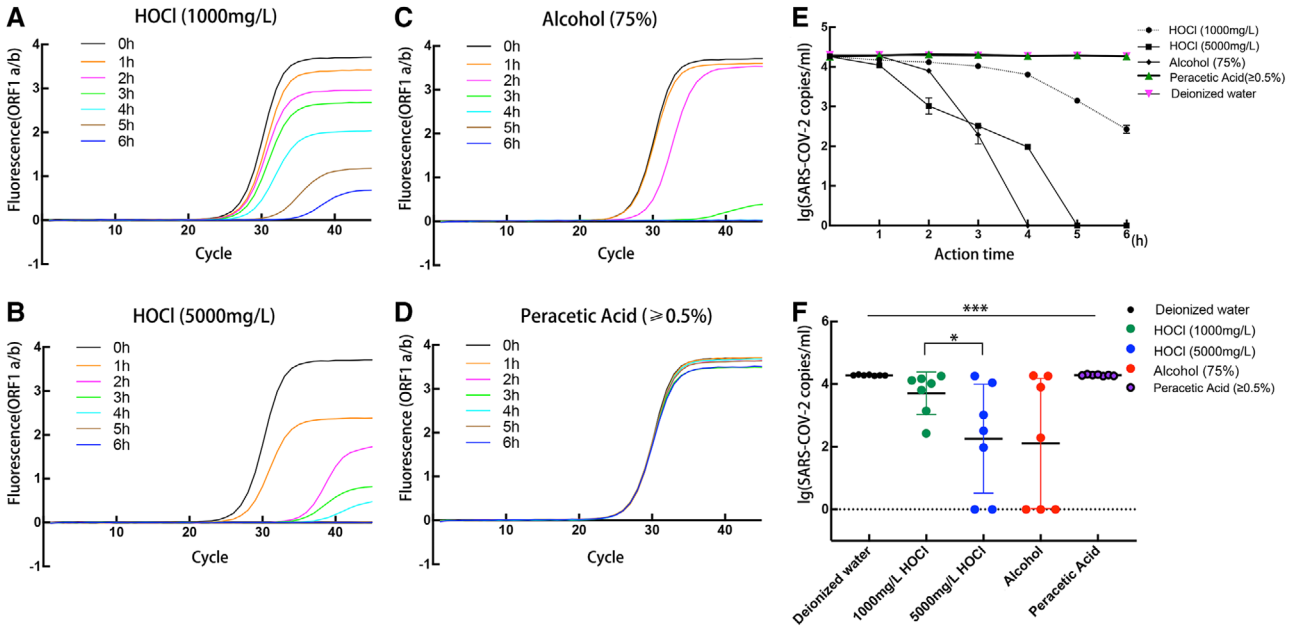

Figure 2. The effect of different environmental disinfectants on detection ability of SARS-CoV-2.

SARS-CoV-2 can hardly be detected anymore, see Figure 2C. In addition to HOCl, the 75% ethanol disinfectant also hindered the detection of SARS-CoV-2 nucleic acid, resulting in a decrease in the concentration of SARS-CoV-2 after 3 h of exposure ($Z = -1.964$, $p < 0.001$). Meanwhile, it was found that the influence of HOCl on SARS-CoV-2 NAAT gradually increased with prolonged exposure time, while the influence of ethanol changed more greatly since SARS-CoV-2 could hardly be detected after exposure ethanol for 4 h. However, peracetic acid had no effect on the detection of SARS-CoV-2 after exposure for 0 to 6 h ($Z = 0.232$, $p > 0.05$), as shown in Figure 2D.

The ABCD respectively represent the amplification reaction fluorescence curves of the SARS-CoV-2 S2 with different disinfectants and different times. E and F shows the change in the SARS-CoV-2 S2 copies detected when its amplification system was placed with different disinfectants at different times and the abscissa represent the value of the SARS-CoV-2 S2 copies after taking log10, $^{*}p < 0.05$,$^{**}p < 0.01$,$^{***}p < 0.001$.

4 Discussion

SARS-CoV-2 is a novel beta-coronavirus with high homology to SARS-CoV [17] that has attracted much attention worldwide. Laboratory viral NAAT, such as real-time reverse-transcription polymerase chain reaction (RT-PCR), has been recommended by the WHO as a standard confirmation protocol for SARS-CoV-2 detection [3]. NAAT shows better performance than immunoassays, as it can identify viral RNA in the early stage of infection. Since 1988, PCR technology has been extensively used in clinical laboratories due to its advantages of high sensitivity and ease of use [4]. However, RT-PCR often leads to a false-negative result, leading to infected patients undergoing repeated sampling. Our RT-PCR results unexpectedly found that many positive quality control products showed negative results in daily testing. Korlević, P. and Yu, M. [18,19] found that HOCl can destroy the DNA structure when it remains on the surface of various substances. This destruction can damage the bases in DNA [20] and the tertiary structure of proteins [21]. For the above reasons, we tried to determine the influence of hypochlorite disinfectant on the detection ability of SARS-CoV-2 by RT-PCR.

SARS-CoV-2 copies significantly decreased with increasing HOCl concentration and ambient humidity by HOCl evaporated. We simulated a SARS-CoV-2 nucleic acid testing laboratory. The PCR-Mastermix was subjected to HOCl concentrations of 5000, 1000 and 0 mg/L for different time periods, and the changes in the SARS-CoV-2 viral load were detected by NAAT. The number of SARS-CoV-2 copies significantly decreased with increasing HOCl concentration. When we changed the ambient humidity by changing the volatile surface area of the HOCl, we found that the larger the volatile area of the HOCl was at a constant concentration of 1000 mg/L HOCl, the more easily the PCR amplification reaction system was damaged. Many researchers have also proven that HOCl can be used as an effective nucleic acid detergent to reduce or even avoid nucleic acid cross-contamination [18,22,23]. Whiteman et al. found that HOCl attacks nucleotides and individual DNA bases *in vitro*, and HOCl at various concentrations was added to a reaction mixture containing calf thymus DNA in 50 mM phosphate buffer [24]. Whiteman et al. demonstrated that HOCl induces DNA base modification by damaging DNA with reactive oxygen species [25]. Moreover, the destruction increased as the chlorine concentration increased [22,26].

To investigate which substances in the PCR amplification system are damaged by disinfectants, the PCR amplification system was divided into four experimental groups and two control groups. When the six groups acted together with disinfectants, these disinfectants had differential effects. The SARS-CoV-2 detection ability of the two control groups with deionized water and empty tubes did not change with prolonged exposure, which excluded inhibition of subsequent PCR reactions by HOCl aerosols dissolved into deionized water and deposition of HOCl aerosols into empty PCR tubes. After the dNTPs, primers and probes interacted with HOCl for 2 h, SARS-CoV-2 could hardly be detected by RT-PCR. HOCl has been shown to attack nucleotides and individual DNA bases *in vitro* [24–28] and to inactivate various DNA repair enzymes [29,30]. HOCl and hypochlorite (OCl^-), its conjugate base, have been shown to efficiently chlorinate cellular DNA and RNA nucleobases [31,32]. N.R. Stanley et al. [33] found the ability of HOCl to form chlorinated base products on nucleosides, nucleotides, DNA, and in cellular systems. Jean Cadet et al. [20] summarized and reviewed dedicated to DNA damage on recent aspects of the formation and measurement of oxidatively generated damage in cellular DNA including single pyrimidine and purine base lesions, purine 5', 8-cyclonucleosides, DNA-protein adducts, and interstrand cross-links with the HOCl and other hydroxyl radical. Kang et al. [34] found that inflammation-mediated neutrophil-derived HOCl can damage DNA to induce transition mutations and perturb epigenetic signals. The destruction of PCR enzymes by environmental HOCl increased with the extension of the interaction time, which can also be seen in our study. Pattison [21] also proved that hypochlorous acid can mediate protein oxidation by destroying the tertiary structure of proteins. HOCl reacts rapidly with biological materials by targeting related proteins [35]. Excessive HOCl production can lead to host tissue damage, which is implicated in human diseases such as atherosclerosis, cystic fibrosis, and arthritis. In our study, we also found that the primers and probes damaged by HOCl in the PCR system were more severe than those damaged by enzymes or extracted RNA, which could be seen in group a curve dropped more than group d as shown in Figure 1E. We speculate that the destruction of nucleotide bases by HOCl may be random. The damage for short sequences of primers or probes is complete as long as one the base damaged, while the damage site of long sequences of RNA may not be within the range of the specific gene base sequence. This hypothesis needs to be verified by our subsequent experiments.

After determining the impact of the use of HOCl disinfectants on the detection amount of SARS-CoV-2, we also explored the impact of other disinfectants, such as 75% ethanol and peracetic acid, on the detection of SARS-CoV-2 by NAAT. We found that 5000 mg/L HOCl showed a greater influence than 1000 mg/L HOCl. An ethanol disinfectant (75%) also affected the detection of SARS-CoV-2, resulting in a false-negative concentration of SARS-CoV-2 after exposure for 4 h. However, peracetic acid had no effect on the detection of SARS-CoV-2 nucleic acid even after exposure for 6 h. Despite a lack of experimental evidence *in vitro*, Wu et al. [36–38] suggested that reactive oxygen species produced during ethanol intake may induce oxidative DNA damage in mice and that rat gastric mucosa exposed to ethanol also showed DNA fragmentation. The DNA damage caused by peracetic acid was significantly weaker than that caused by chlorine. Zhang et al. [39] discovered that chlorine had a more pronounced impact on the functionality of plasmid DNA because it oxidizes or destroys bacterial components. Even though peracetic acid efficiently kills bacteria, bacterial plasmids and other mobile genetic elements might still be intact and functional after peracetic acid disinfection.

Above all, we found that if PCR reagents and high concentration of HOCl stay in the same enclosed space, the virus copy number will be reduced or even false negatives during the SARS-CoV-2 detection by RT-PCR. We recommend that laboratories minimize the use of disinfectants during the NAAT of SARS-CoV-2, use a lower concentration of HOCl as much as possible under the reasonable protection level of personnel, and regularly ventilate to maintain the humidity range as low as possible while acceptable to the human body. Additionally, we believe that it is a good choice to disinfect the environment and surface of objects in the laboratory as soon as possible after the experiment. With the rapid development of the epidemic, accurate and rapid detection of the SARS-CoV-2 is particularly important. The effective operation of laboratory quality management can greatly reduce the risk of missed detection of COVID-19 patients due to experimental errors and ensure the prevention and control of the epidemic.

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The authors have declared no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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