



## Ozone Water Is an Effective Disinfectant for SARS-CoV-2

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Dear Editor,

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative pathogen of the pandemic coronavirus disease 2019 (COVID-19), which has infected nearly 90 million people and resulted in 2 million human deaths by the end of the year 2020. SARS-CoV-2 can survive in aerosols or on the surfaces of various materials for up to 72 h (Ong *et al.* 2020; van Doremalen *et al.* 2020). In China, although the human-to-human transmission pathway is under well control, the fomite transmission pathway now accounts for a significant fraction of new infection cases. Therefore, environmental disinfection of public areas and workspaces potentially contaminated by SARS-CoV-2 is an important measure to control the spread of COVID-19 by fomite transmission. A disinfectant that is safe and readily available at a low cost is urgently needed to meet environmental disinfection requirements.

Ozone is a highly reactive oxidant gas composed of three oxygen atoms and widely used in industry and medical waste disinfection (Elvis and Ekta 2011; Smith *et al.* 2017). A broad spectrum of viral pathogens (Sato *et al.* 1990), including SARS-CoV (Zhang *et al.* 2004), can be effectively inactivated by ozone treatment. The purpose of this study was to evaluate whether ozone water is a potential disinfectant to eliminate the environmental contamination of SARS-CoV-2.

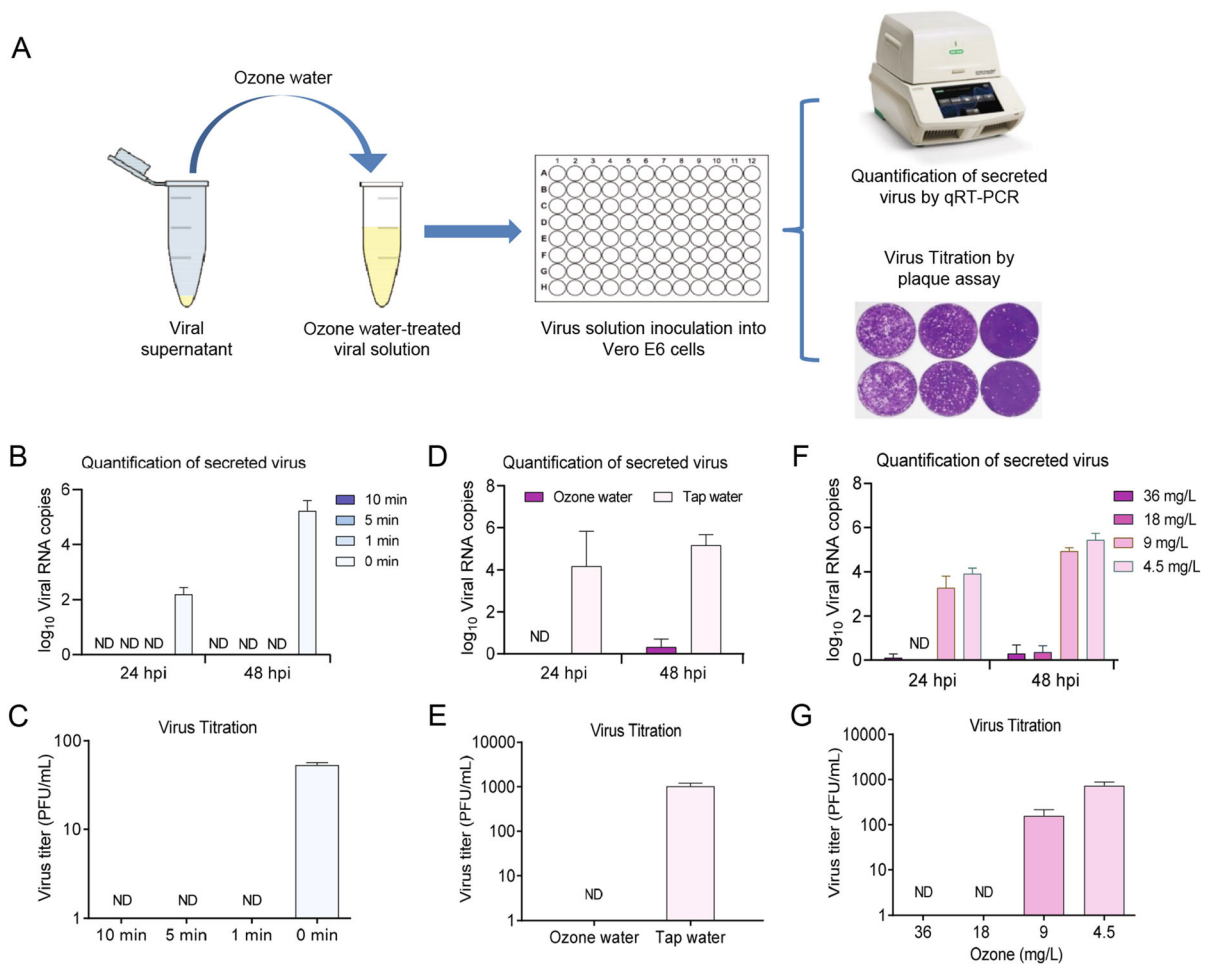
The scheme of the experiment is showing in Fig. 1A. Briefly, 50  $\mu$ L viral supernatant was incubated with 225  $\mu$ L ozone water or unozonated tap water. After different incubation periods, the disinfection reaction was terminated by adding 225  $\mu$ L stopping buffer (complete culture media with 5 g/L sodium thiosulfate). The resulting mixture was then inoculated into Vero E6 cells. At 24 and 48 h post-infection (hpi), the genomic RNA of secreted viruses was quantified by reverse transcription-polymerase chain reaction (qRT-PCR) assay using a Taqman probe targeting the *N* gene of SARS-CoV-2 (Primers: 5'-TAACCAG AATGGAGAACGCAGTG-3'/5'-TGAGTGAGAGCGGTG AACCAAGAC-3'; Probe: 5'-ATCAAACAACGTCGGCC CCAAGGT-3'/HEX + BHQ2) (Zheng *et al.* 2020). In parallel, the viral titers were determined by viral plaque assay at 96 hpi.

A recent report showed that the Ct values of SARS-CoV-2 from various contaminated materials were generally between 25 to 32 (Ong *et al.* 2020). Based on this information and the observed correlation between Ct values and plaque-forming units (PFU) of SARS-CoV-2 (Uhteg *et al.* 2020), we estimated the viral PFU from common environmental contamination is approximately ranging from 0 to 10<sup>2</sup> PFU/mL. To check whether ozone water can inactivate environmental SARS-CoV-2 contamination, we

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**Fig. 1** Ozonated water inactivates SARS-CoV-2. **A** Scheme of the experiment. The viral supernatant was incubating with ozone water. After disinfection reaction, the mixture was then inoculated into Vero E6 cells. The secreted viruses in the supernatant were collected at 24 hpi and 48 hpi, respectively, and subjected to qRT-PCR assay. The titers of viruses were determined by viral plaque assay at 96 hpi. **B**, **C** Contact time required for ozone water inactivation of SARS-CoV-2. Viral solution ( $4.0 \times 10^3$  PFU/mL) was incubating with ozone water (36 mg/L) for 0, 1, 5, 10 min, respectively. The disinfection efficacy was evaluated by qRT-PCR (**B**) and viral plaque assay (**C**). **D**, **E** Disinfection capacity of ozone water. A higher PFU viral solution

( $4.0 \times 10^4$  PFU/mL) was incubated with ozone water (36 mg/L ozone) or tap water (0 mg/L ozone), respectively. Similar tests were performed to quantify viral RNA and titers by qRT-PCR (**D**) and plaque assay (**E**). **F**, **G** Ozone concentration required for ozone water inactivation of SARS-CoV-2. Serially diluted ozone water (36, 18, 9, 4.5 mg/L) was incubating with the viral solution ( $4.0 \times 10^4$  PFU/mL) for 1 min, and the disinfection efficacy was determined by qRT-PCR (**F**) and viral plaque assay (**G**), as mentioned above. The data points indicated the averages of triplicate experiments, and the error bars represent the standard error of the mean (SEM). ND: not detected.

diluted SARS-CoV-2 stock to  $4.0 \times 10^3$  PFU/mL, a 10-times higher PFU than the estimated upper limit of environmental virus contamination. The viral solution was incubated with ozone water (36 mg/L ozone) for 0, 1, 5, or 10 min, respectively. No viral genomic RNA or viral plaques were detected after ozone water treatment for 1 min (Fig. 1B, 1C), suggesting that ozone water could effectively inactivate SARS-CoV-2 in less than 1 min. When viral PFU increased to  $4.0 \times 10^4$  PFU/mL, ozone water (36 mg/L ozone) could still fully inactivate 50  $\mu$ L viral solution in less than 1 min compared to tap water-treatment (Fig. 1D, 1E).

To further determine the minimal ozone water concentration required to inactivate SARS-CoV-2, ozone water was serially diluted and incubated with the viral solution ( $4.0 \times 10^4$  PFU/mL) for 1 min to inactivate the virus. We found that SARS-CoV-2 was fully inactivated by ozone water harboring 36 mg/L and 18 mg/L ozone, as neither viral genomic RNA nor viral plaques were detected (Fig. 1F, 1G). However, when ozone concentration was below 9 mg/L in ozone water, the genomic RNA copy number of SARS-CoV-2 was detected in the range of  $4.0 \times 10^3$  and  $5.0 \times 10^5$  copies/mL (Fig. 1F), and the virus titer was between  $1.0 \times 10^2$  and  $7.0 \times 10^2$  PFU/mL

(Fig. 1G). The above results implied that ozone concentration above 18 mg/L could effectively eliminate SARS-CoV-2 infectivity within 1 min.

In summary, we demonstrated that ozone water is an effective disinfectant in the elimination of SARS-CoV-2. Compared to the traditional chemical disinfectant, ozone water offers several advantages. Firstly, ozone water is eco-friendly and will not cause a secondary hazard to the environment after its administration. The residual ozone in the water can be decomposed rapidly and converted to oxygen within a short period (typically within 30 min) under natural conditions. This feature is highly valuable for food disinfection. Secondly, an ozone water generator's price is usually as low as 8,000 Yuan in China, which can convert tons of tap water into ozone water in less than 30 min. Such ease of production at a low cost makes ozone water far superior to traditional chemical disinfectants, particularly when a large amount of disinfectant is needed to disinfect a large area, *e.g.*, a cold warehouse for imported food, which is usually contaminated with SARS-CoV-2. In contrast, traditional disinfectants like ethanol, the price is usually over 10,000 Yuan per ton in China. Thirdly, SARS-CoV-2 can be inactivated by ozone water within 1 min, which is very efficient. In addition to ozone water, other studies also showed that ozone gas is highly effective in eliminating SARS-CoV-2 (Criscuolo *et al.* 2021). In our opinion, ozone gas has a considerable advantage in disinfecting virus contamination in the aerosol, whereas ozone water is more effective in surface disinfection simply by immersing contaminated material in the ozone water. However, both forms of ozone must be administered with caution to prevent lung injury due to the inhalation of ozone gas.

Although further study is needed to evaluate the disinfection efficacy of ozone water in real-world conditions, our study provides the first step in the proof of principle before applying ozone water in environmental settings.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Animal and Human Rights Statement** This article does not contain any studies with human or animal subjects performed by any of the authors.

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