奈良県立医科大学
Nara Medical University

Test Report

Addressed to: FUJI FAINES Co., Ltd.

Evaluation of the inactivating effect of ozone gas generator

on SARS-CoV-2

February 18, 2021

Department of Microbiology and Infectious Diseases

Nara Medical University

We would like to kindly inform you of the subject regarding our research with your company in cooperation with MBT Consortium Association and BIOTEK ENVIRONMENTAL SCIENCE LTD.

Notes

1. Purpose of the study

To assess inactivation effects of ozone gas on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

2. Tested products

Ozone gas generator（Trade name: O3 Max Air, Model number: OY-FF90-A）

3. Test pathogen: SARS-CoV-2

VeroE6 cells were infected with SARS-CoV-2, and cells exhibiting confirmed cytopathic effects were harvested and cryopreserved at -80 °C. The samples were subjected to two freeze-thaw cycles and were subsequently centrifuged. Supernatants were concentrated and purified using an ultrafiltration membrane. The resulting ultrafiltrates were used as virus test solutions and were cryopreserved at -80 °C until testing.

4. Test details

- The virus solution was smeared onto a 20 μl petri dish and dried.
- The plates were placed in an ozone-proof airtight acrylic box with the device generating ozone gas.
- The plates were then exposed at a concentration of 1.0 ppm ozone for 2 hours at relative humidity of 45% and 80%.
- The ozone concentration of 0.1 ppm was set based on the measured ozone concentration under the actual operating conditions of the model by an independent organization, and the experiment was conducted by reproducing the actual model in a box.
- Only one specimen was placed in the enclosed space in each experiment, and the specimen was removed after the end of the test time.
- After exposure, 2 ml of SCDLP medium with sodium thiosulfate was dropped onto the specimen, and the virus was collected using a cell scraper.
- Virus infectivity titers (PFU/mL) were determined by plaque assays using the recovered fluid.

The inactivating effect was calculated as follows:

$$\text{Inactivating effect (Mv)} = \log(Ct/C_0) - \log(Nt/N_0)$$
$$= \log Ct/Nt$$

Ct: Infection titer after t hours of control

C_0: Infection titer after 0 hours of control

Nt: Infection titer after t hours of test product

N_0: Infection titer after 0 hours of test product

The rate of decrease was calculated from the logarithmic decrease as follows:

Rate of decrease rate = $(1 - 1/10^{\text{logarithm reduction value}}) \times 100\%$.

All tests were conducted at the biosafety level-3 (BSL3) laboratories of the Nara Medical University under appropriate pathogen containment measures.

5. Results

The results were shown in Tables 1and 2 and in Figures 1.

After 2 hours of ozone gas exposure to 9.06×10^6 PFU/mL of SARS-CoV-2, the infectivity titers decreased to 7.50×10^2 PFU/mL (99.990% decrease) at relative humidity of 45% and below the detection limit of 1.00×10^2 PFU/mL (> 99.998% decrease) at relative humidity of 60%, respectively.

Table 1. Changes in viral titer by test sample

Duration (hours)	0	2
Control	9.06E+06	8.00E+06
Ozone gas（relative humidity of 45%）	9.06E+06	7.50E+02
Ozone gas（relative humidity of 60%）	9.06E+06	＜1.00E+02

Detection limit: < 1.00E+02

Table 2. Viral inactivating effect of the test sample and rate of reduction

	Ozone gas （relative humidity of 45%）	Ozone gas （relative humidity of 60%）
Virus inactivation effect (Mv)	4.03	4.90
Decrease	99.990%	＞99.998%

The rate of decrease (%) is rounded down to the fourth decimal place.

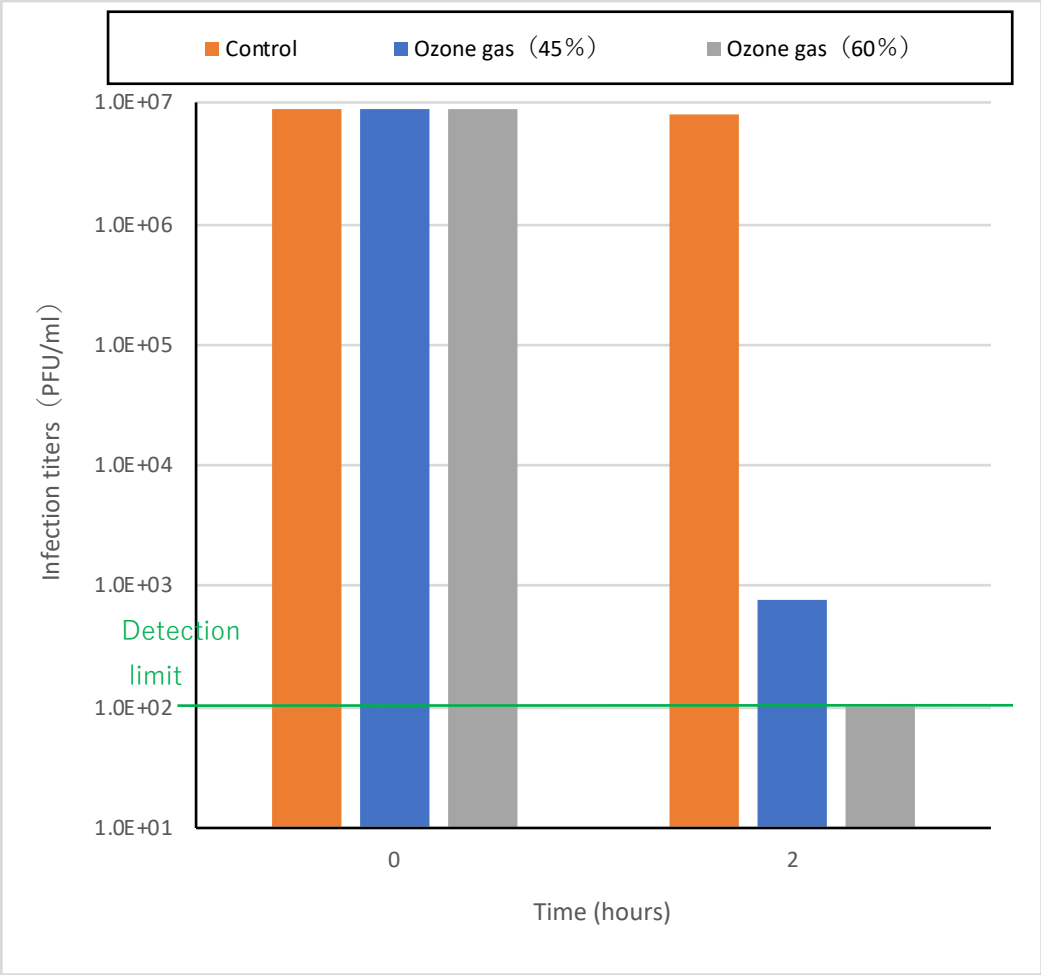

Figure 1. Changes in viral titer

Figure 2. Experimental scene（left）and equipment（right）

6． Conclusions

The ozone gas assessed here inactivated SARS-CoV-2. Our findings indicated that the ozone gas tested in this study might be effective for the prevention of transmission of SARS-CoV-2 via surfaces; however, this study did not assess potential effects of the tested products on airborne viruses or on the human body.

We certify that the results of the tests conducted were as described in this report.

Department of Microbiology and Infectious Diseases
Nara Medical University