A study to examine the virus inactivation effect of the test material

—Study Report—

Study number: 217183N

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1. Study title

   A study to examine the virus inactivation effect of the test material

2. Study number

   No.217183N

3. Study purpose

   This study was conducted to confirm the virus inactivation effect of the test material when reacted with the novel coronavirus (SARS-CoV-2).

Name, address, and name of the head of the testing facility

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Address:	561-21 Arakuchi-machi, Maebashi, Gunma, Japan
Name of the head:	Kazuhiro Kubo, President and CEO

Name of study director

Tomohide Kamiya

Name of study personnel

Nobusato Endo

5. Study schedule

Study was contracted on:	September 21, 2021
Study started on:	November 8, 2021
Study ended on:	November 30, 2021

6. Test material

   Test material 1: Bamboo vinegar (undiluted)

   Test material 2: Bamboo vinegar (diluted by 10 times)

   *Sterile phosphate buffer solution was used as the control material.

7. Microorganism used in this study

SARS-CoV-2 (novel coronavirus)

*A human-derived virus strain isolated from saliva. After isolation and culture using vero cells, real-time PCR (the method described in the notification from the Ministry of Health, Labour and Welfare) was performed to confirm the amplification of the SARS-CoV-2 gene.

Cultured cell: Vero cell (cell lineage derived from kidney epithelial cells of African green monkey)

8. Study design

Condition	Treatment	Sensitization time
Control	1 mL of virus solution was added to 10 mL of phosphate buffer solution	0, 1, and 60 minutes after the test start
Test 1	1 mL of virus solution was added to 10 mL of the Test material 1	1 and 60 minutes after the test start
Test 2	1 mL of virus solution was added to 10 mL of the Test material 2	1 and 60 minutes after the test start

9. Test method

The test was performed in reference to "Method for virus neutralization test" in "Introduction to Experiments in Virology, 2nd revised edition" (Maruzen Publishing, in Japanese).

10. Test procedure

1. Preliminary test

Before performing the test, each test material was serially diluted (10-fold), inoculated into cultured cells, and cultured for 5 days at 37°C and 5% CO_2. If the cultured cells did not show normal shape, the material was judged to be cytotoxic; the dilution factor at which cytotoxicity was confirmed was excluded from the test judgment in this study.

As a result, the cell growth was poor in the 10-fold diluted Test material 1, whereas no poor cell growth was observed in the 10-fold diluted Test material 2. Therefore, it was determined that the mixture of the Test material 1 and the virus solution needed to be diluted 10-fold before inoculated into the cells. The virus was added at the concentration of 10^6 $TCID_{50}$/mL.

2. Main test, mixing of test solution

According to the study design, 10 mL of the test materials or phosphate buffer solution were dispensed, and the virus solution was added.

After the addition of 1 mL of the virus solution, the mixture was kept still for a predetermined time at room temperature (25°C).

3. Main test, inoculation to the cells

After the completion of the sensitization, 10-fold serial dilutions of the mixture were prepared for each test condition, and 100 μL of each of these solutions were inoculated into cells cultured in 96-well plates.

Judgment was made by observing the cultured cells microscopically after 5 days of incubation at 37°C and 5% CO_2; viral replication was confirmed by the presence of CPE (cytopathic effect) appeared in the cultured cells, and the concentration at which CPE was observed was calculated.

4. Evaluation

On the basis of the test results, percent reduction in the test condition (%, relative to control) was calculated for each test time point to confirm the effectiveness.

In this study, the percent reduction was calculated by the following formula.

$$\text{Percent reduction (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

11. Results

The results of the test using SARS-CoV-2 are shown in Table 1 and Figure 1.

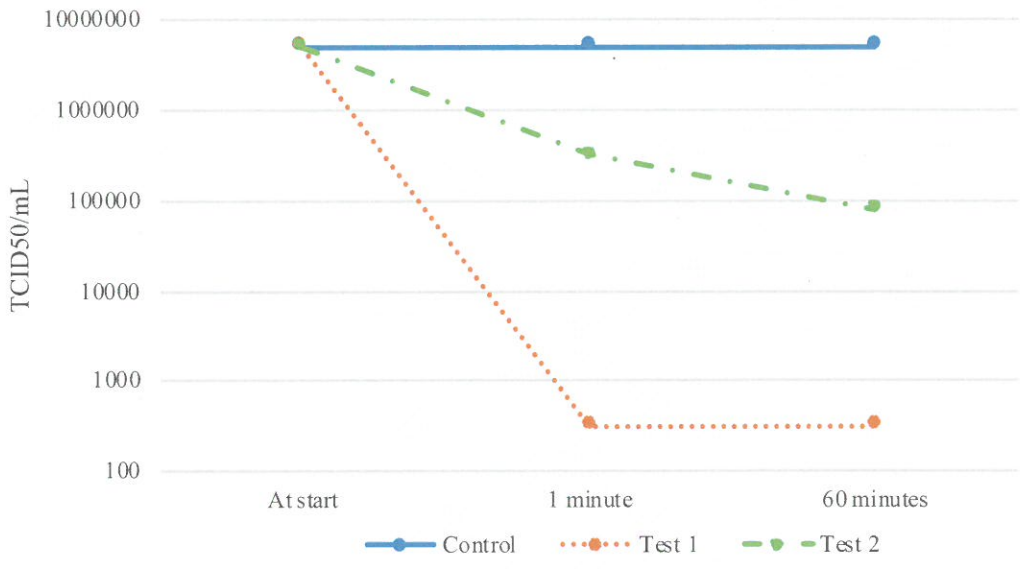
In the control condition, there was no change in viral titer throughout the test period, from the test start to 60 minutes after the start ($10^{6.7}$ $TCID_{50}$/mL).

In the Test 1 condition, the virus titer decreased to $<10^{2.5}$ $TCID_{50}$/mL (percent reduction: 99.99% or more) at 1 minute after the test start; in the Test 2 condition, the virus titer decreased to $10^{5.5}$ $TCID_{50}$/mL (percent reduction: 93.60%) at 1 minute and $10^{4.9}$ $TCID_{50}$/mL (percent reduction: 98.42%) at 60 minutes.

Table 1. SARS-CoV-2 test results ($TCID_{50}$/mL)

Condition	At start	1 minute later	60 minutes later
Control	$10^{6.7}$	$10^{6.7}$	$10^{6.7}$
Test 1		$<10^{2.5}$	$<10^{2.5}$
Test 2		$10^{5.5}$	$10^{4.9}$

Figure 1. SARS-CoV-2 test result



12. Discussion

In this study, inactivation effect of the test materials against SARS-CoV-2 (novel coronavirus) were examined.

The results showed that the inactivation effect was more than 99.99% at 1 minute for the Test material 1, and 93.60% at 1 minute and 98.42% at 60 minutes for the Test material 2.