Virus inactivation study of the test material

—Study report—

Study number: 217025N

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1. Study title

   Virus inactivation study of the test material

2. Study number

   No.217025N

3. Purpose

   This study was conducted to confirm the inactivation effect of the test materials against the novel coronavirus (SARS-CoV-2).

4. Organizations involved in the study management

   Sponsor

Name:	Thanks Ai Corporation
Address:	6-1-6 Goryo, Higashi-ku, Kumamoto City, Kumamoto, 861-8035, Japan

   Testing facility

Name:	Shokukanken, Inc
Adderss:	561-21 Arakuchi-machi, Maebashi, Gunma, 379-2107, Japan
Facility manager:	Kazuhiro Kubo (CEO)

   Study director

   Tatsuya Suzuki

   Study personnel

   Nobusato Endo

5. Study schedule

Study was contracted on:	April 13, 2021
Study started on:	June 10, 2021
Study ended on:	July 6, 2021

6. Test materials

   Test material 1: Perfect Mineral Ai (undiluted solution)

   Test material 2: Perfect Mineral Ai (10-fold diluted solution)

   * Sterile phosphate buffer solution was used as control material.

7. Virus used for the study

SARS-CoV-02 (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	Add 1 mL of virus solution to 10 mL of phosphate buffer solution	0, 15, and 60 seconds
Test 1	Add 1 mL of virus solution to 10 mL of test material 1	15 and 60 seconds
Test 2	Add 1 mL of virus solution to 10 mL of test material 2	15 and 60 seconds

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 material was diluted 10 times, 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, cytotoxicity was confirmed in the 10-fold diluted solution. Therefore, the detection limit in this study was set at $10^{2.5}$ $TCID_{50}$/mL.

2. Main test, mixing of test solution

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

After the addition 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

A 10-fold dilution of the mixture was prepared for each test condition, and 100 μL of these mixtures 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 (degeneration of the cells) appeared in the cultured cells, and the concentration at which CPE was observed was calculated.

4. Evaluation

On the basis of the test results, percentage reduction in the test condition (%, relative to control) was calculated for each test time point to confirm the effectiveness.
In this study, the percentage reduction was calculated by the following formula.

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

11. Results

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

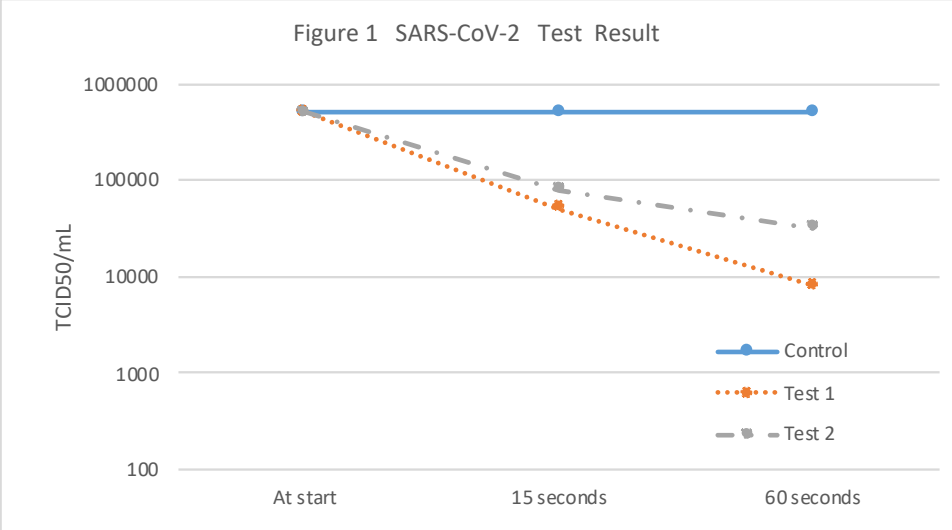
In the control condition, there was no change in virus titer during the first 5 minutes after the start of the test ($10^{5.7}$ $TCID_{50}$/mL).

In the "Test 1" condition, virus titers were $10^{4.7}$ $TCID_{50}$/mL (90.0% decrease) at 15 seconds and $10^{3.7}$ $TCID_{50}$/mL (99.0% decrease) at 60 seconds.

In the "Test 2" condition, virus titers were $10^{4.9}$ $TCID_{50}$/mL (84.2% decrease) at 15 seconds and $10^{4.5}$ $TCID_{50}$/mL (93.7% decrease) at 60 seconds.

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

Condition	At start	15 seconds	60 seconds
Control	$10^{5.7}$	$10^{5.7}$	$10^{5.7}$
Test 1		$10^{4.7}$	$10^{3.7}$
Test 2		$10^{4.9}$	$10^{4.5}$



12. Discussion

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

As a result, inactivation effect of test material 1 was determined to be 90.0% at 15 seconds and 99.0% at 60 seconds; inactivation effect of test material 2 was determined to be 84.2% at 15 seconds and 93.7% at 60 seconds.