VIRUS INACTIVATION EFFECT TEST OF TEST SOLUTION
STUDY REPORT

STUDY NUMBER:207282N

Shokukanken Inc.
561-21 Araguchi-cho, Maebashi City, Gunma,JAPAN

DATE: December 14, 2020

1. TITLE

Virus inactivation effect test of test solution

2. STUDY NUMBER

207282N

3. SPONSOR

Name: Thanks AI Corporation

Address: 6-1-6 Goryou, Higashi-ku, Kumamoto-City, Kumamoto, JAPAN

4. TEST FACILITY

Name: Shokukanken Inc.

Address: 561-21 Araguchi-cho, Maebashi City, Gunma, JAPAN

Test Site

Manager: Kubo Kazuhiro

5. PERSONNEL RESPONSIBLE FOR STUDY

Study

Director: Shohei Matsumoto

Analysis

Operator: Kondo Miki

6. DATE

Initiation Date: July 20, 2020

Experimental Completion Date: December 14, 2020

7. TEST VIRUS

Influenza virus：swine influenza virus H1N1 IOWA stock (IFV)

Cultured cells：MDCK Cell (Canine kidney-derived cell line)

Porcine epidemic diarrhea virus P-5V (PEDV)

※Swine infectious coronavirus

Cultured cell: Vero cells (cell line derived from African epithelial kidney epithelium)

8. TEST SAMPLE(LIQUID)

Name: Perfect Mineral Ai

※The test sample used was a stock solution and a 10-fold diluted solution.

9. TEST GROUP

Group	Sample	Inspection time	Number of iterations
			Virus
Control	Sterilized saline	0, 1min, 6hrs	1
Test1	stock solution	1min, 6hrs	1
Test2	10-fold diluted solution	1min, 6hrs	1

10. TEST METHOD

(1) Mixing of test virus solution and measurement of virus infectivity

Before performing the test, the test sample was serially diluted 10-fold, inoculated into vero cells, and cultured at 37° C and 5% CO_2 for 5 days. When vero cells did not show a normal shape (CPE), it was determined that there was cytotoxicity due to the material, and in this test, the dilution ratio in which cytotoxicity was confirmed was excluded from the test.

As a result, cell degeneration was confirmed in the stock solution.

① 1 mL of the samples was dispensed into the each test tube.

② 0.1 mL of Virus solution was mixed in the sample.
Immediately after mixing, the mixture was stirred for 1 second with a mixer and then left standing at 25°C.

③ For the Control group, the sample was taken from the test tube immediately after mixing, 1 minutes after mixing and 6hours after mixing, dispensed into another container, and serially diluted 10-fold with MEM medium.

④ For the Test group, samples were taken from the test tube at 1 minutes after mixing and 6hours after mixing, dispensed into another container, and serially diluted 10-fold with MEM medium.

⑤ Cells were inoculated with the diluted solution and then cultured at 37 ° C and 5% CO 2 for 5 days.

⑥ The viral infectivity ($TCID_{50}$) was measured based on the presence or absence of CPE expression in the Cells after culturing.

(2) Evaluation

From the test results, the reduction rate of the Test Group with respect to the Control Group was calculated, and the effect was confirmed.

$$\text{Reduction rate (\%)} = \frac{\text{Control titer -Test titer}}{\text{Control titer}} \times 100$$

11. RESULT

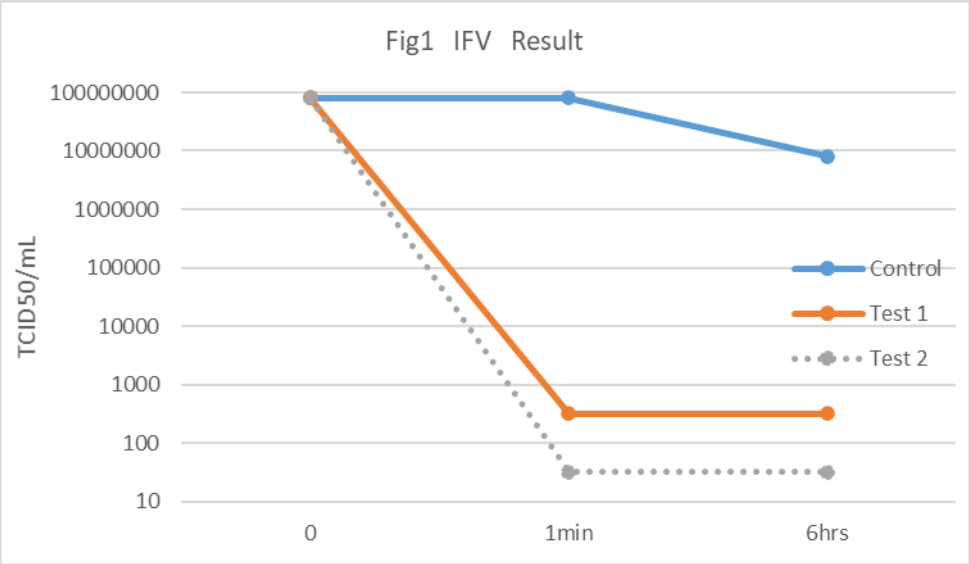
1) IFV

The virus titer in the Test group 1 was less than $10^{2.5}$ $TCID_{50}$ / mL (limit of ditection) 1 minute after inoculation and Test group 2 was less than $10^{1.5}$ $TCID_{50}$ / mL (limit of ditection) 1 minute after inoculation.

Compared to the Control group, the reduction rate of the Test sample 1 and 2, 1 minute after inoculation was more than 99.999% and 99.9999% .

Table1 IFV result($TCID_{50}$/mL)

Group	0min	1min	6hrs
Control	$10^{7.9}$	$10^{7.9}$ (80000000)	$10^{6.9}$ (8000000)
Test 1		$<10^{2.5}$ (<320)	$<10^{2.5}$ (<320)
Test 2		$<10^{1.5}$ (<32)	$<10^{1.5}$ (<32)



2) PEDV

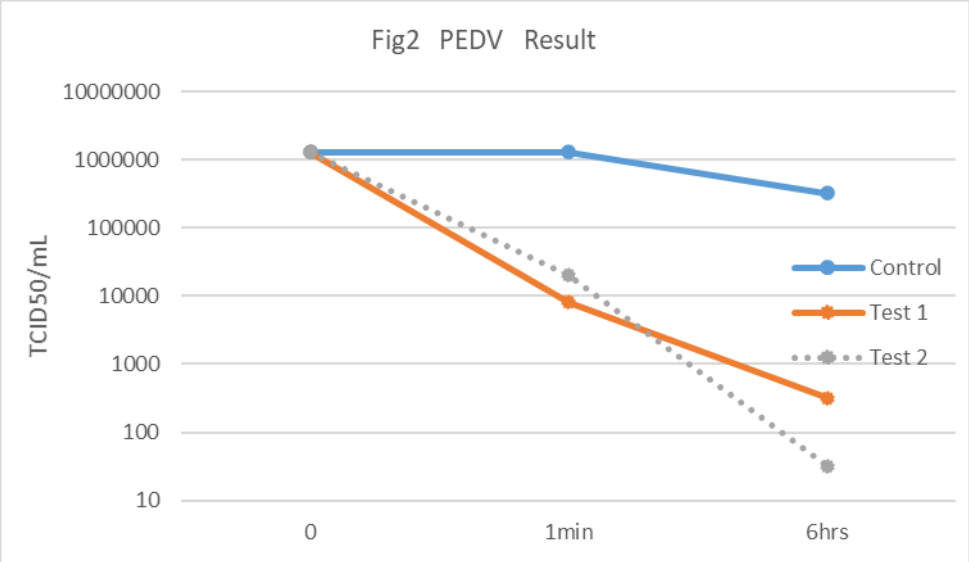
The virus titer in the Test group 1 was $10^{3.9}$ $TCID_{50}$ / mL 1 minuite after inoculation, less than $10^{2.5}$ $TCID_{50}$ / mL (limit of ditection) 6 houres after inoculation.

The virus titer in the Test group 2 was $10^{4.3}$ $TCID_{50}$ / mL 1 minuite after inoculation, less than $10^{1.5}$ $TCID_{50}$ / mL (limit of ditection) 6 houres after inoculation.

Compared to the Control group, the reduction rate of the Test sample 1 and 2, 6 hours after inoculation was more than 99.90% and 99.99% .

Table2 PEDV result($TCID_{50}$/mL)

Group	0min	1min	6hrs
Control	$10^{6.1}$	$10^{6.1}$ (1300000)	$10^{5.5}$ (320000)
Test 1		$10^{3.9}$ (8000)	$<10^{2.5}$ (<320)
Test 2		$10^{4.3}$ (20000)	$<10^{1.5}$ (<32)



12. CONCLISION

This test was conducted to confirm the virus inactivating effect of the test solution on IFV and PEDV.

As a result of the test, a significant decrease in the viral infectivity of IFV and PEDV were observed 1 minute after the test product and Virus were mixed.