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Test report

Inactivation of classical swine fever virus by ALTANT

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[E-Tech Co., Ltd. translated this report from "Japanese version" to "English version"]

[Purpose]
Evaluate the inactivating effect of “ALTANT” on classical swine fever virus.

[Materials and methods]
1. Virus
Classical swine fever virus GPE-strain (infection value 107.5$TCID_{50}$/ml)

2. examined goods
“ALTANT”

3. Infectious titer measurement cells
Pig kidney-derived cell line (SK-L cell)

4. Protein detection reagent for infectious titer measurement
Nano Glo HiBiT Lytic Detection System (Promega)

5. Virus inactivation test
900 μl of the test product was placed in a test tube, 100 μl of GPE-strain diluted 5-fold with sterile distilled water (DW) was added thereto, and the mixture was mixed with vortex and reacted at 25℃.
After 15 seconds, 1 minute, and 5 minutes, 100 μl of each was collected, and 0.3 sodium thiosulfate and 400 μl of horse serum were added as a stop solution to prepare a sample for infectious titer measurement.
In addition, sterile DW was used instead of the test product as a control.

The infectious titer measurement sample and the control sample were each diluted 10-fold with MEM (including 10% horse serum), and 50 μl of the 10-fold serial dilution was added to 4 holes of the 96-well plate, and the cells derived from porcine kidney (strained cells derived from pig kidney) SK-L cells) were simultaneously injected in 100 μl.
After 4 days, the reaction was carried out with the Nano Glo HiBiT Lytic Detection System (Promega), the luciferase activity was measured, and then the virus infectivity titer ($TCID_{50}$/ml) was calculated.

6. Confirmation after stop
90 μl of the test product or sterile DW was placed in a test tube, and 10 μl of 0.3% sodium thiosulfate and 400 μl of horse serum were added as a stop solution and mixed with a vortex.

Add 10 μl of GPE-strain 5 times diluted with sterile DW to the reaction solution after shutdown, dilute each 10 times with MEM (including 10% horse serum), and add 50 μl of 10 times diluted solution to 4 holes of 96-well plate , 100 μl of porcine kidney-derived cell (SK-L cells) was simultaneously injected therein.

After 4 days, the reaction was carried out with the Nano Glo HiBiT Lytic Detection System (Promega), the luciferase activity was measured, and then the virus infectivity titer ($TCID_{50}$ /ml) was calculated.

[Result]

1. Virus inactivation test (Fig. 1, left and center)

In the test product, the virus fell below the detection limit in 15 seconds.

The virus infection rate has been reduced to 1/1000 compared to DW.

2. Check the stop solution (Fig. 1, left and right)

As a result of adding the virus after the reaction with the stop solution, the results were similar to those when DW was used in the virus inactivation test.

Therefore, it was confirmed that the reaction of the test product was stopped by the stop solution.

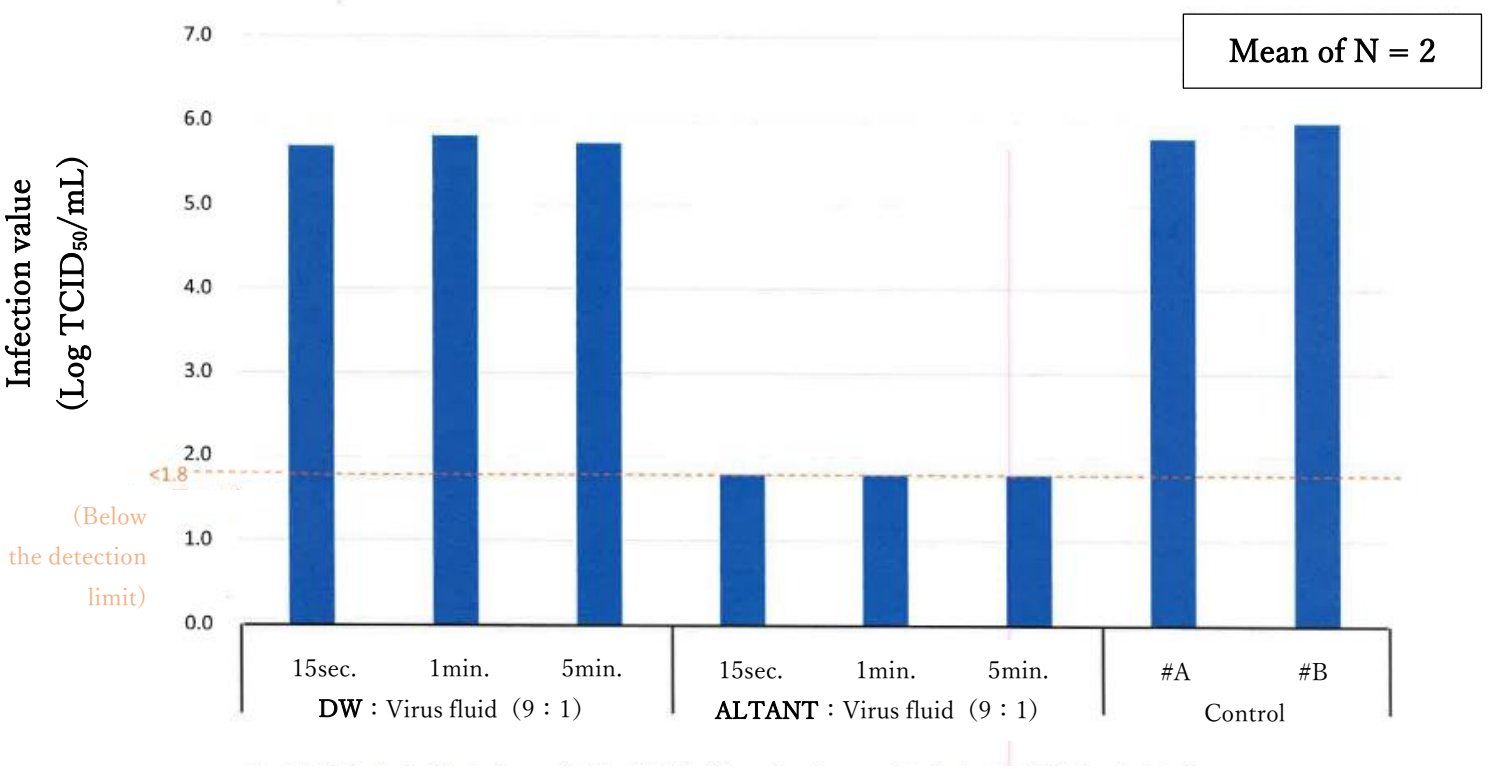
[Summary and consideration]

"ALTANT" was found to instantly inactivate the classical swine fever virus.

When using pigs in the field, it is desirable to additionally investigate the effects of organic matter.

Fig. 1

Inactivation of classical swine fever virus GDE-/ Hibit strain by "ALTANT"



#A: After mixing DW, sodium thiosulfate and serum, virus solution was added and the infectious titer was measured.

#B: After mixing altanto, sodium thiosulfate and serum, virus was added and the infectious titer was measured.