Test Report

Inactivation test of feline calicivirus by "ALTANT"

2017.9.22

Kitasato Research Center for Environmental Science

1. Test purpose

The inactivation effect of Feline calicivirus (substitution for norovirus) by "Altant" provided by your company.

2. Requester

name: E · Tech Co., Ltd.

location: Hyogo Prefecture Kobe City Chuo-ku Minatojima 9-1 Kobe Incubation Office 316

3. Laboratory

name: Kitasato Research Center for Environmental Science

location: 252-0329Kanagawa prefecture Sagamihara-shi Minami-ku Kitasato 1-15-1

Responsible: Virus Department Virus Division

4. Test period

2017.9.5～2017.9.8

5. Grained goods

1) "ALTANT"

2) IPA (control)

Each stock solution was used

6. Test Condition

working temperature: 25± 2 ℃

working time: control: 0(initial), 15 seconds、1minute、 5minutes

ALTANT、IPA; 15seconds、1 minute、5minutes

7. Test virus

Feline calcivirvs, F-9,ATCC VR-782

8. Cells for infectivity measurement

CRFK: Crandell- Rees feline kidney

9. Test method

1) How to prepare virus solution

The virvs was infected with CRFF,and when about 90% or more of the cell culture area showed cytopathic effect, it was frozen in a freezer at -30° C.

Thereafter, freeze-thaw operation was performed, and the supernatant was centrifuged at 2,380×g for 10minutes. The supernamant was collected,and the virus solution concentrated with an ultrafiltration membrane was stored at -80% C as a storage virus.
For the test,virus solution diluted 10-fold with PBS(phosphate buffered saline) was used as test virus solution.

2) Virus Inactivation test

The virus Inactivation effect test was conducted according to the following procedure.
0.1ml of the test virus solution was added to 0.9ml of the test product in a test tube,mixed gently by a test tube,mixed gently by a test tube mixer,and allowed to act at room temperature for a predetermined time.
The stopping of the test on the virus was diluted 100-fold with 0.1% sodium thiosurfate-added SCDLP broth medium to prepare a sample for measuring the virus infectivity value.
In addition,PBS was used in place of the specimen for working time 0(initial) and "control". The effectiveness of suspension was confirmed by a separate test. The procedure and results are shown on page 12.

3) Measure virus infect ivity value

CRFK cells were seeded in a 12-well plate and cultured for 4days in a CO2incubator.
Prior to ingesting the virus, the culture supernamant was removed and replaced with fresh medium.
Next,the stock solution of the virus infectivity value measurement sample was serially diluted 10times with PBS.
Wells from which the culture solution was removed wer inoculated with 0.1ml of a 10-fold serial dilution of stock solution for infectivity titration sample or PBS, and cells were infected with virus at 37° C for 1hour.
After virus inoculation, the plate were shaken every 15minutes to prevent drying and absorb the virvs evenly across the cells.
After 1 hour,a plaque forming meditm was added to each well and allowed to solidify,followed by culturing in a CO2 incubator.
After cultivation,1ml of PBS (4% formalin／PBS) with 4% formalin was added to each well of a 12-well plate and allowed to stand at room temperature for 30minutes. Thereafter,4% formalin／PBS and agarose were removed and 5% crystal violet 1ml of methanol added was added to each well to stain the cells.
The wells were washed with tap water,air-dried, aod the number of plaques formed by the growth of the virus was counted to obtain the virus infectivity value(PFU／ml) per

1ml of the sample for measuring the virus infectivity value. In addition, when the solution after stopping the operation of the test product shows toxicity to cells for infectivity measurement, it becomes difficult to measure the infectivity value and the detection limit value also changes, so a separate confirmatary test was conducted. Procedures and results are shown in section 12.

4) Calculation of log infectivity value log reduction value.

Based on the initial infectivity value of the control and the infectivity value after the action of the test product, the infectivity value log reduction value(LRV was calculated using the following calculated values) In addition, LRV is represented by one digit after the decimal point (rounded down to the second place)

A formula

$LRV = \log_{10}$(Initial infectivity value of control ÷ Infectivity value after test article action)

10. Test results

The test results are shown in Table 1 and Table 2. Also, as reference data, pictures of plaques are shown in Photo-1.

The test result of the virus inactivation test is the virus infectivity value per 1ml of the sample for measuring the virus infectivity value log reduction value (LRV : log reduction value).

Early viral infectivity was 7.2×10^5PFU／ml.

No significant fluctuation in virus infectivity was observed ,even when "control(PBS)" was allowed to act for a maximum of 5minutes.

On the otherhand, when the virus was allowed to act on "ALTANT" for15 seconds, 1minute and 5minutes, it become less than the detection limit value(10 PFU／ml) in 15 seconds.

The LRV at each action time was > 4.8 in 15seconds.

Also, when IPA was given a virus for 15seconds, 1minute, and 5minutes, the LRV of each action time was 0.5, 0.9 and 1.2, respetively.

11. Comment

In this study, we evoluated the inactivation effect of feline calcivirvs(substitution for norovirus) by the company supplied by your company.

As a criterion for disinfecting effect according to EN14476:2005 (Chemical disinfectants and antiseptics. Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine.

In the present study," ALTANT" had LRV greater than 4.0 with action for 15seconds, and a virus inactivating effect was observed.

In general, it is reported that the disinfecting effect is affected by the virus inactivating effect by organic matter in the environment.

Depending on the use environment of the test, disinfection effect may be affected by organic substances, and it is important to consider appropriate usage.

From now on, consider the effect of virus inactivation under conditions loaded with organic matter.

Table-1

Grained goods	working time			
	0(initial)	15sec	1min	5min
control(PBS)	7.2×10^5	7.8×10^5	8.0×10^5	7.9×10^5
Altant		＜10	＜10	＜10
IPA		2.2×10^5	8.5×10^4	4.3×10^4

Infectious value unit : PFU/ml

Test virus infectivity : 4.7×10^8 PFU/ml

Detection limit value : 10 PFU/ml

Table-2

Grained goods	The virus infectivity value log reduction value (LRV : log reduction value) a)		
	15sec	1min	5min
control(PBS)	0.0	0.0	0.0
Altant	>4.8	>4.8	>4.8
IPA	0.5	0.9	1.2

a) Log_{10} (Early viral infectivity / Infectivity value after action for a predetermined time)

12. Efficacy confirmation test of ation stop solution

1) The purpose

The effectiveness of the stopping solution used for stopping the in activating effect of the test virus by the test product was confirmed.

2) Method

As a stop solution for the test product,a method of diluting 100times with a 0.1% sodium thiosurfate- added SCDLP broth medium was adopted.

0.1ml of PBS was added to 0.9ml of the test product in stead of the virus solution, and the solution diluted with the action stopping solution was designated as "test sample".

1ml of test sample was in oculated with 0.1m of virus solution and allowed to act at room temperature for 5minutes.

This solution was used as a stock solution and virus infectivity titer was measured.

The effectiveness of the infectivity value did not decrease by more than 0.5log10 as compared with the control(PBS).

3) Result

The results are shown in Table3.

The infectivity value when the virus was applied to the "Efficacy test sample" was within the criteria compared to "control(PBS)" . Based on the above result,it was judged that the stop solution was effective for the test specimen.

T able-3　Confirming the validity of the stop solution

Confirmation of Efficacy-stop effectiveness a)	Virus infectivity value	Infection value log reduction value b)	Effectiveness of outage c)
ALTANT	3.3×10^5	0.0	Effectiveness
IPA	5.0×10^5	0.0	Effectiveness
control(PBS)	3.3×10^5		

Infectious value unit : PFU/ml

a) Samples to be tested were diluted 100-fold with 0.1% sodium thiosurfate added SCDLP broth medium

b) $\log_{10}$(Infectivity value of control/ infectivity value of test sample

c) A case where it did not decrease by 0.5$\log_{10}$ or more relative to the control was judged as effective.

13. Cytotoxicity confirmation test

1) the purpose

When the tested product shows cytotoxicity to the cells to be cultured of the virus to be tested, it is difficult to measure the virus infectivity value. Therefore, the toxicity to CRFK cells was confirmed using the test product after the inactivation, Detection limit value was examined.

2) Method

0.1 ml of PBS was added to 0.9 ml of the test product instead of the virus solution, and the solution diluted with the action stop solution was designated as "sample for cytotoxicity confirmation". This solution was inoculated into a CRFK cell previously cultured on a 96-well plate with a sample stock solution for measurement or diluted virus solution in an amount of 25 μL per well, and allowed to stand in a CO 2 incubator at 37 ° C. for 1 hour, then inoculated The solution was removed, 100 μL / well of DMEM supplemented with 1% FBS was added and cultured for 1 day in a CO 2 incubator.

After culturing, the cells were stained with crystal violet and cytotoxicity was confirmed by the degree of staining of each well. Cytotoxicity is determined by determining the viable cell ratio of cells cultured by adding a sample for confirming cytotoxicity with the viable cell ratio set to 100% when cultured with addition of PBS, and when it is less than 50%, there is cytotoxicity .

3) Results

The test results are shown in Table 4. In this test, cytotoxicity was not observed even in the stock solution of the sample for cytotoxicity confirmation. Therefore, the detection limit value in this test was 10 PFU / mL.

TABLE-4 Toxicity of test article to CRFK cells

Sample for confirming cytotoxicity a)	Viable cell ratio b) (mean ± standard deviation)		Determination of cytotoxicity c)
	Stock solution	10 times diluted solution	
ALTANT	95 ± 0	118 ± 2	No toxicity
IPA	111 ± 3	116 ± 3	No toxicity

a) Samples to be tested were diluted 100-fold with 0.1% sodium thiosulfate added SCDLP broth medium

b) The average value and standard deviation of 4 wells are shown.

c) When the viable cell ratio was less than 50%, it was judged that there was cytotoxicity.

reference data

Plaque of feline calicivirus

photo1 Inactivation effect of test article on feline calicivirus