Japan Food Research Laboratories

Test Report

No. 209060705-001

July 2, 2009

Client: Advanced Textiles Development Center, Toray Industries, Inc.

Sample: Antibacterial/antifungal/antivirus/anti-allergen processed sheet 0906-1

Title: Virus Inactivation Test

The following are the test results of the above samples submitted to the JFRL on June 8, 2009.

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Virus Inactivation Test

1. Client

Advanced Textiles Development Center, Toray Industries, Inc.

2. Sample

Antibacterial/antifungal/antivirus/anti-allergen processed sheet 0906-1

3. Purpose of the test

Perform an inactivation test on sample viruses

4. Overview of the test

A viral suspension of influenza virus or feline calicivirus (substitute virus of norovirus) was poured onto a sample cut into two pieces measuring about 3 cm x 3 cm and stacked together (hereinafter referred to as "sample"). Viral infectivity was measured after storing the sample at room temperature for 24 hours.

It should be noted that a preliminary test was conducted in advance to examine the cytopathic effect of the sample.

5. Test results

The results are shown in Table 1.

In addition, with regard to the working solution of the sample diluted 100-fold with a cell maintenance medium, it was confirmed through preliminary tests that cytopathic effects due to the sample was not observed.

It should be noted that feline calicivirus is widely used as a substitute virus for norovirus, which cannot be cultured in cells.

Table 1 Results of measurement of viral infectivity of sample working solution

Test virus	Measurement	Target	log $TCID_{50}$/ml[*1]
Influenza virus	Immediately after inoculation	Control	7.0
	24 hours later[*3]	Sample[*4]	<2.5
		Control (1)	6.7
Feline calicivirus[*2]	Immediately after inoculation	Control	6.8
	24 hours later[*3]	Sample[*4]	<2.5
		Control (2)	6.5

$TCID_{50}$: Median tissue culture infectious dose. 50% tissue culture infectious dose

<2.5: No detection

Control (1): Plastic petri dish

Control (2): Standard cloth (cotton)

Viral suspension: Diluted 10-fold with PBS

*1) $TCID_{50}$ logarithmic value per I ml of working solution

*2) Substitute virus for norovirus

*3) Storage at room temperature

*4) Sample cut into two pieces measuring about 3 cm x 3 cm and stacked together

6. Test method

1) Test virus

Influenza A virus (H1N1)

Feline calicivirus F-9 ATCC VR-782 (feline calicivirus)

2) Cells used

Influenza virus: MDCK (NBL-2) cells ATCC CCL-34 strain [Dainippon Pharmaceutical Co., Ltd.]

Feline calicivirus: CRFK cells [Dainippon Pharmaceutical Co., Ltd.]

3) Medium used

A. Cell growth medium

Eagle MEM medium “Nissui” (1) [Nissui Pharmaceutical Co., Ltd.] with 10% fetal bovine serum added was used.

B. Cell maintenance medium

Influenza virus: 1,000 ml

10% NaHCO3: 14 ml

L-glutamine (30 g/l): 9.8 ml

100×MEM vitamin solution: 30 ml

10% albumin: 20 ml

0.25% trypsin: 20 ml

Feline calicivirus:

Eagle MEM medium “Nissui” (1) with 2% fetal bovine serum added was used.

4) Preparation of viral suspension

(1) Cell culture medium

Cell growth medium was used for cell culture, and the cells used were cultured in a single layer in a tissue culture flask.

(2) Virus inoculation

After monolayer culture, the cell growth medium was removed from the flask and inoculated with the test virus. Next, a cell maintenance medium was added and cultured in a carbon dioxide incubator (CO_2 concentration: 5%) at 37 °C ± 1 °C for 1 to 5 days.

(3) Preparation of viral suspension

After culturing, the morphology of the cells was observed using an inverted phase contrast microscope, and it was confirmed that morphological changes (cytopathic effect) occurred in the cells. Next, the culture solution was centrifuged (3,000 rpm, 10 minutes), and the resulting supernatant was used as a virus suspension.

5) Preparation of sample

The sample was cut into two pieces measuring about 3 cm x 3 cm and stacked together to be used as a sample.

6) Test operation

0.2 ml of the viral suspension was poured onto the sample and stored at room temperature.

7) Washing-out of the virus

After 24 hours of storage, the viral suspension of the sample was washed out with 2 ml of cell maintenance medium.

8) Measurement of viral infectivity

Cell culture medium was used, and the cells used were monolayer cultured in a tissue culture microplate (96 wells). Then the cell growth medium was removed, and 0.1 ml of cell maintenance medium was added. Next, the working solution and 0.1 ml of the diluted solution were inoculated to every 4 wells and cultured in a 37 °C carbon dioxide incubator (CO_2 concentration: 5%) for 4 to 7 days. After culturing, the presence or absence of cell morphological change (cytopathic effect) was observed using an inverted phase contrast microscope; the 50% tissue culture infectious dose ($TCID_{50}$) was calculated by the Reed-Muench method and converted to the viral infectivity per ml of working solution.