Test report

No. 204070398-002

3 August, 2004

Applicant Mitsubishi Paper Mills, Ltd.

Specimen Aller Sweep

Title Virus inactivation test

The result of the test to the specimen above is as follows on 2 July, 2004.

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Virus inactivation test

1. Applicant

   Mitsubishi Paper Mills, Ltd.

2. Specimen

   Aller Sweep

3. The purpose of the test

   To investigate the efficiency of the inactivation against the influenza virus.

4. Test outline

   A drug in which floating the influenza virus was dropped into a sample (hereinafter referred as a "sample") cut into a size 3*3 cm, and the virus infection was measured over time after it was stored at room temperature.

5. The result of the test

   Figure-1 shows the result.

Figure-1 the result of virus infectivity measurement of washout sample

Test virus	Measurement	Target	Log $TCID_{50}$/ml*1
Influenza virus	Soon after vaccination	Contrast	5.2
	2 hours later	Specimen	3.5
	6 hours later	Specimen	2.5
		Contrast	5.5
	24 hours later*2	Specimen	2.7
		Contrast	5.3

$TCID_{50}$: median tissue infectious dose

*logarithmic value per 1ml of washout sample

Contrast: plastic petri dish

6. Test method

(1) Test virus

Influenza virus type A (H1N1)

(2) Test cell

MDCK (NBL-2) cell ATCC CCL-34 line (Dainippon Pharma Co., Ltd.)

(3) Medium

①cell growth medium

Used Eagle MEM (included 0.06mg/ml of Kanamycin) with 10% of newborn calf serum.

②cell maintenance medium

Used below medium of composition.

Eagle MEM	1,000ml
10% $NaHCO_3$	12ml~22ml
L-glutamine (30g/l)	9.8ml
100*vitamin liquid for MEM	30ml
10% Albumen	20ml
2.5% Trypsin	2ml

(4) Preparation of virus-floating drug

①Culturing the cell

The cell for the test is monolayer cultured in tissue culturing flask, using cell growth medium.

②Vaccination of the virus

After the monolayer culture, the cell growth medium was removed from the flask and the test virus was inoculated. Then, the cell maintenance medium was added and they were cultured in a carbon dioxide incubator (CO2 concentration 5%) at 37 degree for 2~5 days.

③Preparation of virus-floating drug

After the culture, we observed the form of the cell by using inverted phase contrast microscope and we could confirm that the cell change the form (Cytopathic Effect). Then, the cultured liquid was centrifuged and its supernatant liquid was used as a virus suspension.

(5) The preparation of sample

We made the specimen by cutting them approx. 3*3cm and sterilizing with wet heat.

(6) Test operation

Drop 0.2ml of virus floating liquid to the sample and store it at room temperature.

(7) Washout the virus

After storing the sample for 2, 6 and 24 hours, virus floating liquid was washed by 2ml of cell maintenance medium.

(8) Measurement of virus infection rate

First, MDCK cell was monolayer-cultured by tissue culture microplate (96 holes), using cell growth medium. Then, removed it and cell maintenance medium was added by 0.1ml. Second, the sample washout liquid and 0.1 ml of the diluted liquid were inoculated into each 4 holes and it was cultured by carbon dioxide gas incubator (CO2 concentration 5%) at 37 degree for 4~7 days. After that, we observed the form of the cell changing the form (Cytopathic Effect) by using inverted phase contrast microscope and calculate 50% of culture infectious dose ($TCID_{50}$)by Reed-Muench method and converted them into the rate of infection of the Virus per 1ml.