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Japan Food Research Laboratories

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January 06, 2011

REPORT

Client: TOKYO ELECTRON Co., Ltd.
1-1-21 Karakida, Tama-shi, Tokyo 206-0035, Japan

Sample(s): KTM-100GL (without hot air)

Title: Virus Inactivation Test

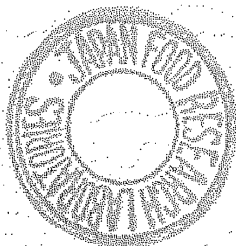
Received date of sample(s): December 10, 2010

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Jan. 13, 2011

Noriko Imaizumi
Principal Investigator

Date





Virus Inactivation Test

1. Client
TOKYO ELECTRON Co., Ltd.

2. Sample
KTM-100GL (without hot air)

3. Purpose
This test aims to evaluate virus inactivation of the sample.

4. Outline of methods
Feline calicivirus suspension (0.1 ml) was added dropwise to each petri dish (diameter 60 mm), and the petri dishes were placed at about 5 and 7 cm from the ultraviolet lamp in around the center of the sample. Then, they were stored at room temperature under the rays of the ultraviolet lamp, and virus infectivity was determined. Feline calicivirus is a commonly used as a surrogate for Norovirus, which has not been cultured routinely.

5. Results
Table 1 shows the results of the test.

Table 1. Virus infectivity of virus suspensions after operation of the sample

Test organism	Condition	log TCID ₅₀ /ml ^{*1}	
Feline calicivirus ^{*2}	No operation	6.0	
	5 cm ^{*3}	Approximately 10 seconds	2.3
		Approximately 20 seconds	<1.5
	7 cm ^{*3}	Approximately 10 seconds	3.3
		Approximately 20 seconds	1.8

TCID₅₀: Median tissue culture infectious dose

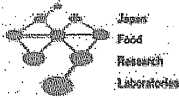
*1 Logarithm of TCID₅₀ per 1 ml of the virus suspension

*2 Surrogate virus for Norovirus

*3 Distance from the light source

Virus suspension: 10-fold dilution (diluted with purified water)

<1.5: Not detected



6. Methods in detail

1) Test virus

Feline calicivirus F-9 ATCC VR-782

2) Test cell

CRFK cells (Dainippon Pharmaceutical Co., Ltd.)

3) Culture media

(1) Cell culture medium

Eagle's MEM "Nissui"(1) (Nissui Pharmaceutical Co., Ltd.) added 10 % of fetal bovine serum

(2) Cell support medium

Eagle's MEM "Nissui"(1) added 2 % of fetal bovine serum

4) Preparation of virus suspensions

(1) Cell incubation

The test cell was monolayer-cultured in a tissue culture flask using the cell culture medium.

(2) Virus inoculation

After monolayer culture, the culture medium was removed from the flask, and the test virus was inoculated. Next, the cell support medium was added, and the virus was cultured in a CO₂ incubator (CO₂: 5 %) at 37 °C ± 1 °C for 1 to 5 days.

(3) Preparation of virus suspensions

Following culture, the cell form was observed with an inverted phase-contrast microscope. After morphological changes (cytopathic effects) were confirmed, the culture solution was centrifuged (3000 r/min, 10 minutes). The supernatant was diluted to 10-fold with purified water to make a virus suspension.

5) Test procedure

Virus suspension (0.1 ml) was added dropwise to each petri dish (diameter 60 mm). The petri dishes were placed at about 5 and 7 cm from the ultraviolet lamp in around the center of the sample. Under the illumination of the ultraviolet lamp, they were stored at room temperature.

6) Infectivity assays

The test cell was monolayer-cultured in a tissue culture microplate (96-well) using the cell culture medium. After removal of the culture medium, 0.1 ml of the cell support medium was added. Next, 0.1 ml of the diluted virus suspension was poured to four wells and cultured in a CO₂ incubator (CO₂: 5 %) at 37 °C ± 1 °C for 7 to 10 days. After culture, the cells were observed by an inverted phase-contrast microscope to determine whether any morphological changes (cytopathic effects) had occurred. Then, median tissue culture infectious dose (TCID₅₀) was calculated by Reed-Muench method and converted into virus infectivity liter per 1 ml of the virus suspension.

End of Report