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REPORT

Client: THALGO JAPON Co., Ltd.
19 Ichiban-cho, Chiyoda-ku, Tokyo 102-0082, Japan

Sample(s): KALUNITE SOLID Saturated solution (Lot. 2020)

Title: Virus Inactivation Test

Received date of sample(s): July 23, 2020

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Signed for and on behalf of JFRL

JAPAN FOOD RESEARCH LABORATORIES

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Section of Analysis Documentation

Oct. 13, 2020

Date

Virus Inactivation Test

1. Client

THALGO JAPON Co., Ltd.

2. Sample

KALUNITE SOLID Saturated solution (Lot. 2020)

3. Outline of the method

Influenza virus suspension was added to the sample and used as the test solution. After the designated storage periods, the virus infectivity titer of the test solution was determined. The method for determining the virus infectivity titer was validated by a preliminary test.

4. Results

1) Preliminary test (confirmation of the conditions for neutralization)

The preliminary test confirmed that the test solution should be diluted with cell support medium so that the virus infectivity titer could be determined without the effect of the sample.

2) Determination of virus infectivity titers

Table 1 shows the results. Tables 2 and 3 show the cells and media used in the test and the test conditions, respectively.

Table 1. Virus infectivity titers of test solutions

Test organism	Object	log $TCID_{50}$/mL			
		Initial	After 1 min.	After 5 min.	After 10 min.
Influenza virus	Sample	–	<3.5	<3.5	<3.5
	Control (purified water)	7.0	–	–	6.8

$TCID_{50}$: Median tissue culture infectious dose
Storage temperature: Room temperature
<3.5: Not detected

Table 2. Cells and media used in test

Test cell	MDCK (NBL-2) cells JCRB 9029 strain	
Cell culture medium	Eagle's MEM "Nissui" (1) containing 10 % fetal bovine serum (Nissui Pharmaceutical Co., Ltd.)	
Cell support medium	Eagle's MEM "Nissui" (1)	1000 mL
	10 % $NaHCO_3$	14 mL
	L-glutamine (30 g/L)	9.8 mL
	100 × Vitamins for MEM	30 mL
	10 % albumin	20 mL
	0.25 % trypsin	20 mL

Table 3. Test conditions

Test virus	*Influenza A virus* (H1N1) A/PR/8/34 ATCC VR-1469
Virus suspension	The virus culture solution after cell incubation was centrifuged and the supernatant liquid was used.
Test solution	The virus suspension (0.1 mL) was added to the sample (1 mL).
Reaction conditions	1, 5 and 10 minutes (Room temperature)
Neutralization conditions	The test solution was diluted 1000-fold with the cell support medium.
Control	Purified water
Determination method for virus infectivity titer	$TCID_{50}$ method

End of Report