JFRL

Japan Food Research Laboratories
Accredited by the Japanese Government
52-1 Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan
http://www.jfrl.or.jp/

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REPORT

Client: FLAX Co., ltd.
4-1 Hommokumotomachi, Naka-ku, Yokohama-shi, Kanagawa 231-0822, Japan

Sample(s): Hypochlorous acid water Generation bottle「ZIA pocket」

Title: Virus Inactivation Ability Confirmation Test of Generated Water

Received date of sample(s): August 21, 2018

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Signed for and on behalf of JFRL

JAPAN FOOD RESEARCH LABORATORIES

T. Arai
Takeko Arai
Section of Analysis Documentation

Dec. 14, 2018
Date

Virus Inactivation Ability Confirmation Test of Generated Water

1. Client

FLAX Co., ltd.

2. Sample

Hypochlorous acid water Generation bottle 「ZIA pocket」
Sodium chloride was provided by the client.

3. Outline of the method

Purified water and sodium chloride were added to the sample and mixed. The sample was then allowed to operate and the generated water was used as the test water.

The test water was added with *Influenza virus* suspension (hereafter called "the test solution"). At a designated measurement point, the virus infectivity titer of the test solution was determined. The method for determining a virus infectivity titer was validated by a preliminary test.

4. Results

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

It was confirmed that the sample should be diluted with cell support medium so that the virus infectivity titer could be determined without the effect of the sample (shown in Table 3, Neutralization conditions).

2) Virus infectivity assay

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

Table 1: Virus infectivity titers of the test solutions

Test organism	Object	log $TCID_{50}$/mL			
		Initial	After 30 sec.	After 1 min.	After 5 min.
Influenza virus	Test water	−	<1.5	<1.5	<1.5
	Control (1)	−	<1.5	1.7	1.7
	Control (2)	6.3	−	−	6.0

$TCID_{50}$: Median tissue culture infectious dose
Test water: 180 mL of purified water and 1 g of sodium chloride were added to the sample and mixed. The sample was then allowed to operate for 3 minutes.
Control (1): 1 g of sodium chloride was added to 180 mL of purified water.
Control (2): Purified water
Storage temperature: Room temperature
<1.5: Not detected
Virus suspension: A culture solution was diluted 10-fold with purified water.

Table 2: Cells and media used in the test

Test cell	MDCK (NBL-2) cells ATCC CCL-34 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) 10 % $NaHCO_3$ L-glutamine (30 g/L) 100 × Vitamins for MEM 10 % albumin 0.25 % trypsin	1000 mL 14 mL 9.8 mL 30 mL 20 mL 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. The supernatant liquid was diluted 10-fold with purified water.
Test water	180 mL of purified water and 1 g of sodium chloride were added to the sample and mixed. The sample was then allowed to operate for 3 minutes.
Test solution	1 mL of the test water was added with 0.1 mL of the virus suspension.
Reaction conditions	Time: 30 seconds, 1 and 5 minutes Temperature: Room temperature
Neutralization conditions	The test solution was diluted 10-fold with the cell support medium.
Control (1)	1 g of sodium chloride was added to 180 mL of purified water.
Control (2)	Purified water
Determination method for virus infectivity assay	$TCID_{50}$ method

End of Report