March 15,2010
KRCES Report #21_0184(1)

Prepared for:
BAN-YU Co., Ltd

Test Report

Antiviral efficacy of a glass plate treated with non-photo catalyst, BY-50

Kitasato Research Center of Environmental Sciences
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1. Aim of the test

To investigate the antiviral efficacy of a glass plate treated with non-photo catalyst, BY-50.

2. Client

Company: BAN-YU Co., Ltd

Address: 7F, 2-4-10 Kawaramachi, Chuo-ku, Osaka-shi, Osaka, Japan

3. Test organization

Kitasato Research Center of Environmental Sciences

Address: 1-15-1 Kitasato, Minami, Sagamihara, Kanagawa, Japan

4. Test sample and condition

Glass plate treated with non-photo catalyst, BY-50 (the glass plate which passed for 116days after treating, treated date: October 26th 2009).

Testing sample and reaction time are summarized in Table 1.

Table 1 Test condition

Test sample	Reaction time (min)	
	0	30
Glass plate treated with BY-50	○	○
Control glass plate		○

5. Test virus

Influenza A virus (H1N1)

6. Preparation of virus

Influenza A virus was inoculated into the allantoic cavity of embryonated chicken eggs. These eggs were incubated at 37℃. After 3 days, the virus multiplying in the allantoic fluid was harvested and purified by the sucrose density gradient centrifugation method.

7. Methods

1) Test procedure

A test piece (25mm×60mm) was placed into a polystyrene case, and then 0.02mL of virus solution with infectivity titer of approx. $8.4\times10^{8}TCID_{50}$/mL was dripped onto the surface of test piece. The samples were covered with a 25mm×60mm of the polypropylene film to increase contact efficiency between the virus and the test sample. These were incubated for 30 minutes at room temperature. After incubation, the virus was recovered in phosphate buffered saline (PBS).

The reaction time "0" means the time when the virus was recovered immediately after it was dripped on the untreated sample.

2) Measurement of infectivity

Viral infectivity titers in test samples were determined by observation of a cytopathogenic effect of influenza virus in Madin-Darby canine kidney (MDCK) cells. Fifty μL of the 10-fold serial dilution of samples and 50 μL of MDCK cell suspensions were added into 96-well microwell plates. After an incubation for 4 days at 37℃ in a CO_2 incubator, virus-induced cytopathogenic effect was observed using an inverted microscope. The virus titer was calculated by the Reed-Muench method as virus titers ($TCID_{50}$/mL). These $TCID_{50}$ values were then transformed [$\log_{10}$] to express a log reduction value (LRV).

8. Test results

Test results are summarized in Table 2 and Table 3. When the control glass plate was reacted with the virus for 30 min at room temperature, initial virus infectivity $1.6\times10^{6}TCID_{50}$/mL was decreased to 7.2×10^{4} $TCID_{50}$/mL (1.4 $\log_{10}$).

The infectivity of virus on test glass plate treated with non-photocatalyst BY-50, was decreased to $8.2\times10^{2}TCID_{50}$/mL (3.3 $\log_{10}$), for 30 min. LRVs, between the control glass plate and the glass plate treated with non-photocatalyst BY-50, showed 1.9 $\log_{10}$ for 30 min. On the other hand, LRVs of the glass plate treating with BY-50 (KRCES Report #21_0110(1) and KRCES Report #21_0123(1)), showed 1.3 $\log_{10}$ and 1.8 $\log_{10}$ for 30 min, respectively.

As the results, the LRVs of test sample (116 days after treatment) were equivalent to previous report.

Table 2: Effect on viral infectivity

Test sample	Reaction time (min)	
	0	30
Glass plate treated with BY-50	1.6×10^{6}	8.4×10^{2}
Control glass plate		4.5×10^{4}

Units: $TCID_{50}$/mL

Table 3: Log reduction value in viral infectivity at each reaction time

Test sample	LRV	
	A	B
Glass plate treated with BY-50	3.3	1.9
Control glass plate	1.4	***

A) LRV from the initial viral infectivity.
Formula for calculating LRV: $\log_{10}$ (Initial viral infectivity÷viral infectivity for 30 min)

B) Differences of LRV between test sample and control glass plate.
Formula for calculating LRV: $\log_{10}$(viral infectivity of control÷viral infectivity of treated glass)

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May 24, 2012

REPORT

Client: BAN-YU CO., LTD.
2-4-10 Kawara-machi, Chuo-ku, Osaka-shi, Osaka 541-0048, Japan

Test sample(s): BY-50

Title: Acute Oral Toxicity Test in Mice

Received date of test sample(s): April 25, 2012

This report has been translated into English from the Japanese report No. 12042531001-01 (May 24, 2012).

Y. Kawamoto

Yasuharu Kawamoto
Study Director
Section of Biological Safety Research

May 31, 2012
Date

JAPAN FOOD RESEARCH LABORATORIES

Acute Oral Toxicity Test in Mice

Abstract

The test sample, BY-50, was tested for acute oral toxicity in male and female mice. To the experimental animals, the test sample was administered orally at a dose of 2000 mg/kg b.w. (body weight) in a single dose, and the experimental period was 14 days. The control animals were administered water for injection as vehicle control. As a result, the test sample caused no death in any of the mice during the observation period. Consequently, the LD50 value (single dose, oral administration) of BY-50 is considered to be more than 2000 mg/kg b.w. in male and female mice.

Client

BAN-YU CO., LTD.

Test sample

BY-50

Testing period

From April 25 to May 24, 2012

Test facility

Tama Laboratory, Japan Food Research Laboratories
6-11-10 Nagayama, Tama-shi, Tokyo 206-0025, Japan

Study director

Yasuharu Kawamoto
Section of Biological Safety Research
Department of Biological Safety Research
Tama Laboratory, Japan Food Research Laboratories

1. Purpose

The acute oral toxicity in male and female mice of BY-50 is evaluated.

2. Test sample

BY-50

Character: colorless transparent liquid

3. Preparation of test dilution

The test sample was diluted in water for injection to make a 100 mg/mL test dilution.

4. Experimental animals

Male and female mice of ICR strain, at an age of 5 weeks, were purchased from Japan SLC, Inc. Before test, they were acclimated to laboratory conditions for about 1 week to verify that no abnormalities were shown in general conditions. And then, they were housed in plastic cages (five animals per cage) under standard laboratory conditions (Temperature: 23 °C ± 2 °C, Light-dark cycle: 12/12 hours). Feed (Labo MR Stock diet, Nihon Nosankogyo K.K.) and tap water were provided *ad libitum* throughout the experiment.

5. Procedures

Male and female mice were allocated into experimental and control groups each consisting of five mice.

The mice were not fed for about 4 hours before administration. After measurement of body weight, the animals in the experimental group were administered orally the test dilution at a dose of 20 mL/kg b.w. (at a dosage of 2000 mg/kg b.w. test sample) in a single dose using a stomach tube. The animals in the control group were administered water for injection, as vehicle control, at a dose of 20 mL/kg b.w. in the same manner.

The clinical observation was carried out frequently on the day of the administration and once a day for the following 13 days. The body weight was measured after 7 and 14 days of the administration, and the mean body weight values of the experimental and control groups were statistically analyzed by t-test ($\alpha = 0.05$).

At the completion of the test, all of the mice were sacrificed for necropsy.

6. Results

1) Death of animals

Neither male nor female mice died during the experimental period.

2) Clinical observations

No abnormalities were observed in either male or female mice during the experimental period.

3) Body-weight changes (Tables 1 and 2)

In male mice, no significant differences in body weight were detected between the experimental and control groups after 7 and 14 days of the administration.

In female mice, no significant difference in body weight of the mice was detected between the experimental and control groups after 7 days of the administration. However, the body weight of the experimental group after 14 days was significantly lower ($p<0.05$) than that of the control group.

4) Necropsy

No remarkable changes were found in any male or female mice.

7. Conclusion

The acute oral toxicity of BY-50 was determined in male and female mice.

Oral administration of 2000 mg/kg b.w. of the test sample caused no death in any of the mice during the observation period.

Consequently, the LD50 value (single dose, oral administration) of the test sample is considered to be more than 2000 mg/kg b.w. in male and female mice.

8. Reference

• OECD Guidelines for the Testing of Chemicals 420 (2001).

Table 1. Body-weight changes (male)

Group	Body weight (g)		
	Pre-administration	7 days	14 days
Experimental group	33.3 ± 1.4 (5)	38.0 ± 1.5 (5)	41.0 ± 1.9 (5)
Control group	33.6 ± 1.7 (5)	38.1 ± 2.8 (5)	41.1 ± 2.9 (5)

Values represent mean ± SD.
Values in the parentheses show the number of animals.

Table 2. Body-weight changes (female)

Group	Body weight (g)		
	Pre-administration	7 days	14 days
Experimental group	26.0 ± 0.8 (5)	29.6 ± 1.1 (5)	30.4 ± 1.5* (5)
Control group	26.3 ± 0.9 (5)	29.4 ± 1.0 (5)	32.2 ± 0.9 (5)

Values represent mean ± SD.
Values in the parentheses show the number of animals.
* A significant difference is detected between the experimental and control groups ($p<0.05$).

End of Report

ORIGINAL

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QUALITY TESTING REPORT

Applicant BAN-YU COMPANY LIMITED

TEST No. 10237101(3/5)
Issue Date: April-08-2011

Sample : Ceramic water solution BY-50

Test item : The test of the antibacterial efficacy

Test bacteria Methicillin resistant *Staphylococcus aureus* IID 1677

Test method : JIS L 1902:2008 Quantitative test

Measurement of number of living bacteria : Pour plate method

Sample was washed with JAFET standard detergent in accordance with JIS L 0217.

Test results :

The number of inoculated bacteria (log)	[a]	4.3	2.6
The number of inoculated bacteria in untreated control sample after 18h incubation (log)	[b]	6.9	

Test sample	The number of the bacteria in test sample (log)		log a−log c	(log b−log a) − (log c−log o)
processed goods (original)	[o]	3.8	1.7	3.8
	[c]	2.6		

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Supervised by K. Imaizumi

Notece — This test result is applied to the submitted sample, not to the lot.

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QUALITY TESTING REPORT

Applicant BAN-YU COMPANY LIMITED

TEST No. 10237101(4/5)
Issue Date: April-08-2011

Sample : Ceramic water solution BY-50

Test item : The test of the antibacterial efficacy

Test bacteria *Escherichia coli* NBRC 3301

Test method : JIS L 1902:2008 Quantitative test

Measurement of number of living bacteria : Pour plate method

Sample was washed with JAFET standard detergent in accordance with JIS L 0217.

Test results :

The number of inoculated bacteria (log)	[a]	4.4	3.5
The number of inoculated bacteria in untreated control sample after 18h incubation (log)	[b]	7.9	

Test sample	The number of the bacteria in test sample (log)		log a−log c	(log b−log a) − (log c−log o)
processed goods (original)	[o]	3.6	3.1	5.8
	[c]	1.3		

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QUALITY TESTING REPORT

Applicant BAN-YU COMPANY LIMITED

TEST No. 10237101(1/5)
Issue Date: April-08-2011

Sample : Ceramic water solution BY-50

Test item : The test of the antibacterial efficacy

Test bacteria *Staphylococcus aureus* NBRC 12732

Test method : JIS L 1902:2008 Quantitative test

Measurement of number of living bacteria : Pour plate method

Sample was washed with JAFET standard detergent in accordance with JIS L 0217.

Test results :

The number of inoculated bacteria (log)	[a]	4.4	2.7
The number of inoculated bacteria in untreated control sample after 18h incubation (log)	[b]	7.1	

Test sample	The number of the bacteria in test sample (log)		log a−log c	(log b−log a) − (log c−log o)
processed goods	[o]	4.4	1.1	3.8
(original)	[c]	3.3		

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Notece — This test result is applied to the submitted sample, not to the lot.

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QUALITY TESTING REPORT

Applicant BAN-YU COMPANY LIMITED

TEST No. 10237101(2/5)
Issue Date: April-08-2011

Sample : Ceramic water solution BY-50

Test item : The test of the antibacterial efficacy

Test bacteria *Klebsiella pneumoniae* NBRC 13277

Test method : JIS L 1902:2008 Quantitative test

Measurement of number of living bacteria : Pour plate method

Sample was washed with JAFET standard detergent in accordance with JIS L 0217.

Test results :

The number of inoculated bacteria (log)	[a]	4.3	3.1
The number of inoculated bacteria in untreated control sample after 18h incubation (log)	[b]	7.4	

Test sample		The number of the bacteria in test sample (log)		log a-log c	(log b-log a) - (log c-log o)
processed goods	[o]	4.4	2.0	5.2	
(original)	[c]	2.3			

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Notece — This test result is applied to the submitted sample, not to the lot.