

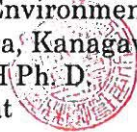
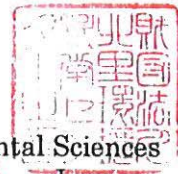
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Prepared for:  
BAN-YU Co., Ltd

## Test Report

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

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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 26<sup>th</sup> 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°C. 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.  $8.4 \times 10^8$ TCID<sub>50</sub>/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  $\mu$ L of the 10-fold serial dilution of samples and 50  $\mu$ L of MDCK cell suspensions were added into 96-well microwell plates. After an incubation for 4 days at 37°C in a CO<sub>2</sub> 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<sub>50</sub>/mL). These TCID<sub>50</sub> 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  $1.6 \times 10^6$ TCID<sub>50</sub>/mL was decreased to  $7.2 \times 10^4$  TCID<sub>50</sub>/mL (1.4 log<sub>10</sub>).

The infectivity of virus on test glass plate treated with non-photocatalyst BY-50, was decreased to  $8.2 \times 10^2$ TCID<sub>50</sub>/mL (3.3 log<sub>10</sub>), for 30 min. LRVs, between the control glass plate and the glass plate treated with non-photocatalyst BY-50, showed 1.9 log<sub>10</sub> 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<sub>10</sub> and 1.8 log<sub>10</sub> 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 <sup>6</sup> | 8.4×10 <sup>2</sup> |
| Control glass plate            |                     | 4.5×10 <sup>4</sup> |

Units: TCID<sub>50</sub>/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:  $\log_{10}(\text{Initial viral infectivity} \div \text{viral infectivity for 30 min})$ 

B) Differences of LRV between test sample and control glass plate.

Formula for calculating LRV:  $\log_{10}(\text{viral infectivity of control} \div \text{viral infectivity of treated glass})$