/Test Report/

Evaluation of Antiviral Activity of All Titanium AT-254 Coating

(Coating Material-Based Test)

Report No.: KHK21_0046
Date: August 31, 2009

Kitasato Environmental Science Center
1-15-1 Kitasato, Sagamihara-shi, Kanagawa, Japan
President: Toshihiro Ito

1. Test Objective

To evaluate the antiviral activity of a glass plate coated with All Titanium AT-254 against Influenza A virus.

2. Test Requester

Company: BLESS Co., Ltd.

Address: 7-15-10-2F, Nishi-Shinjuku, Shinjuku-ku, Tokyo, Japan

3. Testing Organization

Organization: Kitasato Environmental Science Center

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

Test Representative: Virology Division, Yasuhiro Nojima

4. Test Period

July 31, 2009 to August 4, 2009

5. Test Virus

Influenza A virus (H1N1)

6. Test Method

(1) Preparation of Test Virus Suspension

Influenza A virus was inoculated into the allantoic cavity of embryonated chicken eggs and cultured using a flask incubator. The allantoic fluid was harvested and purified via density gradient centrifugation to obtain the virus suspension.

(2) Test Specimens

Form: Glass plates

Types: All Titanium AT-254 coated glass plate

Control (Uncoated glass plate)

(3) Antiviral Test Procedure

The test specimen was placed in a humidified petri dish (Figure 2, Photo 1), and 0.2 mL of virus suspension with an infectivity titer of 1.4×10^7 $TCID_{50}$/mL was dropped onto its surface.

Immediately afterward, the specimen was covered with a 4 cm^2 polypropylene film to enhance contact efficiency between the test virus and the specimen, and was left at room temperature for the designated time.

After the exposure period, the test specimen along with the film was placed into a stomacher bag, and 10 mL of phosphate-buffered saline (PBS) was added to extract the virus from the specimen.

The extracted virus solution was used as the undiluted sample for measuring infectivity.

The 0-minute sample was regarded as the initial viral titer of the virus used in the test.

Viral Infectivity Measurement

The infectivity titer of Influenza A virus was determined using the following procedure.

The virus extracted from the specimen was used as the undiluted sample and was serially diluted tenfold with phosphate-buffered saline (PBS) to prepare dilutions ranging from 10^0 to 10^{-7}.

Fifty microliters (50 µL) of each dilution were added to ten wells per dilution in a 96-well plate.

Then, 50 µL of Madin-Darby Canine Kidney (MDCK) cells, adjusted to a concentration of 8×10^4 cells/mL in

Dulbecco's Modified Eagle's Medium (DMEM) containing 5% fetal bovine serum (FBS), were inoculated into each well.

The plates were incubated for four days in a CO_2 incubator.

After incubation, cytopathogenic effects (CPE) were observed under a microscope, and the viral infectivity titer ($TCID_{50}$/mL) was calculated using the Reed-Muench method.

(4) Test Conditions

Exposure times for virus contact with test samples are shown in Table 1.

Table 1. Test Conditions

Sample	0 min (Initial)	1 min	10 min	1 hr	8 hr
① All Titanium AT-254 Coated Glass	●	●	●	●	●
② Control (Uncoated Glass Plate)	●	***	***	●	●

7. Test Results

The results are shown in Table 2 and Fig. 2. When the virus was applied to the control specimen and incubated for 8 hours, the viral titer decreased by 1.2 $\log_{10}$ from the initial value.

In contrast, the specimen coated with All Titanium AT-254 showed progressive reductions: 0.2 $\log_{10}$ after 1 minute, 0.4 $\log_{10}$ after 10 minutes, 1.1 $\log_{10}$ after 1 hour, and 1.7 $\log_{10}$ after 8 hours. The difference in viral titer between the coated specimen and control after 8 hours was 0.5 $\log_{10}$. This suggests that the All Titanium AT-254 coating may exhibit antiviral effects against Influenza A virus.

Table 2. Viral Titer at Each Contact Time (Influenza A Virus)

Sample	0 min (Initial)	1 min	10 min	1 hr	8 hr
① All Titanium AT-254 Coated Glass	1.4×10^7	1.0×10^7	5.4×10^6	1.2×10^6	2.5×10^5
② Control (Uncoated Glass Plate)	***	***	6.3×10^6	8.4×10^5	

Fig. 2. Changes in Viral Infectivity Over Time (Influenza A Virus)

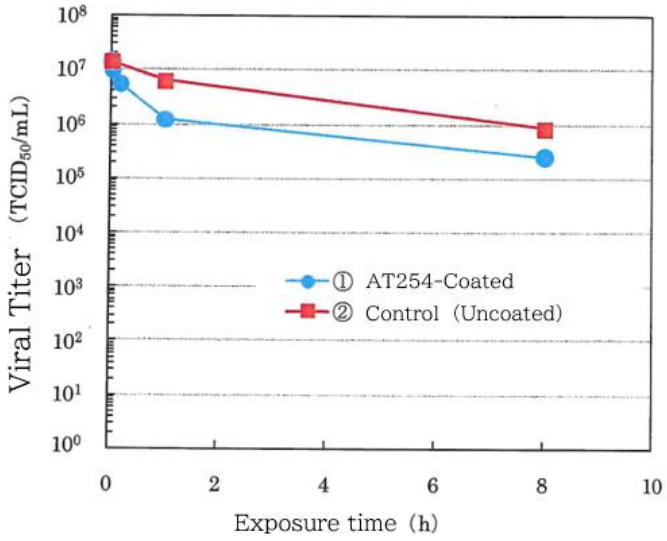


Fig. 1. Test System Overview

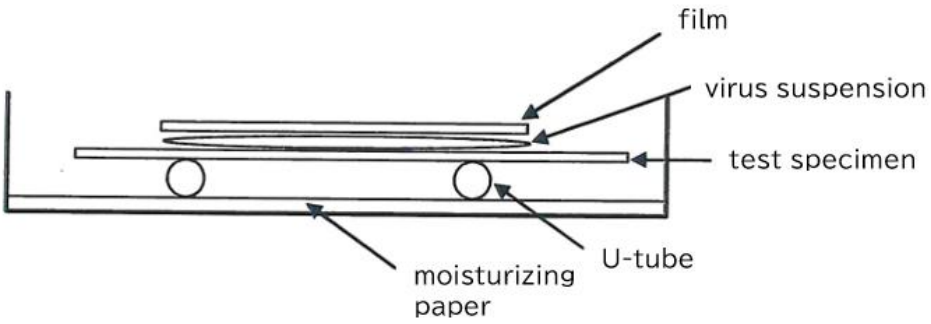
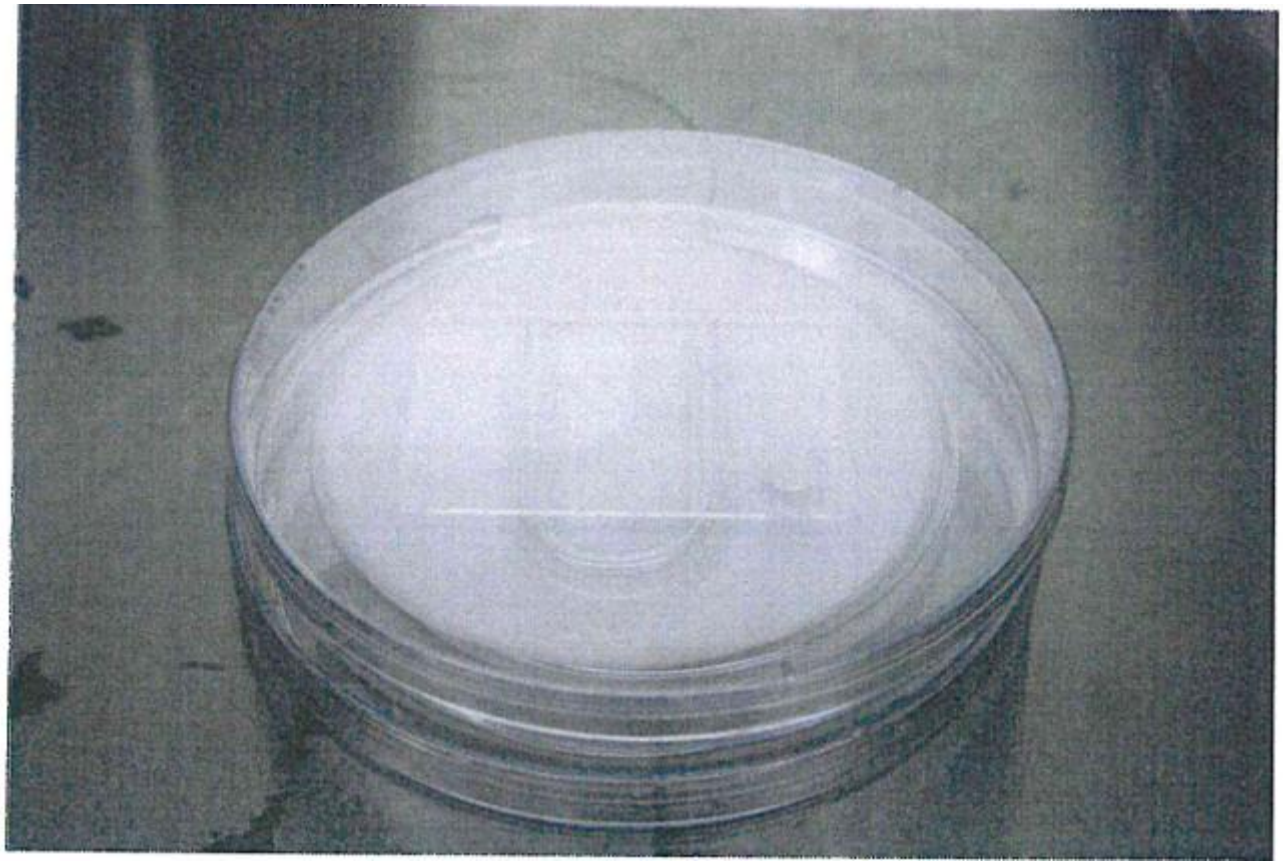
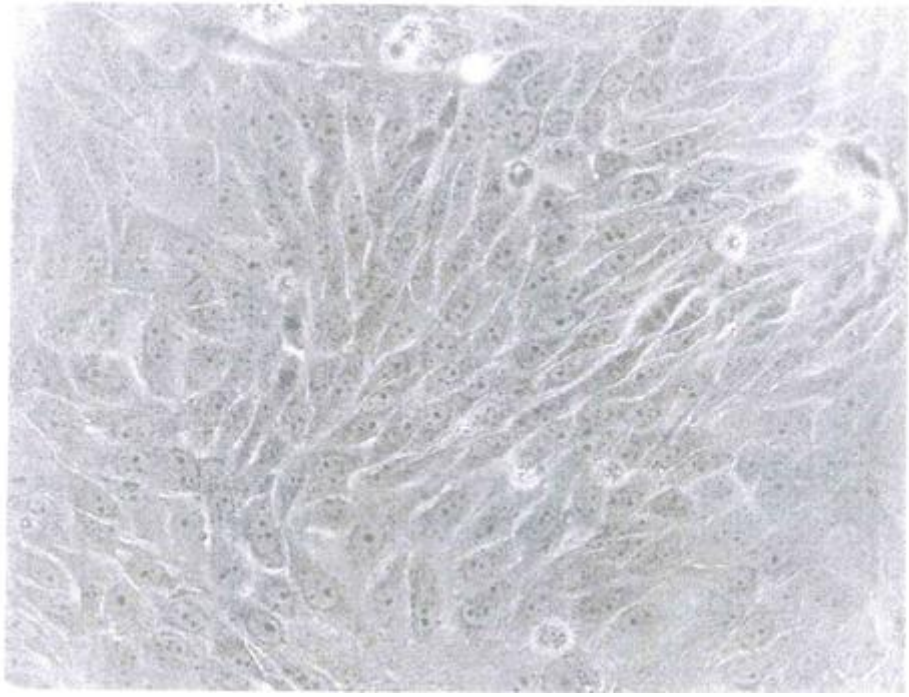


Photo 1. Test System (Humidified Petri Dish)

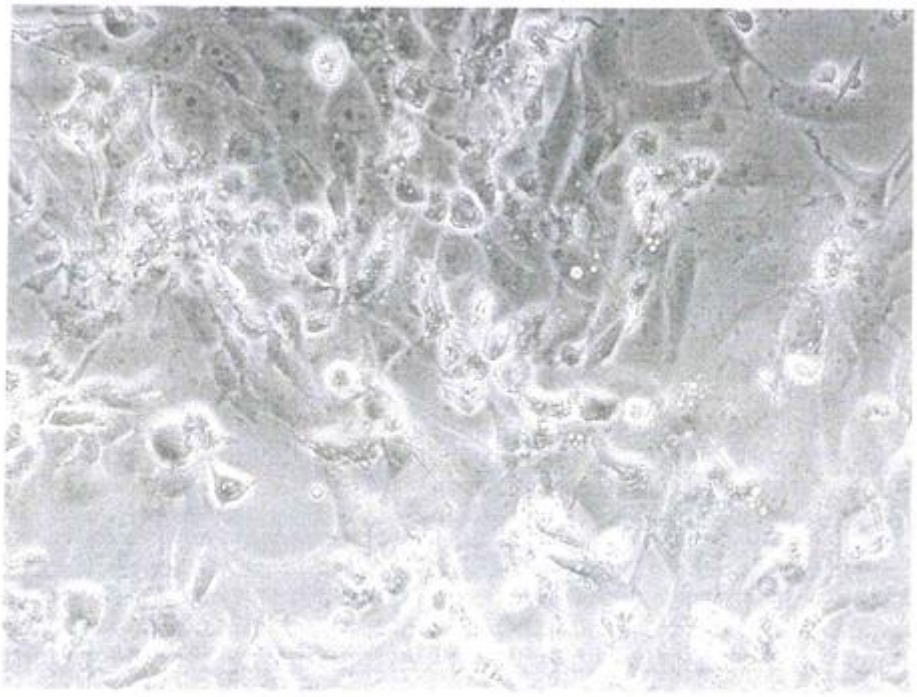


Reference Data

1）Cytopathic effect from Influenza A virus infection



- Non-infected MDCK cells



- Infected cells (4 days post-infection)