-KRCES- Virus Test Report No.21_1154
Feb 15, 2010

To Bioface Co., Ltd

Test Report

Antiviral efficacy of "NANO PLUTINUM®" coated nonwomen fabric against *Influenza A virus* (H1N1)

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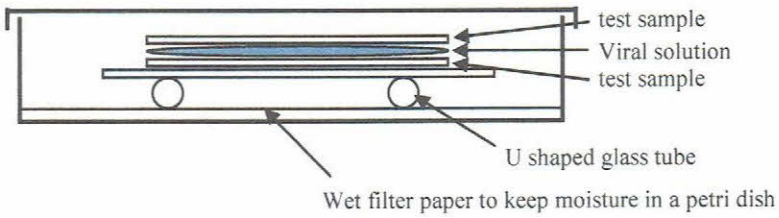

Figure1. Schematic drawing of test instrument

2) Measurement of infectivity

Viral infectivity titers in test samples were determined by observation of a cytopathic 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 transferred into 96-well micro-plates. After an incubation for 4 days at 37°C in a CO_2 incubator, virus-induced cytopathic 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 as log reduction values (LRV).

8. Test results

Test results were summarized on Table 1. When the virus was exposed to the blank sample for 18 hours at room temperature, initial virus infectivity (8.4×10^6 $TCID_{50}$/mL) was decreased to 5.4×10^4 $TCID_{50}$/mL (LRV = 2.2 $\log_{10}$).

On the other hand, the infectivity of the virus on the "Thermal bond nonwoven fabric" was decreased to less than 6.3×10^1 $TCID_{50}$/mL (more than 2.9 $\log_{10}$) and "Previously prepare nonwoven fabric" and "Spun bond nonwoven fabric" were decreased to less than 6.3 $TCID_{50}$/mL (more than 3.9 $\log_{10}$), for 18 h exposure, respectively.

As the result of this test, the differences of LRVs between the blank and the coated nonwoven fabric (number 1, 2 and 3) were more than 2.9 $\log_{10}$, 3.9 $\log_{10}$ and 3.9 $\log_{10}$ for 18 hours, respectively. It was considered that the "NANO PLUTINUM®" coated nonwoven fabric possessed the anti-influenza virus activity within 18 h exposure.

1. Aim of the test

To investigate the antiviral efficacy of "NANO PLUTINUM®" coated nonwoven fabric using *Influenza A virus* (H1N1).

2. Client

Company: Bioface Co., Ltd.

Address: 39-2, Hirakicho, Uji, Kyoto, Japan

3. Testing organization

Virology Division, Department of Virology,

Kitasato Research Center for Environmental Science

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4. Test sample and condition

Test sample: platinum nanoparticle (NANO PLUTINUM®) coated nonwoven fabric

1. Thermal bond nonwoven fabric
2. Previously prepare nonwoven fabric
3. Spun bond nonwoven fabric
4. Blank (non-coated nonwoven fabric)

Exposure time: 0 (initial), 18 hours.

5. Test virus

Influenza A virus (A/PR/8/34, H1N1)

6. Preparation of the test virus

Influenza A virus was inoculated into the allantoic cavity of embryonated chicken eggs. These eggs were incubated at 37°C. After 2 days, the virus multiplying in the allantoic fluid was harvested and purified by the sucrose density gradient centrifugation method.

7. Methods

1) Test procedure

The test samples (50 mm x 50 mm) were placed into a Petri dish (Figure 1) and then 0.2 mL of viral solution was dripped on the test samples. These were incubated for 18 hours 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 the viral suspension was dripped on the blank (non-coated nonwoven fabric).

Table 1: Antiviral efficacy of "NANO PLUTINUM®" coated nonwoven fabric.

Test sample	Viral infectivity		Log reduction of viral infectivity (LRV)	
	0 (Initial)	18 hours	0-18hr [a)]	Compared with the blank [b)]
1. Thermal bond nonwoven fabric	8.4×10^{6}	$< 6.3\times10^{1}$	> 5.1	>2.9
2. Previous prepare nonwoven fabric		< 6.3	> 6.1	> 3.9
3. Spun bond nonwoven fabric		< 6.3	> 6.1	> 3.9
4. Blank (Non treated nonwoven fabric)		5.4×10^{4}	2.2	0.0

Units: $TCID_{50}$/mL

Detection limit: 6.3 x 10^{1} $TCID_{50}$/mL (sample No. 1), 6.3 $TCID_{50}$/mL (Sample No.2, 3 and 4)

Formula for calculating LRV;

a) : Log_{10}(Initial viral infectivity / viral infectivity at each reaction time)

b) : Log_{10}(non treated nonwoven fabric / treated test samples after 18 hours exposure)