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-KRCES- Virus Test Report No.23_2020
Sep 15, 2011

To Bioface Co., Ltd

Test Report

Antiviral efficacy of "NANO PLUTINUM®" against Coxackie B6 virus.

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Approved by: Toshihiro Itoh

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1. Aim of the test

To investigate the antiviral efficacy of "NANO PLUTINUM®" using Coxackie B6 virus.

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

Address: 1-15-1 Kitasato, Minami, Sagamihara, Kanagawa, 252-0329, Japan

4. Test sample and condition

Test sample: platinum nanoparticle solution (NANO PLUTINUM®) (1:2 dilutions)

Exposure time: 0 (initial) and 10 min

5. Test virus

Coxakie B6 virus

Coxsackie virus was used as a surrogate for foot-and-mouth disease virus (FMDV) of Artiodactyls. In general, Coxackievirus has not been recognized as a surrogate for FMDV. In this study, both Coxackievirus and FMDV belong to the picornaviridae, and the structure of the virus is similar, so it was used as a surrogate for FMDV.

6. Preparation of the test virus

Coxakie B6 virus was inoculated on Vero cells. The virus-infected cells were incubated at 37°C for 2～3 days. The cells were checked for cytopathic effects (CPE) every day. When approximately 90% of the cells showed CPE, a cell lysate was prepared by freezing and thawing. The cell lysate was centrifuged at 3,500 rpm for 10 min at 4 °C, and the harvested supernatant was used to prepare the working virus solution.

7. Methods

1) Test procedure

To examine virucidal effect of platinum nanoparticle (NANO PLUTINUM®; sample), 100 μL of virus suspension was added into 900 μL of the sample and this was kept at room temperature for 60 min. After exposure time, the reaction mixture was diluted with 100-fold by phosphate buffered saline (PBS) to avoid the virucidal activity of the sample. Then, 100 μL of the diluted mixture was

Table 1: Antiviral efficacy of "NANO PLUTINUM®".

Test sample	Reaction time		LRV
	0 (initial)	60 min	
NANO PLUTINUM® (1:2 dilution)	8.3×10^4	$< 1.3 \times 10^1$	> 3.8
Control (PBS)		7.7×10^4	0.0

Virus: Coxsackie B6 virus

Units: $TCID_{50}$/mL

Detection limit: 1.3×10^1 $TCID_{50}$/mL

Formula for calculating LRV; Log_{10}(Control (PBS) / NANO PLUTINUM® after 60 min)

immediately serially diluted with 900 μL of PBS (1:10) to measure for virus titer. Additionally, control reaction was carried out by using PBS instead of sample.

2) Measurement of infectivity

Viral infectivity titers in the reaction mixtures were determined by observation of a CPE of Coxackie virus in Vero cells. Fifty μL of the 10-fold serial dilution of the mixtures and 50 μL of Vero cell suspensions were transferred into 96-well micro-plates. After 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

The inactivation efficacy of "NANO PLUTINUM®" supplied by Bioface Co., Ltd. is summarized on Table 1. When the virus was exposed to the control (PBS) for 60 minutes at room temperature, the virus infectivity was 7.7×10^4 $TCID_{50}$/mL. This virus infectivity was scarcely changed compared to the initial virus titer (8.3×10^4 $TCID_{50}$/mL). On the other hand, when the virus was exposed to "NANO PLUTINUM®" for 60 minutes at room temperature, the initial virus infectivity was decreased to less than 1.3×10^1 $TCID_{50}$/mL (LRV = more than 3.8 $\log_{10}$) .

As the result of this test, the differences of LRVs between the test sample and the control were more than 3.8 $\log_{10}$ for 60 minutes. It was considered that the "NANO PLUTINUM®" possessed the antiviral efficacy against Coxackie virus.