

To Bioface Co., Ltd

Test Report

Antiviral efficacy of "NANO PLUTINUM[®]" against
Feline calicivirus (surrogate for norovirus).

-KRCES-Virus Test Report No. 22_1065
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Consent must be obtained from this center prior to public disclosure of the contents of this report. In addition, test results in this report apply to the test sample and do not attest to the quality of the entire batch (lot).

1. Aim of the test

To investigate the antiviral efficacy of "NANO PLUTINUM[®]" using Feline calicivirus (surrogate for norovirus).

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[®]) solution (tenfold dilution of test sample)

Exposure time: 0 (initial) and 10 min

5. Test virus

Feline calicivirus (F-9)

6. Preparation of the test virus

Feline calicivirus (FCV) F-9 strain was inoculated on Crandell-Rees Feline Kidney (CRFK) 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 Nano Platinum solution (sample), 100 µL of virus suspension was added into 900 µL of the sample, and this was kept at room temperature for 10 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 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.

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

Test sample	Exposure time (min)	
	0 (Initial)	10
NANO PLUTINUM®	6.3×10 ⁴	1.2×10 ¹
Control (PBS)		1.4×10 ⁵
LRV	***	4.0

Units: TCID₅₀/mL

Detection limit: 6.3 TCID₅₀/mL

Formula for calculating LRV;

$$\text{Log}_{10}(\text{Control(PBS)} / \text{NANO PLUTINUM}^{\circledR} \text{ after 18 hours exposure})$$

2) Measurement of infectivity

Viral infectivity titers in the reaction mixtures were determined by observation of a CPE of FCV in CRFK cells. Fifty μL of the 10-fold serial dilution of the mixtures and 50 μL of CRFK 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 ($\text{TCID}_{50}/\text{mL}$). 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 PBS (control) for 10 minutes at room temperature, the virus infectivity was $1.4 \times 10^5 \text{ TCID}_{50}/\text{mL}$. This virus infectivity was scarcely changed compared to the initial virus titer ($6.3 \times 10^4 \text{ TCID}_{50}/\text{mL}$). On the other hand, when the virus was exposed to “NANO PLUTINUM[®]” for 10 minutes at room temperature, the initial virus infectivity was decreased to $1.2 \times 10^1 \text{ TCID}_{50}/\text{mL}$ ($\text{LRV} = 3.7 \log_{10}$) at 10 min.

As the result of this test, the difference of LRVs between the test sample and the control was 4.0 \log_{10} for 10 min. It was considered that the “NANO PLUTINUM[®]” possessed antiviral efficacy against FCV.