231021／23_0128

-KRCES-Bio.Test Report No. 23_0128
July 27, 2011

To Bioface Co., Ltd.

Test Report

Antibacterial efficacy of "NANO PLUTINUM®"

-KRCES-Bio.Test Report No. 23_0128
July 27, 2011

Approved by: Toshihiro Itoh

Toshihiro Itoh, Chief Director
Kitasato Research Center for Environmental Science
1-15-1, Kitasato, Minami, Sagamihara, Kanagawa 252-0329, Japan

2) Preparation of test sample

Test sample (solution) was diluted 2-fold with saline (Otsuka Pharmaceutical, Japan) and kept at 25 ± 2℃ until use.

3) Test procedure for antibacterial activity

10 mL of test solution was transferred into 50-mL centrifuge tube and was added with 0.1 mL of test bacterial suspension and mixed well. The mixed solutions were incubated at 25 ± 2℃ for 1 hour. 60 minutes after the incubation, 1 mL of reaction mixture was transferred into 9 mL of neutralizer* solution to inactivate the activity of sample, and this mixture was served for bacterial count. As a control and for the initial bacterial count, bacterial suspension was added with saline instead of test sample, which was incubated and sampled in the same manner with the test sample.

*SCDLP medium (Eiken, Japan) was used as a neutralizer, which was previously tested and reported in KRCES-Bio.Test Report No. 23-0107.

4) Bacterial count

Serial decimal dilutions of bacterial suspension were prepared with saline. 1 mL of each dilution was transferred to a petri dish and mixed with 20 mL of TSA medium by swirling the dish. After solidified, the mixture was incubated at 36 ± 1℃ for 48 hours. Bacterial count per 1 mL of sample solution was estimated by colony count on TSA (detection limit: 10 CFU/1 mL of test solution).

8. Test results

Antibacterial activities of the sample on *E. coli* O157 and O111 were shown in Table1 and 2, respectively. Because both test bacterial counts in control group at 60 minutes did not changes as compared with initial counts, it is considered that the test system was valid.

The test sample reduced the viability of both bacteria less than detection limit (<10), a reduction of 10^4-fold, after 60 minutes exposure.

9. Conclusion

The test sample possessed strong antibacterial activity on both species of *E. coli*.

Concluded

Table1. Antibacterial efficacy of "NANO PLUTINUM®" (*E. coli* O157)

Test solution	Contact time (min)	
	0※ (Initial)	60
Control (Saline)	340,000	400,000
"NANO PLUTINUM®" (2-fold dilution) (PTNS 833-555-22532)		<10

CFU/1 mL of test solution

Test bacteria: *Escherichia coli* (O157 : H7) RIMD509939

Table2. Antibacterial efficacy of "NANO PLUTINUM®" (*E. coli* O111)

Test solution	Contact time (min)	
	0※ (Initial)	60
Control (Saline)	360,000	380,000
"NANO PLUTINUM®" (2-fold dilution) (PTNS 833-555-22532)		<10

CFU/1 mL of test solution

Test bacteria : *Escherichia coli* (O111 : HUT) RIMD05092017

※ ; Just after the test bacterial inoculation in control

1.Aim of the test

To investigate the antibacterial efficacy of "NANO PLUTINUM®" using *E. coli*.

2.Client

Company: Bioface Co., Ltd.

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

3.Testing organization

Biotechnology Division, Department of Microbiology

Kitasato Research Center for Environmental Science

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

4.Test period

July 21 ～ July 25, 2011

5.Test sample and condition

Test sample: platinum nanoparticle (NANO PLUTINUM®) solution
(2-fold dilution of test sample)

Exposure time: 0 (initial) and 60 min

6.Test bacteria

Escherichia coli (O157:H7) RIMD509939
(Enterohemorrhagic *Escherichia coli* O157)

Escherichia coli (O111:HUT) RIMD05092017
(Enterohemorrhagic *Escherichia coli* O111)

7.Methods

1) Preparation of test bacteria

Cryopreserved test bacteria were pre-cultured for 24 hours at 36 ± 1℃ on TSA (Tryptic Soy Agar, Difco) and then sub-cultured for 24 hours at 36 ± 1℃ on TSA. Colonies formed on TSA were scraped off and suspended in sterilized ion-exchange water. Concentration of the bacterial suspension was adjusted to about 10^7 CFU/mL for serving the test.