

# Test Report

**Kitasato Research Center for Environmental Science**

1-15-1 Kitasato, Minami-ku, Sagami-hara-shi, Kanagawa, 252-0329 Japan

[TEL:+81-42-778-9208](tel:+81-42-778-9208) FAX: +81-42-778-4551

To: Nanoclo System Co., Ltd.

# Test Report

**Test (25m<sup>3</sup> space) of efficacy evaluation of disinfection by chlorine dioxide generating agent “nanoclo<sub>2</sub> Place Type”**

北生発 26\_0053 号

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1-15-1 Kitasato, Minami-ku, Sagami-hara, Kanagawa, 252-0329 Japan

Kitasato Research Center for Environmental Science

Chief Director Toshihiro Ito

(Translated by Koichi Kuboyama)

1. Test aims

To evaluate the disinfection efficacy of chlorine dioxide generating agent “nanoclo<sub>2</sub> Place Type” against colon bacillus suspension, by setting up the gas diffusing agent and colon bacillus suspension in a 25m<sup>3</sup> test chamber.

2. Client

N a m e: Nanoclo System Co., Ltd.

Address: 3-6-2 Nishi-Shinbashi, Minato-ku, Tokyo, 105-0003 Japan

3. Test institution

N a m e: Kitasato Research Center for Environmental Science

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

In charge: Biotechnology section of Bacteria department

4. Test term

From May 26, 2014 to May 28, 2014

5. Test object

Chlorine dioxide generating agent “nanoclo<sub>2</sub> Place Type”··· 【Photo 1】

※Quantity of test object:

At the diffusion prior to the test start→4 pieces

After the test start→8 pieces

6. Test conditions

Test duration: 0 (beginning), 24 hours

Temperature: 25 °C

7. Tested bacteria

*Escherichia coli* NBRC 3972 (colon bacillus)

8. Reagent and equipment/instruments

1) Main reagents/culture medium

- Tryptic Soy Agar (Difco) (hereinafter called “TSA medium”)
- Sodium chloride (Wako, Guaranteed reagent, for Normal saline solution preparation)
- Sodium thiosulfate (Wako, 1<sup>st</sup> grade)

## 2) Main equipment/instruments

- 25m<sup>3</sup> test chamber (specially ordered, 3.3 x 3.5 x 2.2 m)
- Stirring fan (BS-B-25, Yamazen)
- Thermometer/Hygrometer (T&D, TR-72Ui)
- Chlorine dioxide gas detecting tube (No.23L, Gastec)
- Incubator (Sanyo, MIR-153, MIR-553)

## 9. Test method

### 1) Preparation of bacterial suspension

Having cultured in advance the freeze-preserved strain in TSA medium at  $36 \pm 1^\circ\text{C}$  for 24 hours, it was cultured in TSA medium at  $36 \pm 1^\circ\text{C}$  for 24 hours more. Scratching off the grown colony by an inoculating loop, it was suspended into normal saline solution. After filtrating it through absorbent cotton, about  $10^7$  CFU/ml was prepared and provided for the test.

### 2) Operation

The test target and a stirring fan were put inside a test chamber of 25m<sup>3</sup>.※ After 16 hours diffusion, the chlorine dioxide gas concentration was adjusted to about 0.04ppm, the target concentration.

The test bacteria suspension of 4ml on a  $\phi$  60mm laboratory dish with lid open was placed about 170cm from the test target. (Phote-1)

After it had been kept for 24 hours at temperature of about  $25^\circ\text{C}$  in the chamber which was tightly sealed, the bacteria suspension was collected from the laboratory dish.

At the same time, the same process was also done in a space without placing any test target as the control.

※Placing 4 pieces of the test target, after 16 hours diffusion, the chlorine dioxide gas concentration didn't reach the target concentration, so that we increased the number of test target to 8 pieces before the test start.

### 3) Measuring number of bacterium

The bacteria solution collected from the laboratory dish, as test raw liquid, was made into 10 fold serial dilution with 0.015% sodium thiosulfate added physiological salt solution.

Pour plates with each 1ml dilution and TSA medium culture were cultured at  $36 \pm 1^\circ\text{C}$  for 48 hours.

After culture, counting the number of colony grown on the medium culture, the number of bacterium per 1ml test liquid was measured.

4) Measurement of chlorine dioxide gas concentration

The chlorine dioxide concentration inside the test chamber was measured by a portable gas concentration measurement instrument which was provided by the client. The place of measurement was set at the chamber side about 50cm high from the floor.

10. Test results

The disinfection efficacy against colon bacillus is shown in table-1.

The chlorine dioxide gas concentration inside the chamber at the beginning and ending of the test is shown in table-2.

As referential data, the chlorine dioxide gas concentration measured and temperature and humidity inside the chamber are also indicated.

The number of bacterium at the beginning was  $1.4 \times 10^7$  CFU/ml.

The number of bacterium "without test object (control)" after 24 hours experiment was  $1.3 \times 10^7$  CFU/ml, which showed no much decrease of the bacteria number.

On the other hand, the number of bacterium with the test object placed inside was  $< 10 \text{ CFU/ml} >$ , which was above 99.99% disinfection efficacy.

As reference, chlorine dioxide gas concentration during the test is shown. It was kept in the range from 0.032 to 0.078 ppm comparing to the target of 0.04 ppm.

Table-1 Disinfection efficacy against colon bacillus

Test condition	Experiment time	
	0(start)	24 hours
Without test object (control)	14,000,000	(A) 13,000,000
Test object placed		(B) < 10 ( >99.99%* )

※Test object: chlorine dioxide generating agent “nanoclo2 Place Type”

※Test bacteria: *Escherichia coli* NBRC 3972

※Number of bacterium unit: CFU/ml

※Test space: 25m<sup>3</sup>

\*Disinfection ratio =  $(1 - (B) / (A)) \times 100\%$   
( <10 being calculated as 10)

Table-2 Chlorine dioxide gas concentration inside 25m<sup>3</sup> test chamber

Measured item	Experiment time (hour)	
	0(start)	24
Chlorine dioxide gas concentration	0.032	0.047

※Measurement instrument: Portable gas concentration measurement instrument  
( J · M · S 、 provided by the client)

※Unit: ppm

※Regarding measured results of chlorine dioxide gas concentration during the tests, please refer to the reference data.

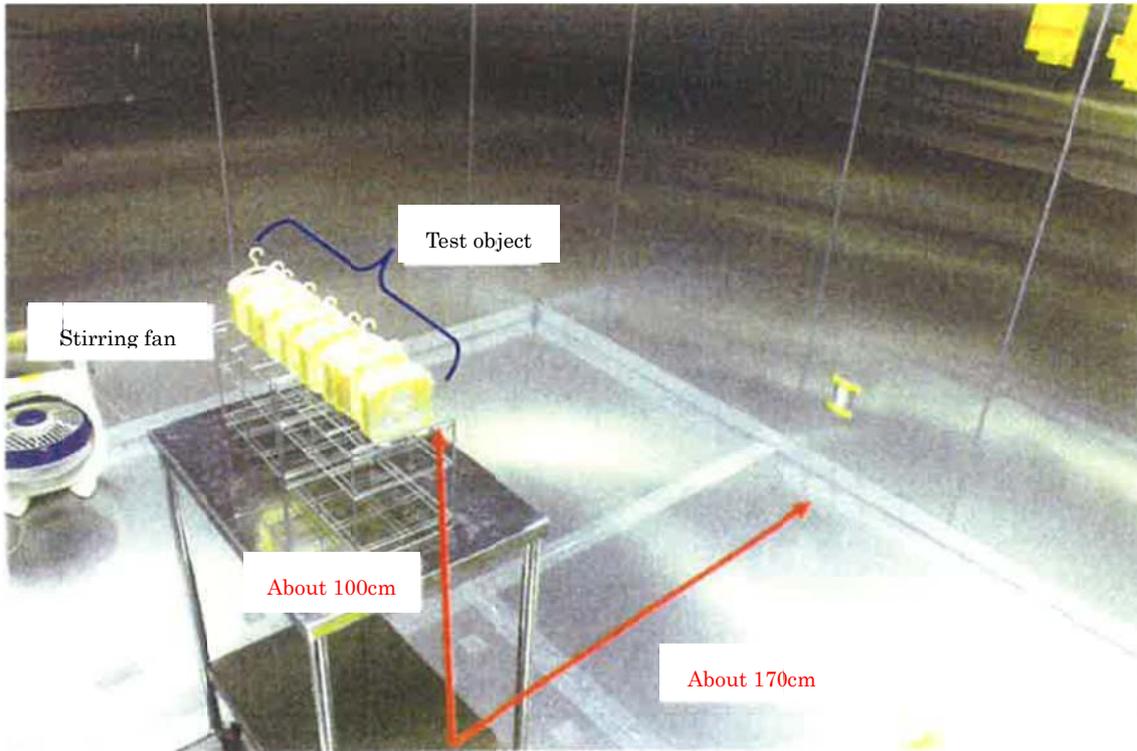


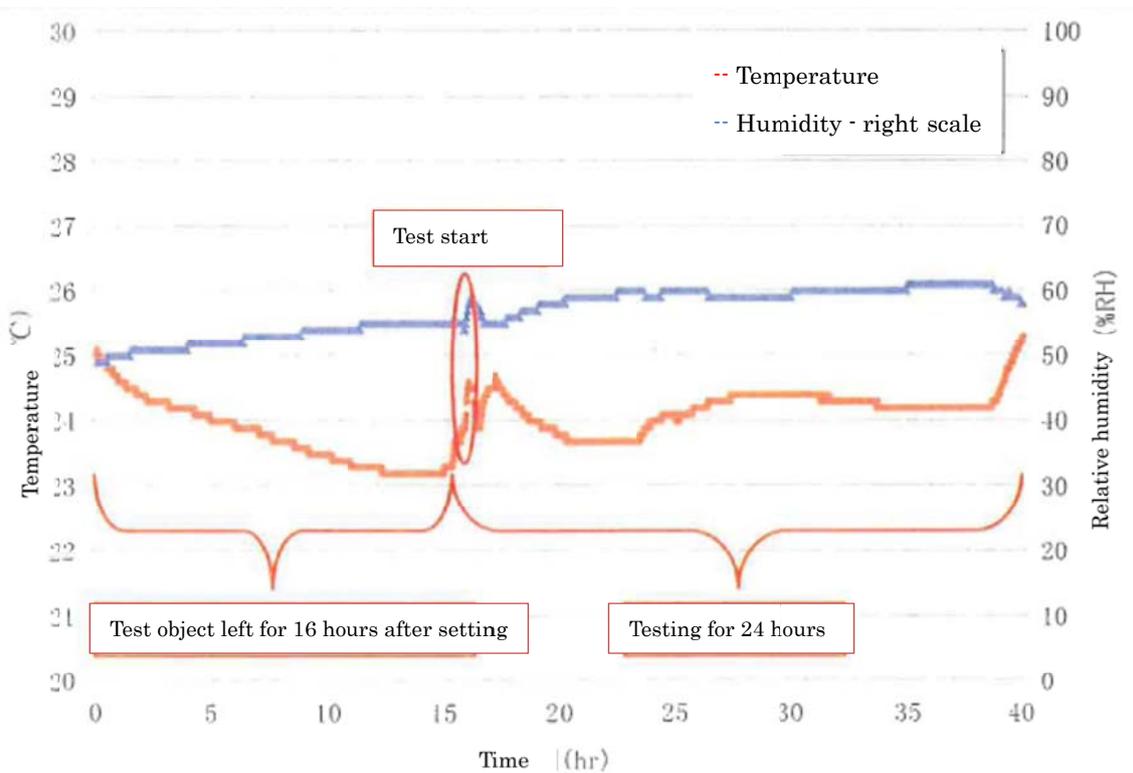
Photo-1 Test conditions inside 25m<sub>3</sub> test chamber

Reference data

Table: Measurement results of chlorine dioxide gas concentration

Date	Time	Chlorine dioxide concentration(ppm)	Remarks
May 27 <sup>th</sup>	9:00	0.026	Increased test object from 4 to 8
	9:05	0.046	
	Test start→9:50	0.032	The door was opened and closed for setting the test target pieces..
	17:00	0.043	
	18:00	0.043→0.038	Ventilated from 0.043 to 0.038ppm
May 28 <sup>th</sup>	9:00	0.078→0.040	Ventilated
	Test finish→9:50	0.047	

- ※ Measurement instrument: Portable gas concentration measurement instrument ( J · M · S 、 provided by the client)
- ※ Test start: Experiment time 0 hour  
Test finish: Experiment time 24 hours



Temperature and humidity variation inside the chamber (5/26/2014 17:50~5/28/2014 9:50)