

Test Report

Kitasato Research Center for Environmental Science

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To: Nanoclo System Co., Ltd.

Test Report

Test (40L space) of efficacy evaluation of disinfection by chlorine dioxide generating agent “nanoclo2 Case in Type”

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

Kitasato Research Center for Environmental Science

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(Translated by Koichi Kuboyama)

1. Test aims

To evaluate the disinfection efficacy of chlorine dioxide generating agent “nanoclo2 Case in Type” against colon bacillus suspension, by setting up the gas diffusing agent and colon bacillus suspension in a 40L 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 “nanoclo2 Case in Type”

※Quantity of test object:

At the diffusion prior to the test start→6g

After the test start→1.6g

About 23 hours after the test start, one test object (6g) was added.

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, 1st grade)

2) Main equipment/instruments

- 40L test chamber (specially ordered)
- 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 was put at the center in a test chamber of 40L.※

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 ϕ 60mm laboratory dish with lid open was placed about 10cm from the test target. (Phote-1)

After it had been kept for 24 hours at temperature of about 25°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 one piece of the test target, after 16 hours diffusion, the chlorine dioxide gas concentration became four times as high as the target concentration. Then, the quantity of solid agent in the test target was reduced from 6g to 0.16g and the operation began.

Because, after 23 hours, the concentration dropped to 0.01ppm, 6g of solid agent was added and the experiment continued for the remaining one hour.

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.

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 1.4×10^7 CFU/ml.

The number of bacterium "without test object (control)" after 24 hours experiment was 1.3×10^7 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 0.01 to 0.13ppm comparing to the target of 0.04ppm.

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 Case in Type”

※Test bacteria: *Escherichia coli* NBRC 3972

※Number of bacterium unit: CFU/ml

※Test space: 40 L

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

Table-2 Chlorine dioxide gas concentration inside 40L test chamber

Measured item	Experiment time (hour)	
	0(start)	24
Chlorine dioxide gas concentration	0.040	0.024

※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.

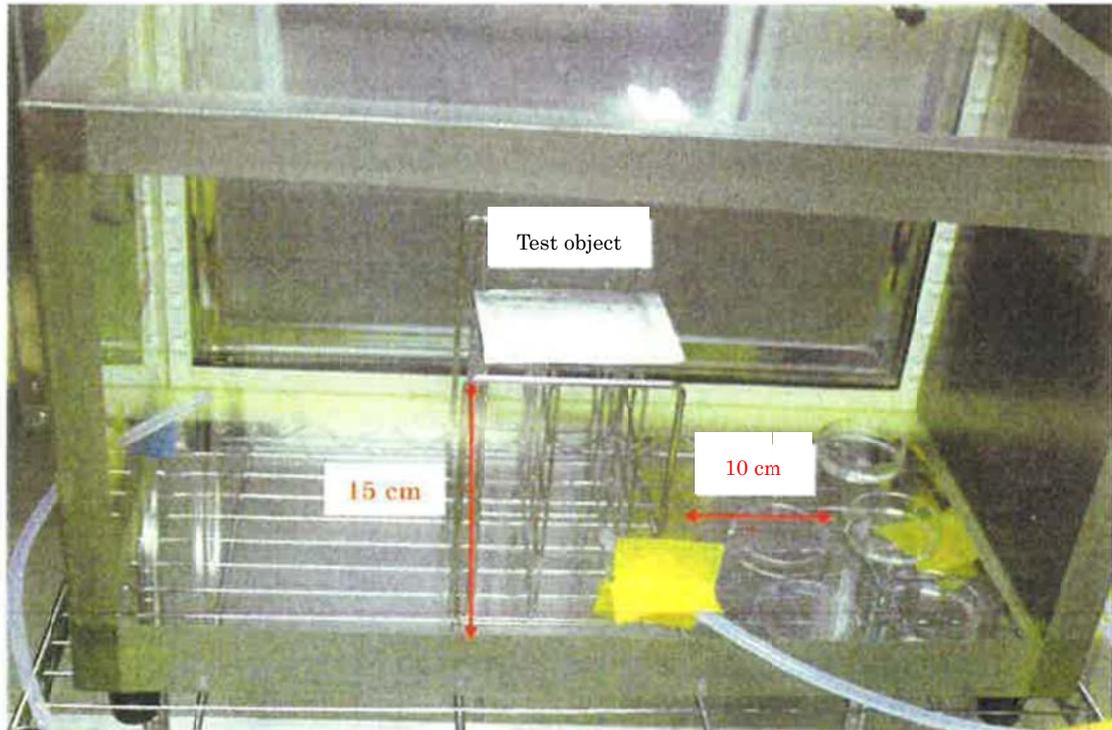


Photo-1 Test conditions inside 40L test chamber

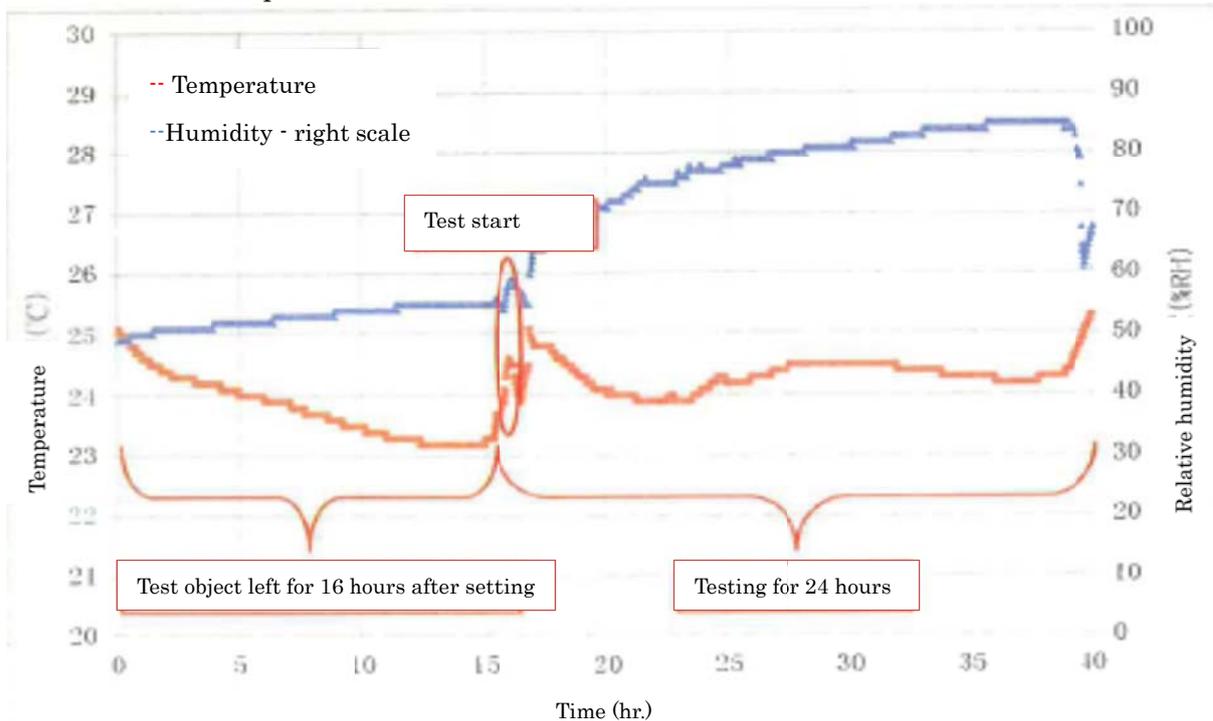
Reference data

Table: Measurement results of chlorine dioxide gas concentration

Date	Time	Chlorine dioxide concentration(ppm)	Remarks
May 27 th	9:00	0.160	
	Test start→9:50	0.040	Solid agent decreased from 6g to 1.6g.
	14:30	0.057→0.041	Ventilated from 0.057 to 0.041.
	17:00	0.037	
	18:00	0.035	
May 28 th	9:00	0.01	Added one solid agent (6g)
	9:20	0.13→0.04	Ventilated to 0.04ppm
	Test finish→9:50	0.024	

※ 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)

Temperature and humidity variation inside the test chamber during the testing