

Test Report

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To: Nanoclo System Co., Ltd.

Test Report

**Test (25 m³ space) of efficacy evaluation of inactivation for viruses
by chlorine dioxide generating agent “nanoclo₂ Place Type”**

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

Kitasato Research Center for Environmental Science

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(Translated by Koichi Kuboyama)

1. Test aims

To evaluate the inactivation efficacy of chlorine dioxide generating agent “nanoclo₂ Place Type” against Influenza A virus, by setting up the agent in a 25m³ test chamber and the Influenza A virus as well in the same test space.

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: Virus Section of Virus Department

4. Test term

From May 26, 2014 to June 2, 2014

5. Test object

Chlorine dioxide generating agent “nanoclo₂ 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. Virus under testing and the compounding method of virus liquid

Influenza A virus, H1N1, A/PR/8/34, ATCC® VR-95™

After chorioallantoic inoculation into embryonated egg, the virus was incubated in an incubator. Taking out chorioallantoic liquid, the liquid was purified by density-gradient-centrifugation, which was used as virus liquid for testing.

8. Test method

1) Test of virus inactivation

Virus inactivation test was conducted according to the process below.

The test target was put at the center in a test chamber of 25m³.※

After 16 hours diffusion, the chlorine dioxide concentration was adjusted to about 0.04ppm, the target concentration.

The test virus suspension of 4ml on a φ 60mm laboratory dish with lid open was placed about 170cm from the test target. (Photo-1)

After it had been kept for 24 hours in the chamber which was tightly sealed, quiet and at temperature of about 25°C, the virus suspension was collected from the laboratory dish and used as infectivity titer measuring test raw liquid. At the same time, the same process was done in a space without placing any test target.

※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.

2) Measurement of virus infectivity titer

The virus infectivity titer measuring test raw liquid was serial-diluted 10 times with PBS (phosphate buffered saline), which is call "diluted virus liquid" hereafter.

And 50 μ L of MDCK(Madin-Darby canine kidney) was suspended into 50 μ L of the virus infectivity titer measuring raw test liquid or the diluted virus liquid and 5% FBS(fetal bovine serum) added DMEM(Dulbecco's modified Eagle's Medium) .

The 50 μ L suspension of MDCK was planted into 96 well plate.

Then it was incubated in a carbon dioxide incubator with 37°C for 4days.

After incubation, we observed the CPE(cytopathic effect) of virus multiplication by inverted microscope, and acquired the virus infectivity titer(TCID₅₀/mL) using Reed-Muench method.

3) Measurement of chlorine dioxide 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.

9. Test results

The test results are shown in table-1. And the chlorine dioxide concentration inside the test chamber during the test is shown in table-2.

The starting virus infectivity titer was 3.9×10^7 TCID₅₀/ml. The virus infectivity titer “without test target (the control)” after 24 hours still standing was 2.9×10^6 TCID₅₀/ml.

On the other hand, the infectivity titer inside the 25m³ test chamber set with “chlorine dioxide generating agent, nanoclo₂ Placed Type” after 24 hours still standing was below the detection limit (6.3 TCID₅₀/ml). The comparison of infectivity titer LRV (log reduction value) with the control after 24 hours experiment was above 5.6 log₁₀. The reduction ratio calculated from the LRV was 99.999% or above.

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

10. Comment

In this test, we placed, inside 25m³ test chamber, “chlorine dioxide generating agent, nanoclo₂ Place Type” that the client provided, and evaluated the disinfection efficacy against A type influenza virus.

As the test results, after 24 hours still standing, 5.6 log₁₀ or above (reduction ratio 99.999% or above) decrease was observed.

It is reported that the virus inactivation effect by chlorine dioxide gas was affected by humidity or organic matters existence.¹⁾ Suitable using methods considering the effects from humidity or organic matters are required when it is actually utilized.

Reference books

- 1) Morino H et al., Inactivation of feline calicivirus by chlorine dioxide gas-generating gel. Yakugaku Zasshi. 133(9):1017-22,2013

Table-1 Disinfection efficacy against A type influenza virus

by “chlorine dioxide generating agent, nanoclo₂ Place Type”

Test object	Experiment time		Infectivity titer LRV(log reduction value) ^{※1} (reduction ratio ^{※2})
	0(start)	24 hours	After 24 hours
Without test object (control)	3.9 x 10 ⁷	2.9 x 10 ⁶	
chlorine dioxide generating agent, “nanoclo ₂ Placed type”		< 6.3	> 5.6 (> 99.999)

Virus used: Influenza A virus (H1N1, A/PR/8/34, ATCC[®] VR-95[™])

Virus infectivity titer of raw virus liquid for testing: 3.9 x 10⁷ TCID₅₀/ml

Infectivity titer unit: TCID₅₀/ml

Detection limit value: 6.3 TCID₅₀/ml

※1: Difference from the control after 24 hour experiment: log₁₀ (infectivity titer of the control / infectivity titer of chlorine dioxide generating agent taken after experiment time)

※2: Figure acquired from conversion of LRV into reduction ratio:
(1-1/10^{LRV}) x 100 (%)

Table-2 Chlorine dioxide gas concentration inside 25m³ 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.

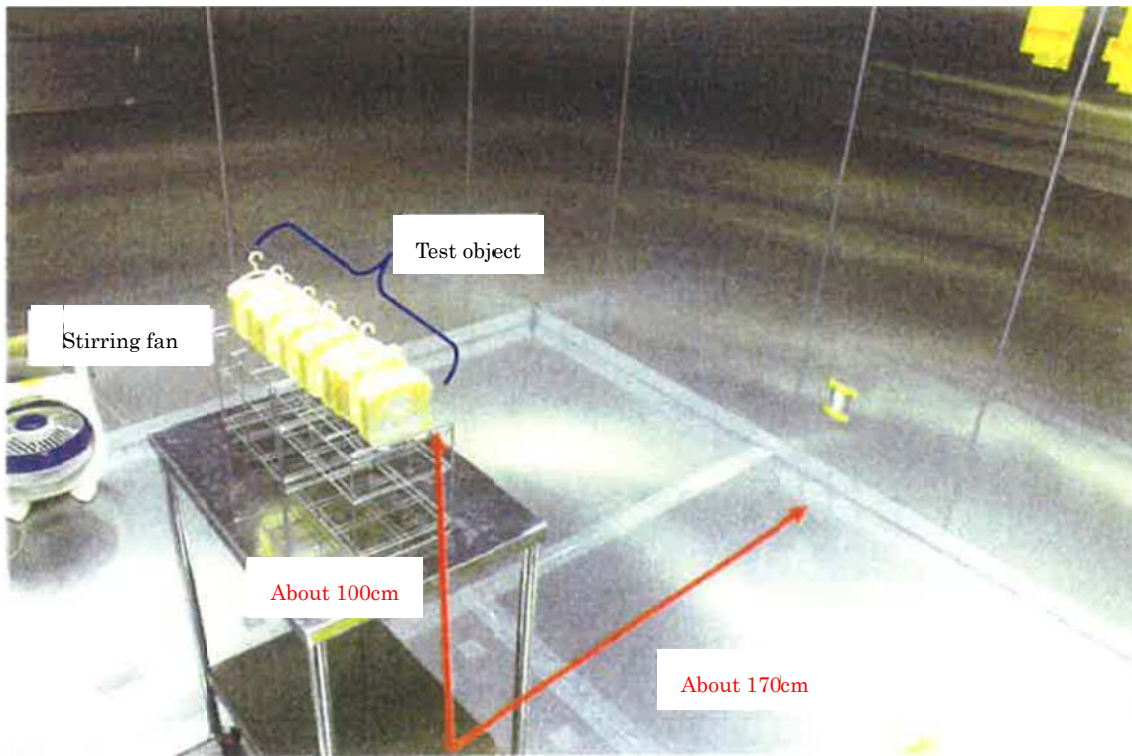


Photo-1 Test conditions inside 25m³ test chamber

Reference data

Chlorine dioxide gas concentration and temperature/humidity inside the test chamber during the testing are shown below:

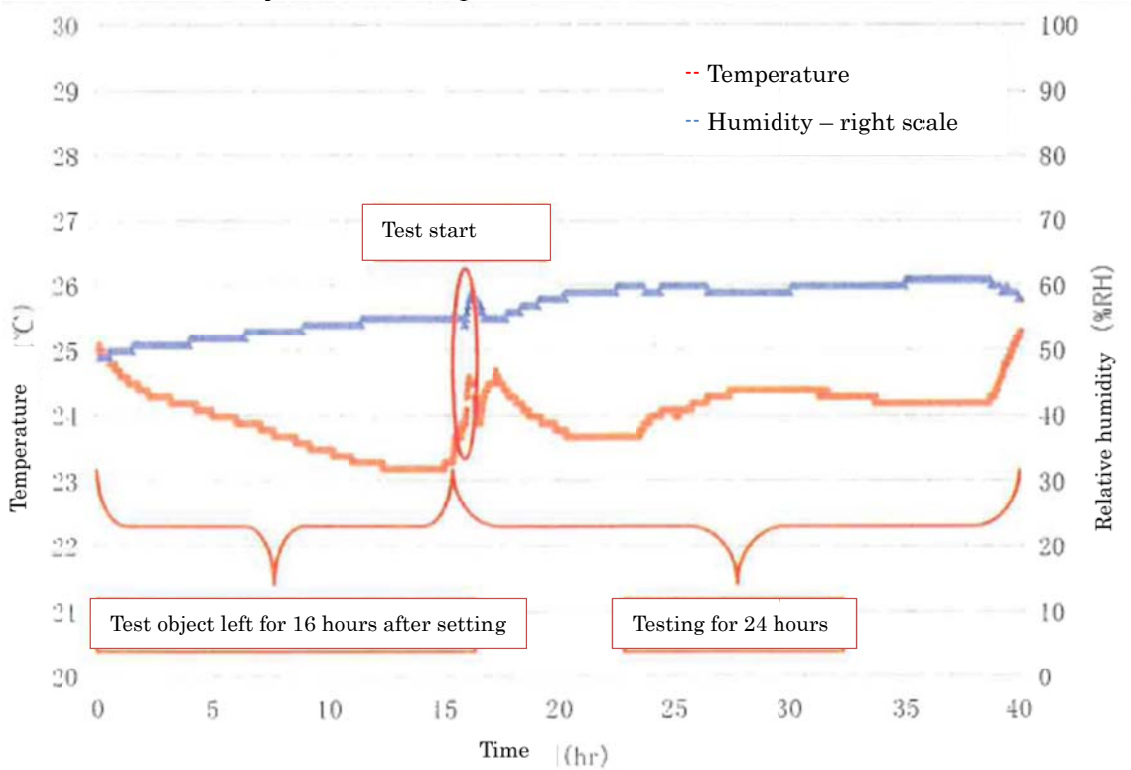
Table-3 Measurement results of chlorine dioxide gas concentration

Date	Time	Chlorine dioxide concentration(ppm)	Remarks
May 27 th	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 th	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: May 27th 9:50 (Experiment time 0 hour)

Test finish: May 28th 9:50 (Experiment time 24 hours)



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

Chart-1 Temperature and humidity variation inside the chamber during the test