

To: RAYCOP JAPAN INC.

Test Report

Test for virus inactivation by a built-in UVC lamp in a “bedding cleaner”

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Kitasato Research Center for Environmental Science
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Two company seals

When the test results are published, we confirm notation of results etc. from a professional point of view. The purpose of confirmation and an application form are published on our home page.
(http://www.kitasato-e.or.jp/?page_id=87)

1. Purpose

Effects of inactivation of type A influenza by a built-in UVC lamp in your company's "bedding cleaner" were evaluated.

2. Client

Name: RAYCOP JAPAN INC.

Address: Akasaka Park Building 11F, 5-2-20, Akasaka, Minato-ku, Tokyo, Japan 107-0052

3. Testing laboratory

Name: General Incorporated Foundation, Kitasato Research Center for Environmental Science

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

Department in charge: Division of virus, Department of virus

4. Test period

From April 7, 2017 to April 12, 2017

5. Specimen and test condition

1) RAYCOP VCEN-100

A specimen is shown in Picture 1.



Picture 1. Specimen

2) Irradiation light source

A built-in UVC lamp in the specimen

3) Irradiation time

For 0 (before irradiation), 5, 10 and 30 seconds

- 4) UV intensity measuring instrument
UV meter MODEL UVC-254 (CUSTOM Corporation)

6. Virus under testing and preparation method of virus fluid

Type A influenza virus (*Influenza A virus*, A/PR/8/34, ATCC VR-1469)

Virus fluid was prepared by inoculating influenza virus into the chorioallantoic membrane cavity of embryonated chicken eggs, culturing it in an incubator for eggs, collecting chorioallantoic fluid, and purifying it by density-gradient centrifugation. The obtained virus fluid was used as virus fluid for testing. The virus fluid was stored in a freezer at -80°C until it was used. When a test was conducted, it was diluted 10 times with phosphate buffered saline (PBS) and utilized.

7. Cell type for measuring infectivity titer

A cell line derived from dog kidney (MDCK: Madin-Darby canine kidney) was used as cells for measuring infectivity titer.

8. Test method

1) Test method

Tests for virus inactivation by irradiation using a built-in UVC lamp in each specimen were conducted by the following procedure.

Samples for measuring infectivity titer were prepared by dropping 1 mL of virus fluid on the lid of a dish made with plastic (IWAKI 3010-060), placing a UVC lamp so that the lamp was located at the shortest distance from the virus fluid level, exposing UV for a fixed time (Figure 1, Picture 2), and collecting the virus fluid

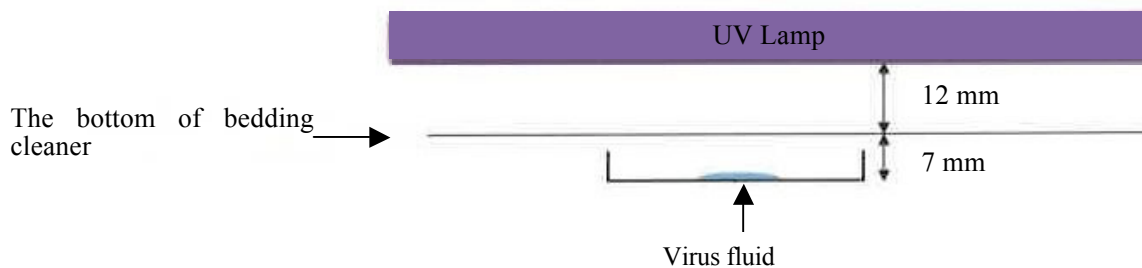


Figure 1. Test system overview



Picture 2. Appearance of UV irradiation

2) Virus quantification method

A 1/10 volume of 10 times concentrated PBS was added into a sample for measuring virus infectivity titer and it was named an undiluted solution of the sample for measuring virus infectivity titer. It was diluted 10 times with PBS stepwisely and inoculated into MDCK cells that were cultured on a 96 well plate in a monolayer culture beforehand, at 25 μ L per well. After letting it sit in a CO₂ incubator at 37°C for 1 hour, the inoculated virus fluid was removed and Minimum Essential Medium that contained 0.42% bovine serum albumin and 5 μ M of trypsin was added at 0.1 μ L per well, and then the cells were cultured in a CO₂ incubator at 37°C for 4 days. After the culture, a cytopathic effect (CPE) was confirmed under an inverted microscope and the infectivity titer was calculated using the Reed-Muench method.

3) Virus inactivation effects

Regarding the effects of virus inactivation by UV irradiation, the difference in virus infectivity titer between before and after irradiation was found as log reduction values (LRV), and the decreasing rate was calculated based on the LRV. The formula is indicated below. A value less than the detection limit (1.3×10^1 TCID₅₀/mL) was calculated as 1.3×10^1 TCID₅₀/mL.

1. Log reduction values = \log_{10} (Infectivity titer before irradiation/Infectivity titer after irradiation)

2. Decreasing rate = $[1 - 1/10^{\text{(infectivity titer log reduction values)}}] \times 100$ (%)

9. Test results

Test results are indicated in Table 1 and Figure 2.

In this test, the distance between the surface of the virus that was dropped on a dish and a UVC lamp was 19 mm in all specimens, and as a result of measurement, UV intensity was 6.63 mW/cm².

Infectivity titer before irradiation was 1.1×10^9 TCID₅₀/mL and infectivity titer after 5, 10, 30 seconds of irradiation was 2.1×10^2 TCID₅₀/mL, 1.4×10^1 TCID₅₀/mL and less than the detection limit (1.3×10^1 TCID₅₀/mL), respectively. LRV and the decreasing rate in each irradiation time was LRV = 6.7 \log_{10} (decreasing rate > 99.99%) for after 5 seconds irradiation, LRV = 7.8 \log_{10} (decreasing rate > 99.99%) for after 10 seconds irradiation, and LRV > 7.9 \log_{10} (decreasing rate > 99.99%) for after 30 seconds irradiation.

10. Comment

The effects of the inactivation of type A influenza by a built-in UVC lamp in a “bedding cleaner” that was provided by your company were investigated in this test. It is known that UV lamps are effective for sterilization and inactivation of many microorganisms and viruses.¹⁾⁻⁴⁾ Since UV is known to be absorbed by various substances⁴⁾, it is presumed that there is a potential where the effective UV dose decreases depending on the organic stain that contains a virus etc. due to absorption of UV. In addition, UV has a low light permeability for various substances and it is only effective on the surface where the light falls. Therefore, it is important to have the ingenuity to avoid making an area where no UV light falls.

Reference

- 1) Toshiharu Kwabata, Tsuneo Harada, Disinfection of Water by Germicidal Lamp, Journal of Science and Technology in Lighting, 36(3), pp. 89-96, 1952
- 2) Edited by Tsuyoshi Hirata, UV exposure-applicability for water disinfection, Gihodo Shuppan Co., Ltd., pp.101-116, 2008
- 3) Kaufman, J. E, IES Lighting Handbook 5th Ed., 1972
- 4) TOSHIBA Lighting & Technology Corporation, Toshiba germicidal lamp technical data, October, 2003 (publication of revision)

Concluded.

Table 1. Test for virus inactivation by irradiation using a built-in UVC lamp in a bedding cleaner

RAYCOP VCEN-100	UV irradiation time (The amount of UV irradiation ^{a)})			
	Before irradiation (0 mJ/cm ²)	For 5 seconds (33.2 mJ/cm ²)	For 10 seconds (66.3 mJ/cm ²)	For 30 seconds (198.9 mJ/cm ²)
UVC lamp	1.1×10^9	2.1×10^2	1.4×10^1	$< 1.3 \times 10^1$
Infectivity titer log reduction values ^{b)} (decreasing rate) ^{c)}		6.7 (> 99.99%)	7.8 (> 99.99%)	> 7.9 (> 99.99%)

Infectivity titer unit: TCID₅₀/mL

Detection limit: 1.3×10^1 TCID₅₀/mL

Distance of UVC lamp: 19 mm, UV intensity in this test: 6.63 mW/cm²

a) The amount of UV irradiation: UV intensity (mW/cm²) × Irradiation time (seconds)

b) Infectivity titer log reduction values: log₁₀ (Infectivity titer before irradiation/Infectivity titer after irradiation)

c) Decreasing rate: $[1 - 1/10^{(\text{Infectivity titer log reduction values})}] \times 100$ (%)

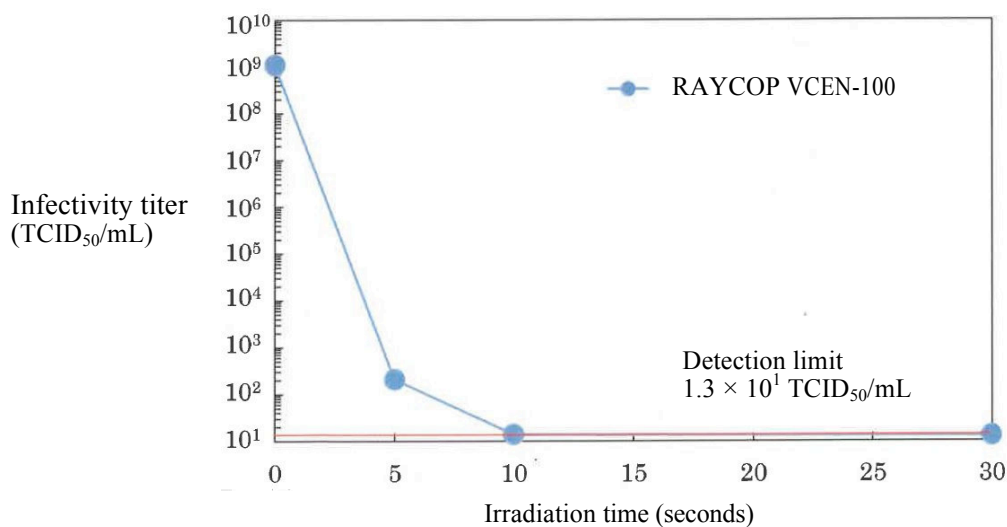


Figure 2. Test for virus inactivation by irradiation using a built-in UVC lamp in a specimen