To: RAYCOP JAPAN INC.

Test Report

Test for virus inactivation by a built-in UVC lamp of Raycop RSC

Issued by KRCES No. 2019_0538

April 21, 2020

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General Incorporated Foundation
Kitasato Research Center for Environmental Science
Director Yamada Haruki
Company seal

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. Subject

Effects of inactivation of type A influenza by a built-in UVC lamp of Raycop RSC were evaluated.

2. Test Number

Request No: 20197101
Report No: KRCES 2019-0538

3. Purpose

Evaluates the effects of inactivation of type A influenza by a built-in UVC lamp of Raycop RSC were evaluated.

4. Client

Name: RAYCOP JAPAN INC.

Address: Gotenyama Trust Twoer 17F, 4-7-35 Kitashinagawa, Shinagawa-ku, Tokyo, Japan 140-0001

5. 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

6. Test period

From April 6, 2020 to April 10, 2020

7. Specimen and test condition

RAYCOP RSC

A specimen is shown in Figure 1.

Figure 1. Specimen

1) Irradiation light source
   A built-in UVC lamp in the specimen

2) Irradiation time
   For 0 (before irradiation), 2, 5 and 10 seconds

3) Irradiation distance: 25 mm

4) UV intensity measuring instrument
UV meter UVX and UV intensity sensor UVX-25 (UVP)

8. Virus under testing and preparation method of virus fluid

Type A influenza virus (*Influenza A virus, H1N1,* 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.

9. 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.

10. 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 2), and collecting the virus fluid after 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 μL per well. After letting it sit in a CO_2 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 μg.mL of trypsin was added at 0.1 mL per well, and then the cells were cultured in a CO_2 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.

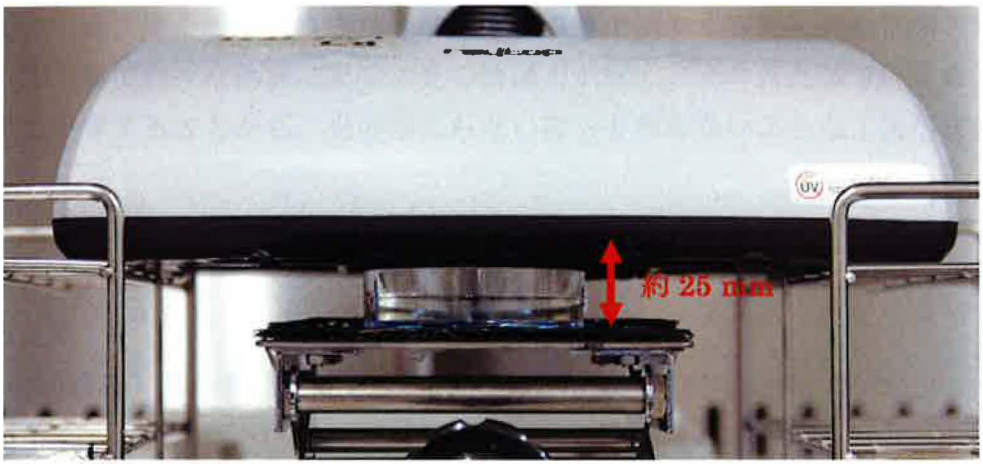

Figure 2. Appearance of UV irradiation

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.

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

2. Decreasing rate= [1- 1/10 (infectivity titer log reduction values)] × 100 (%)

11. Test results

Test results are indicated in Table 1 and Figure 3.

Infectivity titer after 2, 5, 10 seconds of irradiation was 1.7×10^3 $TCID_{50}$/mL, $<1.3 \times 10^1$ $TCID_{50}$/ mL and less than the detection limit (1.3×10^1 $TCID_{50}$/mL), respectively. LRV and the decreasing rate in each irradiation time was 3.8 (decreasing rate > 99.98%) for after 2 seconds irradiation, > 6.0 (decreasing rate > 99.99%) for after 5 seconds irradiation, and LRV > 6.0 (decreasing rate > 99.99%) for after 10 seconds irradiation. In this test, UV intensity was 1.96 mW/cm2.

12. 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 substances[4)], 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 effective on the surface where the light falls. Therefore, it is important to make an effort 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 of Raycop RSC

	UV irradiation time (The amount of UV irradiation [a)])			
	Before irradiation (0 mJ/cm^2)	For 2 seconds (3.92 mJ/cm^2)	For 5 seconds (9.8 mJ/cm^2)	For 10 seconds (19.6 mJ/cm^2)
Infectivity titer	1.3×10^7	1.7×10^3	$<1.3 \times 10^1$	$< 1.3 \times 10^1$
Infectivity titer log reduction values [b)] (decreasing rate) [c)]		3.8 (> 99.98%)	>6.0 (>99.99%)	> 6.0 (> 99.99%)

Infectivity titer unit: $TCID_{50}/mL$
Detection limit: 1.3×10^1 $TCID_{50}/mL$
UV intensity in this test: 1.96 mW/cm^2

a) The amount of UV irradiation: UV intensity (mW/cm^2) × Irradiation time (seconds)

b) Infectivity titer log reduction values: $\log_{10}$ (Infectivity titer before irradiation/Infectivity titer after irradiation)

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

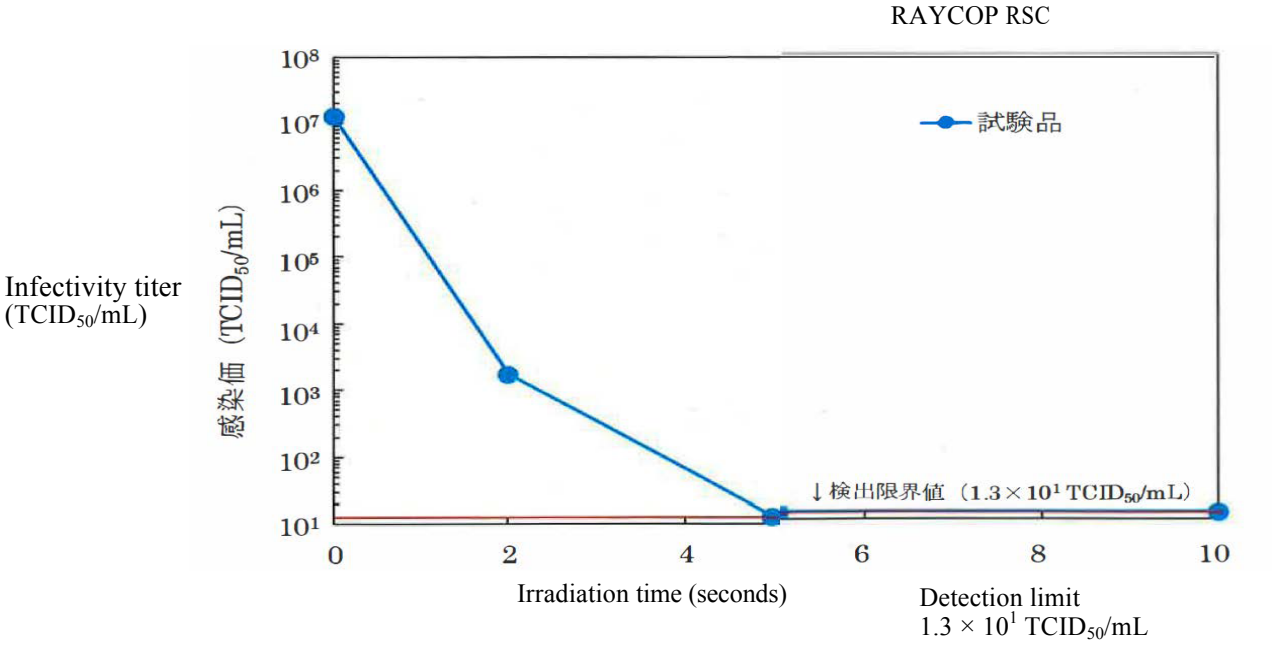

Figure 3. Test for virus inactivation by irradiation using a built-in UVC lamp of Raycop RSC