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For the Attention of RAYCOP JAPAN INC.

TEST REPORT

Test of Viral Inactivation by UVC Lamp Built into “Futon Cleaner”

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1. Objectives

The inactivating effect exerted upon influenza A virus by the UVC lamp built into your company's "Futon Cleaner" was evaluated.

2. Client

Name: RAYCOP JAPAN INC.

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

3. Testing laboratory

Name: Kitasato Research Center for Environmental Science

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

Unit in charge: Virus Division, Virus Department

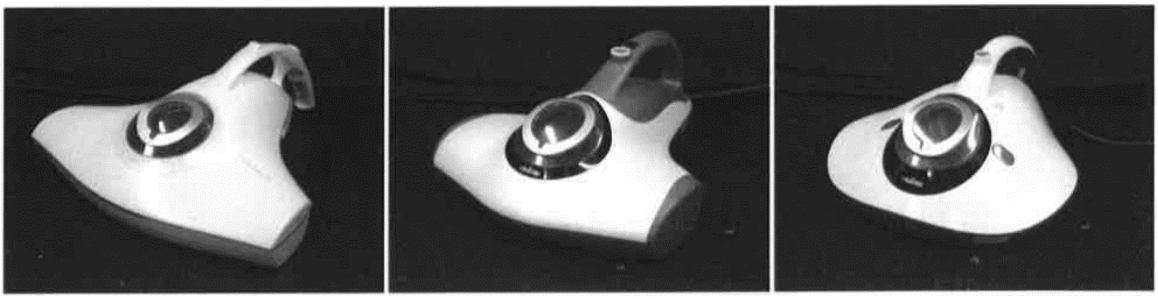
4. Test period

October 13, 2016 to November 9, 2016

5. Test pieces and test conditions

1) RAYCOP RS-2
2) RAYCOP RT-2
3) RAYCOP RE

Test pieces are shown in Photo 1.



RAYCOP RS-2 RAYCOP RT-2 RAYCOP RE

Photo 1. Test pieces

4) Irradiation source

UVC lamp built into each test piece

5) Irradiation time

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

6) Device for measuring intensity of ultraviolet rays
UV Meter MODEL UVC-254 (CUSTOM Corporation)

6. Test virus and method of preparing virus solution

Influenza A virus, A/PR/8/34, ATCC VR-1469

Influenza virus was inoculated into the chorioallantoic membrane cavities of embryonated eggs and cultured in an incubator. The chorioallantoic fluid was collected and purified by density-gradient centrifugation to produce the viral solution for use in testing. The viral solution was stored in a freezer at -80ºC until use and diluted 10-fold in PBS (phosphate buffered saline) at the time of testing.

7. Cell type for measurement of infectivity titer

Madin-Darby canine kidney (MDCK) cell line was used in the measurement of infectivity titer.

8. Test method

1) Test method

The viral inactivation test of the UVC lamp built into each test piece was performed according to the following procedure.

Virus solution in the amount of 1 mL was dropped onto the lid of a plastic dish (IWAKI 3010-060). The UVC lamp was set up at the shortest distance from the surface of the virus solution, and the solution was irradiated with ultraviolet radiation for the specified period of time (Fig. 1, Photo 2). After irradiation, the viral solution was recovered and used as the sample for measurement of infectivity titer.

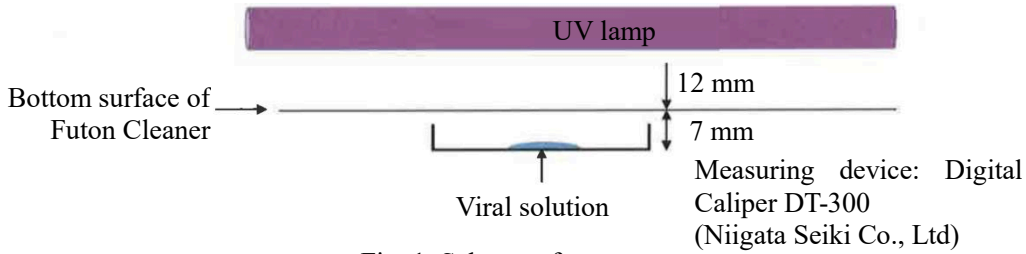


Fig. 1. Schema of test system

RAYCOP RS-2 RAYCOP RT-2 RAYCOP RE

Photo 2. State of ultraviolet irradiation

2) Method of quantifying virus

To the sample stock solution for measurement of viral infectivity titer, we added 1/10th volume of 10-fold-diluted PBS, serially diluted it 10-fold with PBS, and inoculated 25 μL of the solution into each well of a 96-well plate in which a monolayer culture of MDCK cells have been prepared in advance. After letting it stand in a CO_2 incubator at 37ºC for 1 hour, we then removed the inoculated viral solution, added 0.1 mL of Minimum Essential Medium with added 0.42% bovine serum albumin and 5 μg/mL trypsin to each well, and cultured it in a CO_2 incubator at 37ºC for 4 days. After culturing was completed, we checked the cytopathic effect (CPE) under an inverted microscope and calculated the infectivity titer with the use of the Reed-Muench method.

3) Viral inactivation effect

The viral inactivation effect of the ultraviolet irradiation was determined as the log reduction value (LRV) of the difference in viral infectivity titer before and after irradiation, and the reduction rate was calculated on the basis of the LRV. The calculation formula is shown below. Values under the detection limit (1.3×10^1 $TCID_{50}$/mL) were calculated as 1.3×10^1 $TCID_{50}$/mL.

[1] LRV = $\log_{10}$ (infectivity titer before irradiation / infectivity titer after irradiation)

$$[2]\ \text{Reduction rate} = \left(1 - \frac{1}{10^{\text{(Infectivity titer LRV)}}}\right) \times 100\ (\%)$$

9. Test results

The test results are shown tin Table 1 to Table 3 and Fig. 2.

In this test, the distance from the surface of the virus dropped onto the dish to the UVC lamp was 19 mm for all test pieces, and the result of measurement of the intensity of ultraviolet rays was 4.73 mW/cm^2 for RAYCOP RS-2, 4.20 mW/cm^2 for RAYCOP RT-2, and 3.11 mW/cm^2 for RAYCOP RE.

The infectivity titer before irradiation was 8.9×10^7 $TCID_{50}$/mL. In RAYCOP RS-2, the infectivity titers for 5 seconds and 10 seconds of irradiation with the UVC lamp were 7.7×10^2 $TCID_{50}$/mL and 5.3×10^1 $TCID_{50}$/mL, respectively. After 30 seconds of irradiation, the infectivity titer fell below the detection limit (1.3×10^1 $TCID_{50}$/mL). The LRV and reduction rate for each irradiation time were LRV = 5.0 $\log_{10}$ (reduction rate > 99.99%) after 5 seconds of irradiation, LRV = 6.2 $\log_{10}$ (reduction rate > 99.99%) after 10 seconds of irradiation, and LRV > 6.8 (reduction rate > 99.99%) after 30 seconds of irradiation. In RAYCOP RT-2, the infectivity titers after 5 seconds and 10 seconds of irradiation with a UVC lamp were 5.0×10^3 $TCID_{50}$/mL and 1.4×10^2 $TCID_{50}$/mL, respectively. The infectivity titer after 30 seconds of irradiation was below the detection limit (1.3×10^1 $TCID_{50}$/mL). The LRV and reduction rate for each of the irradiation

times (5 seconds, 10 seconds, and 30 seconds) were LRV = 4.2 $\log_{10}$ (reduction rate > 99.99%), LRV = 5.8 $\log_{10}$ (reduction rate > 99.99%), and LRV > 6.8 (reduction rate > 99.99%), respectively. For RAYCOP RE, the infectivity titers after 5 seconds and 10 seconds of irradiation with a UVC lamp were 1.7×10^4 $TCID_{50}$/mL and 8.4×10^2 $TCID_{50}$/mL, respectively. The infectivity titer after 30 seconds of irradiation was below the detection limit (1.3×10^1 $TCID_{50}$/mL). The LRV and reduction rate for each of the irradiation times (5 seconds, 10 seconds, and 30 seconds) were LRV = 3.7 $\log_{10}$ (reduction rate > 99.98%), LRV = 5.0 $\log_{10}$ (reduction rate > 99.99%), and LRV > 6.8 (reduction rate > 99.99%), respectively.

10. Comment

This test investigated the influenza A virus inactivation effect of the UVC lamp built into the "Futon Cleaner" provided by your company. Ultraviolet lamps are known to be effective in killing and inactivating many microorganisms and viruses.[1-4] Ultraviolet rays are known to be absorbed by various substances,[4] and it is assumed that, depending upon the nature of the organic matter (stain) that contains the virus, the ultraviolet rays may be absorbed and the effective amount of ultraviolet radiation reduced. Moreover, since ultraviolet light has limited ability to penetrate certain substances, the viral inactivation effect will be exhibited only on the surfaces illuminated by the light. For this reason, it is important to devise means of ensuring that no areas are left unexposed to the light.

References

1) Kawabata T., Harada T., Sterilization of water with a sterilization lamp, Journal of the Illuminating Engineering Institute of Japan, 36 (3), pp. 89–96, 1952
2) Hirata T., ed., Ultraviolet irradiation – Adaptability to the sterilization of water, GIHODO SHUPPAN Co., Ltd., pp. 101‐116, 2008
3) Kaufman, J.E., IES Lighting Handbook 5th Ed., 1972
4) Toshiba Lighting & Technology Corporation, Technical Data on Toshiba Sterilization Lamps, October 2003 (revised issue)

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Table 1. Test of viral inactivation by UVC lamp built into Futon Cleaner

RAYCOP RS-2	Ultraviolet irradiation time (amount of ultraviolet irradiation[a)])			
	Before irradiation (0 mJ/cm^2)	5 seconds (23.6 mJ/cm^2)	10 seconds (47.3 mJ/cm^2)	30 seconds (141.9 mJ/cm^2)
UVC lamp	8.9×10^7	7.7×10^2	5.3×10^1	$<1.3 \times 10^1$
Infectivity titer LRV[b)] (reduction rate)[c)]		5.0 (> 99.99%)	6.2 (> 99.99%)	> 6.8 (> 99.99%)

Unit for infectivity titer: $TCID_{50}$/mL

Detection limit: 1.3×10^1 $TCID_{50}$/mL

Infectivity titer of test virus: 1.6×10^8 $TCID_{50}$/mL

Distance to UVC lamp, 18.6 mm; intensity of ultraviolet rays in this study, 4.73 mW/cm^2

a) Amount of ultraviolet irradiation: intensity of ultraviolet irradiation (mW/cm^2) × irradiation time (seconds)

b) Infectivity titer LRV: $\log_{10}$ (infectivity titer before irradiation / infectivity titer after irradiation)

c) Reduction rate: $\left(1 - \frac{1}{10^{\text{(Infectivity titer LRV)}}}\right) \times 100$ (%)

Table 2. Test of viral inactivation by UVC lamp built into Futon Cleaner

RAYCOP RT-2	Ultraviolet irradiation time (amount of ultraviolet irradiation[a)])			
	Before irradiation (0 mJ/cm^2)	5 seconds (21.0 mJ/cm^2)	10 seconds (42.0 mJ/cm^2)	30 seconds (126.0 mJ/cm^2)
UVC lamp	8.9×10^7	5.0×10^3	1.4×10^2	$< 1.3 \times 10^1$
Infectivity titer LRV[b)] (reduction rate)[c)]		4.2 (> 99.99%)	5.8 (> 99.99%)	> 6.8 (> 99.99%)

Unit for infectivity titer: $TCID_{50}$/mL

Detection limit: 1.3×10^1 $TCID_{50}$/mL

Infectivity titer of test virus: 1.6×10^8 $TCID_{50}$/mL

Distance to UVC lamp, 18.6 mm; intensity of ultraviolet rays in this study, 4.20 mW/cm^2

a) Amount of ultraviolet irradiation: intensity of ultraviolet irradiation (mW/cm^2) × irradiation time (seconds)

b) Infectivity titer LRV: $\log_{10}$ (infectivity titer before irradiation / infectivity titer after irradiation)

c) Reduction rate: $\left(1 - \frac{1}{10^{\text{(Infectivity titer LRV)}}}\right) \times 100$ (%)

Table 3. Test of viral inactivation by UVC lamp built into Futon Cleaner

RAYCOP RE	Ultraviolet irradiation time (amount of ultraviolet irradiation[a])			
	Before irradiation (0 mJ/cm^2)	5 seconds (15.5 mJ/cm^2)	10 seconds (31.1 mJ/cm^2)	30 seconds (93.3 mJ/cm^2)
UVC lamp	8.9×10^7	1.7×10^3	8.4×10^2	$<1.3 \times 10^1$
Infectivity titer LRV[b] (reduction rate)[c]		3.7 (> 99.99%)	5.0 (> 99.99%)	> 6.8 (> 99.99%)

Unit for infectivity titer: $TCID_{50}$/mL

Detection limit: 1.3×10^1 $TCID_{50}$/mL

Infectivity titer of test virus: 1.6×10^8 $TCID_{50}$/mL

Distance to UVC lamp, 18.6 mm; intensity of ultraviolet rays in this study, 3.11 mW/cm^2

a) Amount of ultraviolet irradiation: intensity of ultraviolet irradiation (mW/cm^2) × irradiation time (seconds)

b) Infectivity titer LRV: $\log_{10}$ (infectivity titer before irradiation / infectivity titer after irradiation)

c) Reduction rate : $\left(1 - \frac{1}{10^{(\text{Infectivity titer LRV})}}\right) \times 100$ (%)

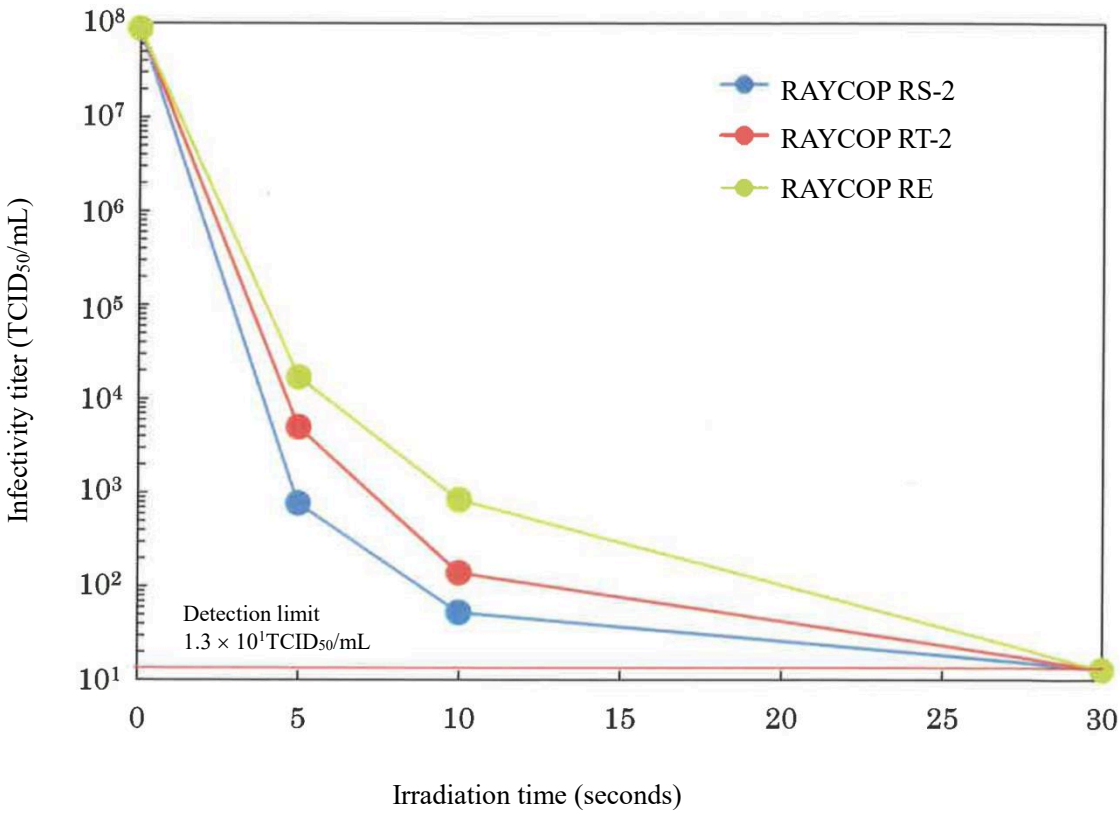

Fig. 2. Test of viral inactivation by UVC lamp built into Futon Cleaner