2019_0032

To USHIO Inc.

Test Report

Performance test for virus inactivation efficacy by UV irradiation

KRCES Report #2019_0032

September 11, 2019

Approved by: Haruki Yamada

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1. Title

   Performance test for virus inactivation efficacy by UV irradiation

2. Test number

   Test request No.: 20197005

   Test report No.: KRCES 2019_0032

3. Test Objective

   To investigate the virus inactivation effect by irradiation of the “light source of emitting a wave length of 222 nm” manufactured by USHIO Inc. in application of the influenza A virus.

4. Client

   Name: USHIO Inc.

   Address: 1194, Sazuchi, Bessho-Cho, Himeji, Hyogo, 671-0224, Japan

5. Testing laboratory

   Name: Kitasato Research Center for Environmental Science

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

6. Test period

   June 3, 2019 - July 13, 2019

7. Test sample and test condition

   1) Test sample

      Light source of emitting a wavelength of 222 nm (Shown in Photo-1)

Photo 1. Light Source of 222nm

2) Test condition

① Irradiation distance: 2 m

Irradiation period: 10, 20, 30 and 40 minutes

② Irradiation distance: 50 cm

Irradiation period: 20, 40, 80 and 160 seconds

3) UV irradiance measuring device

UV irradiance photometer (Model No. UIT-250/VUV-S172 manufactured by USHIO Inc.)

8. Medium, Reagents and apparatus

1) medium

① Dulbecco's Modified Eagle's Medium (DMEM, SIGMA-Aldrich)

② Eargle MEM "NISSUI" ① (MEM, NISSUI)

2) reagents

① Fetal Bovine Serum (FBS, SIGMA-Aldrich)

② Dulbecco's PBS (-) "Nissui" (PBS: Phosphate buffered saline, NUSSUI)

③ Bovine Serum Albumin (BSA: SIGMA-Aldrich)

④ Trypsin (SIGMA-Aldrich)

3) Apparatus

① Micropipette 200 μL, 1,000 μL (GILSON)

② Electronic pipette, 8-channel (10 - 300 μL, Sartorius)

③ Electronic pipette, 8-channel (50 – 1,200 μL, Sartorius)

④ Cell lifter (AS ONE)

⑤ Biological Safty Cabinet (BHC-1902 IIB, Airtech)

⑥ CO_2 incubator (MCO-20AIC, Sanyo)

9. Preparation of test virus

Influenza A virus (A/PR/8/34, ATT VR-1469) was inoculated on Madin-Darby canine kidney (MDCK) monolayer cells. The virus-infected cells were incubated at 37 ºC for 2 to 3 days. The cells were checked for cytopathic effects (CPE) every day. When approximately 90% of the cells showed CPE, the cell culture was freeze at -30 ºC. The cell lysate was prepared by freezing and thawing and culture supernatant was collected by centrifugation at 2,380 × g for 10 minutes. The culture supernatant was concentrated with ultrafiltration membrane. A virus suspension was prepared by ultracentrifugation (108,000 × g at 4 ºC for 3 hours) using a

sucrose cushion method.

10. The cell line for measurement for virus infectivity titer

MDCK was used for the measurement of influenza virus titer.

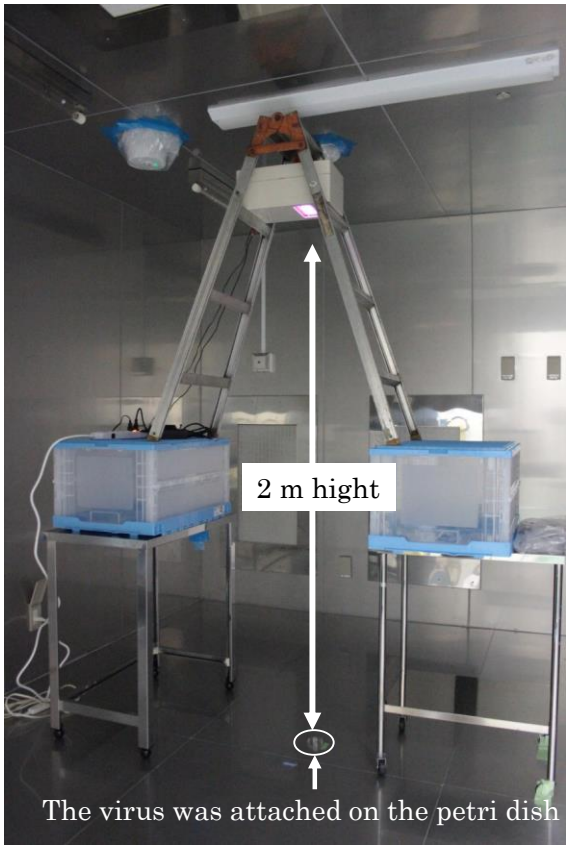
11. Test procedure

1) Preparation of virus adhesion test carrier

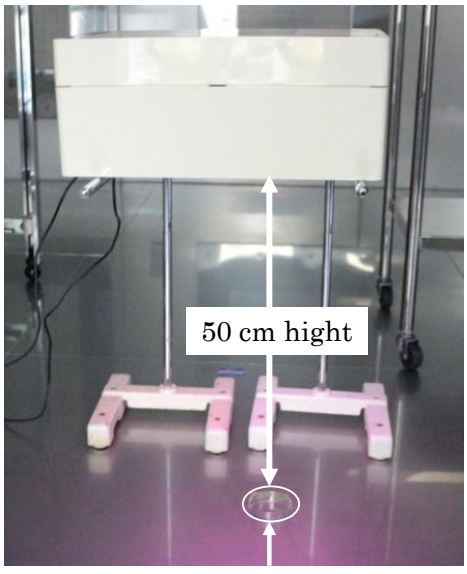
Two μL of the virus suspension was dropped at 5 locations on the petri dish (IWAKI 3010-060) and air-dried for about 30 minutes in a biological safety cabinet to obtain a virus adhesion test carrier.

2) UV irradiation for virus inactivation

The test sample was placed at 2 m or 50 cm at the surface of the petri dish (Photo-2) and UV was irradiated for a predetermined period. After irradiation, add 1 mL of MEM supplemented with 0.42 % BSA into the petri dish and rubbing the surface for about 1 minute by the cell lifter, and finally collected the medium and used for measurement of virus infectivity.



Irradiation from a height of 2 m



Irradiation from a height of 50 cm

Photo 2. UV irradiation from two difference distances

3) Measurement of infectivity titer

Viral infectivity titers of the collected medium were determined by observing CPEs on MDCK cells. MDCK monolayer cell cultures were inoculated with 25 μL of the viral suspension, which was 10-fold serially diluted with PBS. The cultures were incubated for 1 hour at 37 °C in the humidified atmosphere with 5% CO_2 to allow the virus to be adsorbed. After 1 hour incubation, 0.1 mL of the medium (MEM supplemented with 0.42 % BSA and 5 μg/mL trypsin) was added to each well. After 4-days incubation at 37 °C in the humidified atmosphere with 5% CO_2, virus-induced CPEs were observed under a microscope. The virus titer ($TCID_{50}$/mL) was calculated by the Reed-Muench method.

4) The calculation for viral inactivation efficacy

The virus inactivation efficacy by UV irradiation was obtained by calculating the difference in viral infectivity titer before and after irradiation as a log reduction value (LRV) and calculating the reduction rate based on the log reduction value. The calculation formula is shown below.

① Log reduction of viral infectivity = $\log_{10}$ (before irradiation / after irradiation)

② Reduction rate = $\left(1 - \frac{1}{10^{\text{Log reduction value}}}\right) \times 100\ (\%)$

5) Integrated illuminance

The integrated illuminance was calculated by the UV intensity measured by the person in charge of USHIO Inc. and the irradiation time. The calculation formula is shown below.

Integrated illuminance (mJ/cm^2) = UV intensity ($\mu W/cm^2$) × irradiation time (second) / 1000

12. Test results

The test results were shown in Tables 1 to 4.

The viral infectivity titer before irradiation was 1.8×10^5 $TCID_{50}$/mL. When the UV was irradiated from a height of 2 m for 10 and 20 minutes, viral infectivity titer was decreased to 2.1×10^2 $TCID_{50}$/mL and 1.7×10^2 $TCID_{50}$/mL, respectively. When the virus was irradiated with UV for more than 30 minutes, virus infectivity titer was decreased less than the detection limit (1.3×10^1 $TCID_{50}$/mL). On the other hand, when the UV was irradiated from a height of 50 cm for 20, 40 seconds, viral infectivity titer was decreased to 4.0×10^3 $TCID_{50}$/mL and 2.7×10^2 $TCID_{50}$/mL, respectively. When the virus was irradiated with UV for more than 80 seconds, virus infectivity titer was decreased less than the detection limit (1.3×10^1

$TCID_{50}$/mL).

The UV intensity of the irradiated the “Light source of 222 nm” from a height of 2 m and 50 cm was 5 μW/cm^2 and 75 μW/cm^2, respectively.

13. Comments

In this test, the virus inactivation efficacy of influenza A virus using the “Light source of 222 nm” manufactured USHIO Inc. was investigated. As the test results, the reduction rate was showed more than 99.8 % at 3 mJ/cm^2, regardless of the irradiation height. Furthermore, more than 99.99 % of reduction rate was obtained with 6mJ/cm^2 at a height of 50 cm, and with 9 mJ/cm^2 at 2 m, respectively.

A general 254 nm UV lamp is known to be effective in sterilization and inactivation of many microorganisms and viruses [1~4]. In addition, “Light source of 222 nm” was also confirmed to be effective against influenza A virus, as shown in Table 1 ~ 4.

Reference

1) Toshiharu Kawabata, Tsuneo Harada, Disinfection of water by a UV lamp. Journal of the Illuminating Engineering Institute of Japan, 36 (3), pp.89–96, 1952 (in Japanese)
2) Tsuyoshi Hirata, UV the - application for disinfection of water, GIHODO SHUPPAN, pp.101–116, 2008 (in Japanese)
3) Kaufman, J.E, IES Lighting Handbook 5th Ed., 1972
4) Toshiba Lighting & Technology Corporation, Toshiba germicidal lamp, Technical data, 2003 October (revised issue) (in Japanese)

Table 1. Virucidal efficacy by irradiation of "Light source of 222 nm" from a height of 2 m.

Test sample	Irradiation time and integrated illuminance [a]				
	0 (before irradiation) (0 mJ/cm^2)	10 minutes (3.0 mJ/cm^2)	20 minutes (6.0 mJ/cm^2)	30 minutes (9.0 mJ/cm^2)	40 minutes (12.0 mJ/cm^2)
Light source of 222 nm	1.8×10^{5} [b]	2.1×10^{2}	1.7×10^{2}	$< 1.3 \times 10^{1}$	$< 1.3 \times 10^{1}$

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

Units: $TCID_{50}$/mL

Viral infectivity of test virus suspension: 5.9×10^{8} $TCID_{50}$/mL

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

a) Calculate from measurement value (5 μW/cm^2) and irradiation time.

b) Viral infectivity titer after irradiation (unit: $TCID_{50}$/mL)

Table 2. The log reduction value and reduction rate by irradiation "Light source of 222 nm" from a height of 2 m.

Light source of 222 nm	Irradiation time and integrated illuminance [a]			
	10 minutes (3.0 mJ/cm^2)	20 minutes (6.0 mJ/cm^2)	30 minutes (9.0 mJ/cm^2)	40 minutes (12.0 mJ/cm^2)
Log reduction value [b]	2.9	3.0	> 4.1	> 4.1
Reduction rate (%) [c]	99.87	99.90	> 99.99	> 99.99

a) Calculate from measurement value (5 μW/cm^2) and irradiation time

b) Calculation formula = Log_{10} (viral infectivity titer before irradiation / viral infectivity titer after irradiation)

c) Calculation formula = $\left(1 - \frac{1}{10^{\text{Log reduction value}}}\right) \times 100\ (\%)$

Table 3. Virucidal efficacy by irradiation of "Light source of 222 nm" from a height of 50 cm.

Test sample	Irradiation time and integrated illuminance [a]				
	0 (before irradiation) (0 mJ/cm^2)	20 seconds (1.5 mJ/cm^2)	40 seconds (3.0 mJ/cm^2)	80 seconds (6.0 mJ/cm^2)	160 seconds (12.0 mJ/cm^2)
Light source of 222 nm	1.8×10^{5} [b]	4.0×10^{3}	2.7×10^{2}	$< 1.3 \times 10^{1}$	$< 1.3 \times 10^{1}$

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

Units: $TCID_{50}$/mL

Viral infectivity of test virus suspension: 5.9×10^{8} $TCID_{50}$/mL

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

a) Calculate from measurement value (75 μW/cm^2) and irradiation time.

b) Viral infectivity titer after irradiation (unit: $TCID_{50}$/mL)

Table 4. The log reduction value and reduction rate by irradiation "Light source of 222 nm" from a height of 50 cm .

Light source of 222 nm	Irradiation time and integrated illuminance [a]			
	20 seconds (1.5 mJ/cm^2)	40 seconds (3.0 mJ/cm^2)	80 seconds (6.0 mJ/cm^2)	160 seconds (12.0 mJ/cm^2)
Log reduction value [b]	1.6	2.8	> 4.1	> 4.1
Reduction rate (%) [c]	97.48	99.84	> 99.99	> 99.99

a) Calculate from measurement value (75 μW/cm^2) and irradiation time.

b) Calculation formula = Log_{10} (viral infectivity titer before irradiation / viral infectivity titer after irradiation)

c) Calculation formula $= \left(1 - \frac{1}{10^{\text{Log reduction value}}}\right) \times 100\ (\%)$