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EVALUATION OF VIRUCIDE ACTIVITY OF A UV-C LED LAMP SYSTEM BY LIGHT PROGRESS





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TARGET

It is recognized that UV-C light has the ability to stop microbial and viral growth, its physical approach is considered a good compromise between cost and effectiveness, which is why it is progressively becoming more and more widespread in health care and home.

The correct use of UV-C technology takes the following parameters into account: distance from the light source (m), spatial light distribution, radiant power (W), irradiance (W/m^2), inversely proportional to the square of the distance, and radiation times (min). It allows a deep disinfection of objects that are exposed to an adequate dose of UV-C rays, where the dose (J/m^2) is the product of irradiation time and irradiance. Predictive models, that take into account the parameters described above, make it possible to predict the disinfection capacity of systems based on UV-C technology. In particular, once the dose corresponding to a specific reduction in microbial load has been established, they enable the relative UV-C irradiation times to be predicted for each distance, and viceversa.

The aim of this study is to determine the virucide activity, against the SARS-CoV-2, of the UV-C radiation which occurs in a customized UV-C LED Lamp System.

UV-C LED LAMP SYSTEM

The UV-C LED Lamp System, developed by Light Progress, is composed of a bar with 3 LED (Light Emitting Diode) positioned on a Printed Circuit Board (PCB). The distance between each LED is 40mm (Figure 1).

The LED Lamp System has three exchangeable supports that provide fixed distance at 1 cm, 2 cm and 3 cm respectively. In the face opposite to where the LEDs are welded there are two heat sinks to dissipate the heat (Figure 2).



Figure 1 LED Lamp System



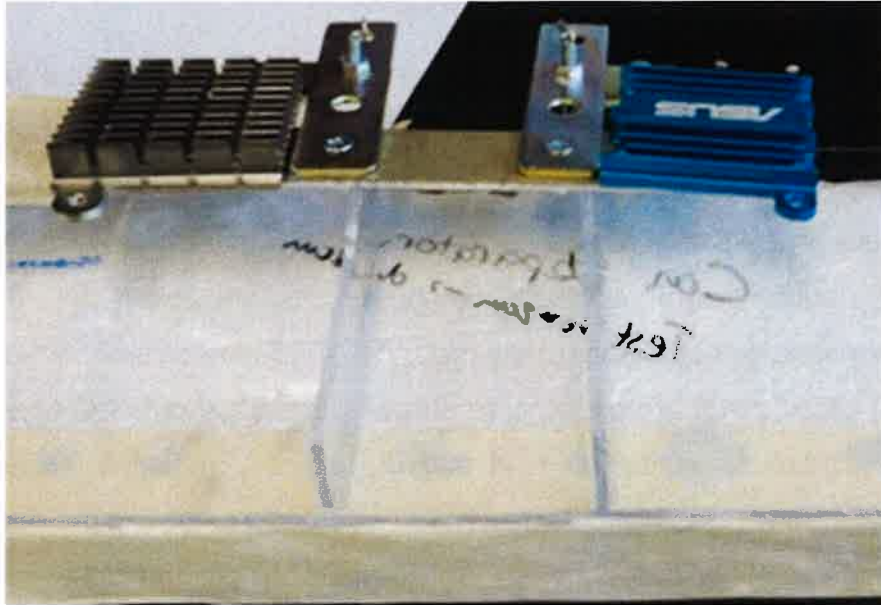


Figure 2 UV-C LED lamp system with the heatsink

PARAMETERS ESTABLISHED FOR THE TESTS

Name of product tested: UV-C LED Lamp System (model: UVLED-STRIP -3b-70°-40)

Period of analysis: 16/06/20 – 19/06/20

Identification of Viral strain: SARS-CoV-2 (Lot: VMR –SARSCP2 VERO E6_28042020)

Distance and irradiation time protocol:

Distance	Irradiation time	Distance	Irradiation time	Distance	Irradiation time
1 cm	1 min	2 cm	2 min	3 cm	3 min
	2 min		3 min		5 min
	3 min		5 min		10 min

Temperature of incubation: 37°C

Repetition of tests: 3 times, for each timing and distance

Temperature of incubation: 37°C

Incubation period: 3 days

OPERATIVE TECHNIQUE

All repetitions were tested for SARS-CoV-2 concentration by TCID₅₀% using VERO E6 C1008 (ATCC CRL-1586) cell line.

Set up

With the exchangeable supports, it was possible to adjust the distances of the slides, inoculated with the virus, at 1 cm, 2 cm and 3 cm, from the 3 LEDs of the UV-C LED Lamp System. The exchangeable supports were built with 2 UV-C shielding walls to avoid that the 3 LED could have overlapping irradiations (Figure 3). The system was turned on by plugging in the power plug.



Figure 3 Removable base system

Experimental method

Three glass slides were positioned under each LED. Over the glass slides, 100 μ L of viral suspension, having a concentration of $10^{7.2}$ TCID₅₀ /mL, were inoculated. The TCID₅₀% assay is used to quantify viral titers by determining the concentration at which 50% of the infected cells display cytopathic effect (CPE).

Three different distances have been evaluated (figure 4). After the irradiation time, the residual virus activity was tested by evaluating the Tissue Culture Infective Dose 50% (TCID₅₀%).

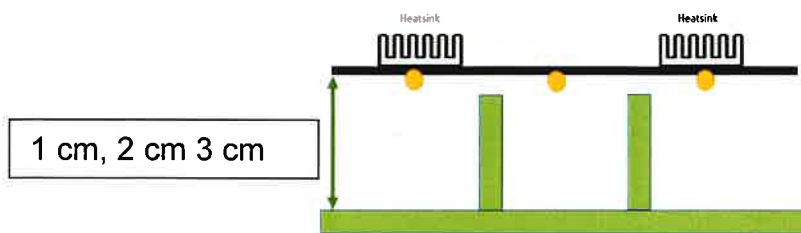


Figure 4 LED and heatsinks set up position with the 3 different distances.

The the glass slides were irradiated according to the protocol.

Examined samples:

- 3 samples (1 per LED) inoculated with viruses and subjected to the action of UV-C with protocol timing;
- 3 samples inoculated but not treated with UV to determine viral titer after recovery and examined immediately after inoculation.

The collected suspensions were used to inoculate a 48-wells plate into which the VERO E6 cell cultures were fixed.

Subsequent decimal dilutions were inoculated for a total of 10 dilutions. Each dilution was inoculated in 4 wells.

The plates were incubated for 3 days at 37°C ±2°C at 5% CO₂ in a humidified atmosphere.

After the exposure time, the residual virus activity was tested by evaluating the Tissue Culture Infective Dose 50% (TCID₅₀%).

Viral titers were determined according to the method developed by Spearman-Kärber where percent reduction of virus is determined according to the following formula:

$$[1-(T/C)]*100$$

where:

T = Log₁₀ of Virus Test Carrier

C = Log₁₀ of Virus Control Carrier





RESULTS

The results are presented in the tables 1 to 3.

Table 1: Tests of the UV-C LED Lamp System at 1 cm from the glass slides and 1, 2 and 3 minutes of UV-C exposition

Distance	Contact time	Repetition & LED	Log suspension virus TCID50%	Log TCID50%	Log reduction TCID50%
1 cm	1 min	1 – LED 1	7.2	1.50	5.7
		2 – LED 2	7.2	1.50	5.7
		3 – LED 3	7.2	1.50	5.7
	2 min	1 – LED 1	7.2	1.50	5.7
		2 – LED 2	7.2	1.50	5.7
		3 – LED 3	7.2	1.50	5.7
	3 min	1 – LED 1	7.2	1.50	5.7
		2 – LED 2	7.2	1.50	5.7
		3 – LED 3	7.2	1.50	5.7

Table 2: Tests of the UV-C Led Lamp System at 2 cm from the glass slides and 2, 3 and 5 minutes of UV-C exposition

Distance	Contact time	Repetition & LED	Log suspension virus TCID50%	Log TCID50 %	Log reduction TCID50%
2 cm	2 min	1 – LED 1	7.2	1.50	5.7
		2 – LED 2	7.2	1.50	5.7
		3 – LED 3	7.2	1.50	5.7
	3 min	1 – LED 1	7.2	1.50	5.7
		2 – LED 2	7.2	1.50	5.7
		3 – LED 3	7.2	1.50	5.7
	5 min	1 – LED 1	7.2	1.50	5.7
		2 – LED 2	7.2	1.50	5.7
		3 – LED 3	7.2	1.50	5.7

* The value of Log TCID50% = 1.50 means total viral inactivation





Table 3: Tests of the UV-C Led Lamp System at 2 cm from the glass slides and 3, 5 and 10 minutes of UV-C exposition

Distance	Contact time	Repetition & LED	Log suspension virus TCID50%	Log TCID50 %	Log reduction TCID50%
3 cm	3 min	1 – LED 1	7.2	1.50	5.7
		2 – LED 2	7.2	1.50	5.7
		3 – LED 3	7.2	1.50	5.7
	5 min	1 – LED 1	7.2	1.50	5.7
		2 – LED 2	7.2	1.50	5.7
		3 – LED 3	7.2	1.50	5.7
	10 min	1 – LED 1	7.2	1.50	5.7
		2 – LED 2	7.2	1.50	5.7
		3 – LED 3	7.2	1.50	5.7

* The value of Log TCID50% = 1.50 means total viral inactivation



DISCUSSION AND CONCLUSIONS

The results showed that testing the customized UV-C LED Lamp System by light Progress against SARS – CoV-2:

- the maximum measurable Log₁₀ reduction, equal to 5.7 (99.9998%), was reached at every time tested, for all distances and all repetitions;
- the irradiation of each of the three UV-C LEDs appears consistent with each other and has an equivalent effectiveness in inactivating the virus.

REFERENCES

ZIMBRO, M.J. ET AL., 2009: *DIFCO & BBL MANUAL –MANUAL OF MICROBIOLOGICAL CULTURE MEDIA-* SECOND EDITION

RAMAKRISHNAN, M.A., Determination of 50% endpoint titer using a simple formula, *World J Virol.* 2016 May 12; 5(2): 85–86.

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