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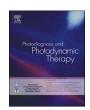
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### UV-C (254 nm) lethal doses for SARS-CoV-2

#### Dear Editor

The rapid and continuous spread of SARS-CoV-2, responsible for COVID-19, has been challenging global health systems and many strategies have been proposed to face the COVID-19 pandemic crisis [1]. In this scenario, ultraviolet lamps emitting ultraviolet C (UV-C) germicidal radiation (peak emission at 254 nm) are in the spotlight to provide efficient and sustainable disinfection of air, liquids and surfaces (e.g., plastics, fabrics, metals, etc). However, UV light with wavelengths greater than 180 nm can cause health adverse effects as eye damage, skin cancer and ageing, and UV-C should be not used in inhabited environments. Herein, we established the inactivation kinetics and reported the UV-C lethal doses (LD) for SARS-CoV-2.

A twenty-four-well plate was seeded with  $2\times10^5$ /mL Vero cells (ATCC CCL-81) for a final volume of 500  $\mu$ L/well. Cells were kept in the DMEM High Glucose (DMEN-HG) culture medium (Sigma-Aldrich, USA) supplemented with 10 % bovine fetal serum, 100 units/mL penicillin and 100  $\mu$ g/mL streptomycin. Subsequently, the plate was incubated at 37 °C with 5% CO<sub>2</sub> for 24 h, and then the culture medium was completely removed and replaced by 750  $\mu$ L of DMEM-HG without supplementation [2,3].

An aliquot of the SARS-CoV-2 stock, previously characterized by Araujo et al. [4], was thawed and  $100\,\mu\text{L}$  were diluted in  $900\,\mu\text{L}$  of DMEM-HG without supplementation. Then,  $200\,\mu\text{L}$  of this dilution were placed in wells of a 24-well plate, which were exposed to the UV-C lamp (UVsurface, Biolambda, Brazil) placed 30 cm above the plate to allow an uniform irradiance over the plate wells  $(2.2\pm0.2\,\text{mW/cm}^2)$ . Light was delivered by 2, 30 and 120 s corresponding to doses of 4.4, 66 e  $264\,\text{mJ/cm}^2$ , respectively. Controls were not submitted to irradiation.

After exposure to UV-C light, aliquots of  $83.4\,\mu\text{L}$  were placed into the plates containing the previously seeded Vero cells and incubated for 1 h at  $37\,^{\circ}\text{C}$  with 5% CO<sub>2</sub> for viral adsorption. Thereafter,  $166.6\,\mu\text{L}$  of DMEM-HG medium containing  $12\,\%$  fetal bovine serum were added and the plate was incubated for  $48\,\text{h}$  at  $37\,^{\circ}\text{C}$  with 5% CO<sub>2</sub>.

After that,  $100 \,\mu\text{L}$  of medium from each well was removed and placed into a lysis buffer solution to proceed with the extraction of the viral RNA using the MagMAX<sup>TM</sup> CORE Nucleic Acid Purification Kit (Thermo Fisher). After extraction, the number of copies of SARS-CoV-2 per mL was obtained using the RT-qPCR technique. Results were normalized in relation to controls for the calculation of viral inhibition rates of each sample. For the viral inactivation kinetics, we used the methodology reported by Sabino et al. [5].

UV-C inactivation kinetics and lethal doses for SARS-CoV-2 are presented at Fig. 1 and Table 1, respectively. We verified that within less than a second, UV-C irradiation was able to inactivate more than 99 % of SARS-CoV-2 viral particles. In fact,  $LD_{90}$  and  $LD_{99.999}$  were achieved at 0.016 and  $108.714 \, \text{mJ/cm}^2$  (0.01 and  $49.42 \, \text{s}$ ) respectively.

In summary, we report the inactivation kinetics and lethal dose analysis of UV-C radiation, emitted by low-pressure mercury lamps at 254 nm, against SARS-CoV-2, in a controlled *in vitro* experiment. Our findings can help scientific community and health authorities to develop safe and effective protocols to reduce the dissemination of SARS-CoV-2 during this global health crisis. Thus, we strongly encourage further studies in more realistic situations.

#### **Declaration of Competing Interest**

The authors report no declarations of interest.

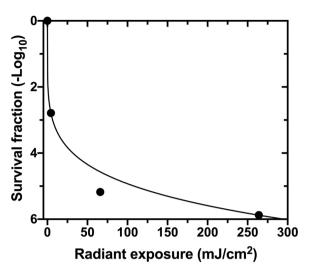
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# References

- [1] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, et al., China novel coronavirus investigating and research team (2020). A novel coronavirus from patients with pneumonia in China, N. Engl. J. Med. 382 (8) (2019) 727–733.
- [2] Y. Shi, D.H. Yang, J. Xiong, J. Jia, B. Huang, Y.X. Jin, Inhibition of genes expression of SARS coronavirus by synthetic small interfering RNAs, Cell Res. 15 (2005) 193–200.
- [3] C. TaŞtan, B. Yurtsever, G. Sir KarakuŞ, D. Dİlek KanÇaĞi, S. Demİr, S. Abanuz, et al., SARS-CoV-2 isolation and propagation from Turkish COVID-19 patients, Turk. J. Biol. 44
- [4] D.B. Araujo, R.R.G. Machado, D.E. Amgarten, F.M. Malta, G.G. de Araujo, C.O. Monteiro, et al., SARS-CoV-2 Isolation From the First Reported Patients in Brazil and Establishment of a Coordinated Task Network, Memórias do Instituto Oswaldo Cruz, 2020, https://doi.org/10.1590/0074-02760200342.

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 $\begin{tabular}{ll} \textbf{Fig. 1.} In activation kinetics of SARS-CoV-2 promoted by UV-C radiation at $254\,\mathrm{nm.} \end{tabular}$ 

Table 1 UV-C lethal doses for SARS-CoV-2.

Viral inactivation (%)	UV-C dose (mJ/cm <sup>2</sup> )	Exposure time (s)
90	0.016	0.01
99	0.706	0.32
99.9	6.556	2.98
99.99	31.880	14.49
99.999	108.714	49.42

[5] C.P. Sabino, M. Wainwright, C. Dos Anjos, F.P. Sellera, M.S. Baptista, N. Lincopan, M.S. Ribeiro, Inactivation kinetics and lethal dose analysis of antimicrobial blue light and photodynamic therapy, Photodiagnosis Photodyn. Ther. 28 (2019) 186–191.

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