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# Rapid SARS-CoV-2 inactivation by mercury and LED UV-C lamps on different surfaces

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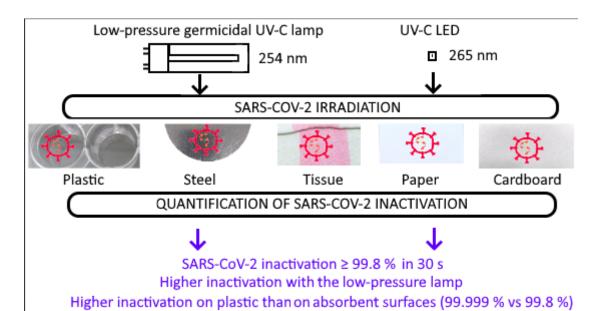
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- 1 Rapid SARS-CoV-2 inactivation by mercury and LED UV-C lamps on different surfaces
- 2 Running title: Rapid SARS-CoV-2 inactivation by UV-C
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8

- 9 **ABSTRACT (99 words)**
- 10 SARS-CoV-2 remains infectious for several hours on surfaces. It can be inactivated by UV-C
- 11 irradiation, but optimal conditions for rapid inactivation on non-plastic surfaces remains
- unclear. We demonstrated that efficient SARS-CoV-2 inactivation (≥ 99.8 %) can be achieved
- by both a UV-C mercury lamp and a UV-C LED in less than 30 seconds. Inactivation on a
- plastic surface was more efficient with the mercury UV-C lamp (p<0.0001). SARS-CoV-2
- inactivation levels were > 99.999 % on plastic,  $\ge 99.8$  % on steel, tissue, paper and cardboard
- after irradiation by both lamps at a distance of 3 cm during 30 sec.

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18 Key words: SARS-CoV-2, irradiation, UV-C, LED, mercury lamp, inactivation

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# MANUSCRIPT (1636 words)

## **BACKGROUND**

The human coronavirus named SARS-CoV-2 is a <i>Betacoronavirus</i> identified for the first time
in late 2019 in Wuhan, China and is now responsible for a major pandemic worldwide. This
virus is transmitted mainly via respiratory droplets and also by direct contact with symptomatic
or asymptomatic patients or with contaminated surfaces [1]. SARS-CoV-2 can remain
infectious for up to 72 h on non-absorbent surfaces like plastic or steel and up to 24 h on
absorbent surfaces like cardboard [1]. UV-C irradiation (200-280 nm) is an effective
disinfection approach to inactivate pathogens on surfaces, especially when detergents are not
suitable (electronic devices or water treatment). UV-C have a strong germicidal activity,
particularly at 265 nm, the maximal wavelength of absorption for nucleic acids [2]. They
inactivate pathogens by inducing the formation of pyrimidine dimers, thus inhibiting the
genome replication [2,3]. UV-C can inactivate SARS-CoV-2 on plastic or wood surfaces in a
few seconds or minutes but have not been evaluated on cardboard or paper [4-8]. UV-C
irradiation can be performed with UV light-emitting diodes (UV LEDs) or classical mercury
lamps [2]. UV LEDs allow more flexibility due to their small size, their low power consumption
and their specific emission wavelengths. Our objective was to evaluate the efficiency of a UV-
C mercury lamp and a UV-C LED for the inactivation of SARS-CoV-2 on non-absorbent
(plastic, steel) and absorbent surfaces (tissue, paper and cardboard).

# **METHODS**

## Cell culture and virus

- 42 Vero cells (ATCC, CCL-81) and Vero-E6 cells (ATCC, CRL-1586) were maintained in
- DMEM medium supplemented with 10% FCS and 1% penicillin-streptomycin at 37 °C, under
- an atmosphere containing 5% CO<sub>2</sub>. A clinical isolate of SARS-CoV-2 was isolated from a

- 45 nasopharyngeal swab collected from a patient suffering from COVID-19 at the Tours
- 46 University Hospital. The virus was amplified using Vero cells expressing Transmembrane
- 47 Protease Serine 2 (TMPRSS2). Viral titer was determined on Vero-E6 cells by the 50% tissue
- 48 culture infective dose (TCID<sub>50</sub>) as previously described [9]. A viral stock of 3.10<sup>5</sup> TCID<sub>50</sub>/mL
- 49 was prepared and stored at -80 °C.
- 50 UV-C lamps

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- 51 The first UV-C lamp was the Puritec HNS-L 2G11 UV-C germicidal mercury lamp (OSRAM,
- Rosny-sous-bois, France), emitting at 254 nm and 185nm with a nominal wattage of 18 W.
- Lamp dimensions were 31.5 cm x 4 cm. The second UV-C lamp was the KL265-50V-SM-WD
- 54 UV-C LED (Klaran, Green Island, USA), emitting at 265 nm and consuming 70 mW of power.
- LED dimensions were 3.5 x 3.5 mm. Irradiations were performed at 3, 5 or 10 cm with the lamp
- positioned directly above the irradiated well for durations of 5, 15, 30 or 60 seconds. UV-C
- doses received by the sample was measured with an optometer X1-5 (Gigahertz-Optik, GmbH).
- 58 UV-C doses (254 nm) received by the sample after irradiation with the mercury lamp at 3, 5
- and 10 cm were 12.6 mJ/s/cm<sup>2</sup>, 6.6 mJ/s/cm<sup>2</sup> and 2.6 mJ/s/cm<sup>2</sup>, respectively. UV-C doses (265)
- nm) received by the sample after irradiation with the LED at 3, 5 and 10 cm were 2.6 mJ/s/cm<sup>2</sup>,
- 61 1.34 mJ/s/cm<sup>2</sup> and 0.68 mJ/s/cm<sup>2</sup>, respectively.
  - Quantification of SARS-CoV-2 inactivation after UV-C irradiation
- The SARS-CoV-2 inoculum (200 μL at 3.10<sup>5</sup> TCID<sub>50</sub>/ml) was deposited on relevant surfaces
- 64 in 12-wells plates. Plastic (bottom of the well), steel (1 cm<sup>2</sup> steel ring), tissue (1 cm<sup>2</sup> punch of
  - cotton cloth), paper (1 cm<sup>2</sup> punch of 80 g/m<sup>2</sup> white paper) and cardboard (1 cm<sup>2</sup> punch of solid
- unbleached board) were used in this experiment. After 5 minutes of contact with the surface,
- of viruses were exposed to UV-C irradiation at 3, 5 or 10 cm for 5, 15, 30 or 60 seconds. Viable
- viruses were collected by a 5 min elution in 200 µL of infection medium and directly deposited
- on Vero-E6 cells (MOI of 0.1). These cells had previously been plated at 3.10<sup>5</sup> cells / well in

removed, the cells were washed with 500 µL of PBS and 1ml of infection medium was added to each well. Twenty-four hours later, the supernatant was collected and the viral titer was determined by endpoint dilution and calculation of the TCID<sub>50</sub>. All measurements were

12-wells plates, 24 h before the experiment. After 1 h of infection, the viral suspension was

- 74 performed in duplicate in three independent experiments. SARS-CoV-2 inactivation (%) was
- 75 calculated as follow: Inactivation (%) =  $(1 \frac{TCID50 \ after \ UV}{TCID50 \ before \ UV})*100$
- 76 SARS-CoV-2 inactivation efficiencies were compared between different UV-C sources,
- different distance and different durations of irradiation, using the Mann-Whitney (two groups)
- or the two-way ANOVA tests (more than two groups) with the GraphPad 9 software.

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#### RESULTS

## SARS-CoV-2 inactivation by UV-C on a plastic surface

- No viable virus was detected after irradiation with the mercury UV-C lamp, even for low UV-
- 83 C doses (12.6 mJ/cm<sup>2</sup>) (Figure 1A). This corresponded to a 6-log<sub>10</sub> decrease in SARS-CoV-2
- TCID<sub>50</sub>. Low doses of UV-C LED light (3.4 to 13 mJ/cm<sup>2</sup>) were associated with a 4-log<sub>10</sub>
- 85 reduction in SARS-CoV-2 TCID<sub>50</sub>. Increased doses of LED UV-C light were associated with a
- 86 slower decrease in SARS-CoV-2 TCID<sub>50</sub> (Figure 1A). We searched for the optimal distances
- 87 (3, 5 or 10 cm) and durations (5, 15, 30 or 60 seconds) of UV-C irradiation for SARS-CoV-2
- 88 inactivation on a plastic surface. SARS-CoV-2 inactivation by the UV-C mercury lamp was >
- 89 99.999 % in all conditions regardless of the distance or duration of irradiation (Figure 1B). In
- 90 contrast, high level SARS-CoV-2 inactivation by the UV-C LED (> 99.999 %) required a close
- position of the lamp (3 or 5 cm) and longer irradiations ( $\geq$  15 seconds) (Figure 1B). The mercury
- 92 UV-C lamp was associated with higher SARS-CoV-2 inactivation rates in all conditions (Figure
- 1B), even when considering similar UV-C doses (3.4 to 156 mJ/cm<sup>2</sup>, p<0.0001) (Figure 1A).

#### SARS-CoV-2 inactivation on steel, tissue, paper and cardboard

In light of previous results, SARS-CoV-2 inactivation by the UV-C lamps on steel, tissue, paper and cardboard was quantified after 15, 30 and 60 seconds of irradiation at a single distance of 3 cm (Figure 2B). These conditions corresponded to UV-C doses between 189 to 756 mJ/cm<sup>2</sup> for the mercury lamp and 39 to 156 mJ/cm<sup>2</sup> for the LED. No viable virus was detected after irradiation with the mercury or the LED UV-C lamp on a steel surface (Figure 2A). In contrast, viable viruses were still detected after irradiation with the mercury or the LED UV-C lamp on absorbent surfaces (tissue, paper or cardboard) (Figure 2A). In contrast with the plastic surface, there were no differences in inactivation rates between both lamps on absorbent surfaces (p = 0.31, Figure 2A). SARS-CoV-2 inactivation was  $\geq 99.8$ % on steel, tissue, paper and cardboard after irradiation by a UV-C mercury lamp or a UV-C LED, at a distance of 3 cm during 30 sec. This condition corresponded to 378 and 78 mJ/cm<sup>2</sup> for the mercury and LED UV-C lamps, respectively (Figure 2B). Short irradiation times (15 seconds) were less effective (90-99 % inactivation, p < 0.05) on absorbent surfaces (Figure 2B).

### **DISCUSSION**

Efficient inactivation of SARS-CoV-2 on contaminated surfaces (> 99.999 % on plastic and  $\geq$  99.8 % on steel, tissue, paper and cardboard) can be achieved by both the UV-C mercury lamp and the UV-C LED at a distance of 3 cm during 30 sec. No viable virus was detected after irradiation with the mercury UV-C lamp for a UV-C dose of 12.6 mJ/cm<sup>2</sup> (10 cm, 5 s), which is in line with most previous studies [8,10,11]. Few studies described lower performances for other mercury lamps [4,12]. This irradiation (12.6 mJ/cm<sup>2</sup>) corresponded to a 6-log<sub>10</sub> decrease in SARS-CoV-2 TCID<sub>50</sub>, rarely observed with other mercury lamps for comparable UV-C doses (3 to 5.5-log<sub>10</sub> decrease) [8,10–12]. These other studies were probably limited more by a lower infectious titer of the viral inoculum than by the performances of the mercury lamps, because no viable viruses were detected after irradiation [8,10.11]

A 4-log<sub>10</sub> reduction in SARS-CoV-2 TCID<sub>50</sub> was observed after irradiation with the UV-C LED light at 3.4 mJ/cm<sup>2</sup> (10 cm, 5 s). These performances were in line with another study, in which a 3.5-log<sub>10</sub> decrease was observed after irradiation with comparable UV-C doses [8]. Another study, by Inagaki *et al.*, demonstrated comparable UV-C LED performances with a 3-log<sub>10</sub> reduction in SARS-CoV-2 TCID<sub>50</sub> after 10 sec of irradiation at 2 cm using a deep UV-C LED (280 nm) [5]. Interestingly, SARS-CoV-2 inactivation on a plastic surface was more efficient with the mercury UV-C lamp than with the LED UV-C lamp, even when considering similar UV-C doses (p<0.0001). This could be due to the germicidal effect of ozone [7], produced after O<sub>2</sub> irradiation at 185 nm with the low-pressure mercury lamp [13].

This study is one of the first description of viral inactivation on steel and absorbent materials by UV-C lamps. Interestingly, inactivation rates were lower on absorbent materials (tissue, paper and cardboard:  $\geq 99.8$  %) than on plastic (> 99.999 %). This was probably because a fraction of the inoculum (200  $\mu$ L) was absorbed inside the materials and shielded from the UV-C light, which is less likely to happen with respiratory droplets (5  $\mu$ L). SARS-CoV-2 inactivation rates on steel were above 99.9 % for each lamp. These inactivation rates were lower than on plastic (99.999 %) but were probably underestimated due to a low viability of SARS-CoV-2 on steel (TCID<sub>50</sub> < 4 log<sub>10</sub>/mL).

The mercury UV-C lamp demonstrated a higher efficacy than the LED on a plastic surface. For this reason, the mercury UV-C lamp could be more relevant in high-risk settings, such as medical care or research laboratories. In contrast, the UV-C LED demonstrated good efficacy on steel and absorbent surfaces and has several advantages over the mercury lamp, especially a lower power consumption (18 mW vs 70 W) and a smaller size (3.5 vs 31 cm). In addition, its performance could probably be improved by combining several LEDs emitting at different

145	wavelengths. For these reasons, the LED could be especially interesting in industry of
146	household applications.
147	
148	STATEMENTS & DECLARATIONS
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150	electronic cards. This society also provided both lamps.
151	Competing interests: This research was funded by BICE45 (Saint-Ay, France), which is
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153	financial interests in BICE45.
154	Author contributions: All authors contributed to the study conception and design. Materia
155	preparation, data collection and analysis were performed by both authors. Both authors wrot
156	and approved the final manuscript.
157	Data availability: Marlet, Julien (2021), "UV-C COVID", Mendeley Data, V1, doi:
158	10.17632/6gxgfgdfwc.1. <a href="https://data.mendeley.com/datasets/6gxgfgdfwc/1">https://data.mendeley.com/datasets/6gxgfgdfwc/1</a>
159	Ethics approval, consent to participate and publication: Sample collection was approved
160	by the Ministère de l'Enseignement Supérieur et de la Recherche (authorization n° DC-2020
161	3961). Informed consent was obtained from all subjects involved in the study.
162	
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# Figure 1: SARS-CoV-2 inactivation on plastic by mercury and LED UV-C lamps

A) SARS-CoV-2 TCID<sub>50</sub> depending on the dose of UV-C received after irradiation on a plastic surface by the mercury lamp (plain black circles) or the LED (empty red circles). Empty black circles, non-irradiated control sample. Red curve represents the non-linear regression by exponential two phases decay for the LED. B) SARS-CoV-2 inactivation rates by UV-C lamps on a plastic surface depending on the duration (5 to 60 s) and the distance of irradiation. Distance of 10, 5 and 3 cm are represented with white/grey/black bars for the mercury lamp and white/orange/red dotted bars for the LED. Results are represented as mean with standard deviation for both panels. \*, p < 0.05; \*\*, p < 0.01.

#### Figure 2: SARS-CoV-2 inactivation on steel, tissue, paper and cardboard by mercury and

#### **LED UV-C lamps**

A) SARS-CoV-2 TCID<sub>50</sub> depending on the dose of UV-C received after irradiation on steel (diamonds), tissue (circles), paper (squares) or cardboards (triangles) surfaces by the mercury lamp (plain symbols) or the LED (empty symbols). B) SARS-CoV-2 inactivation rates by UV-C lamps at 3 cm depending on the irradiated surface (steel, tissue, paper or cardboard) and the duration of irradiation. Durations of 15, 30 and 60 s are represented with white/grey/black bars

- for the mercury lamp and white/orange/red dotted bars for the LED. Results are represented as
- 215 mean with standard deviation for both panels. \*\*, p < 0.01; \*\*\*, p < 0.001; \*\*\*\*, p < 0.0001.

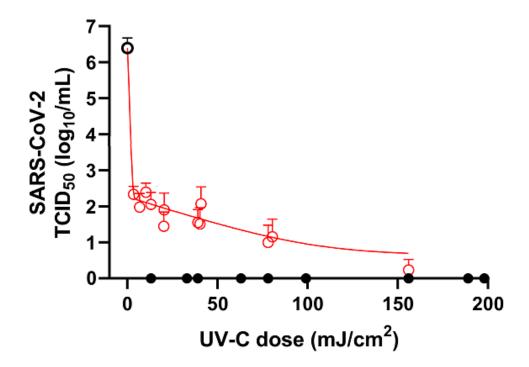


FIG 1A

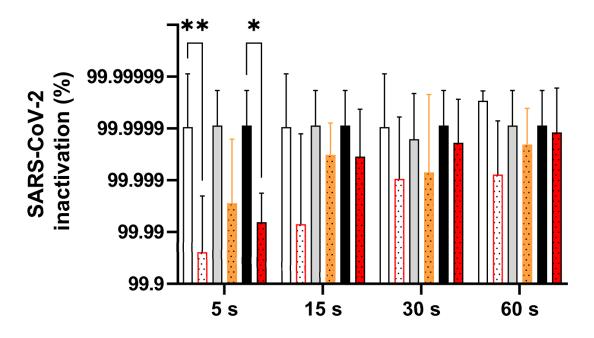


FIG1B

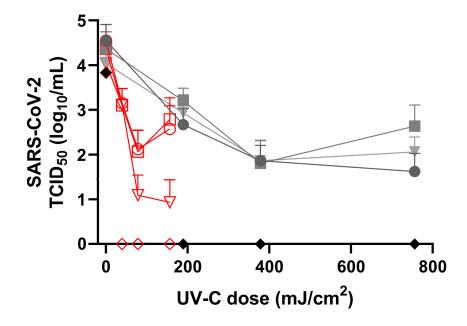


FIG2A

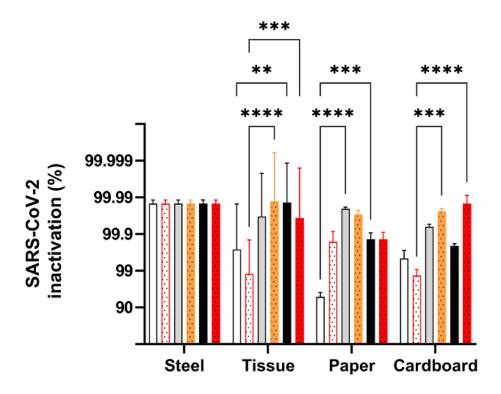


FIG2B