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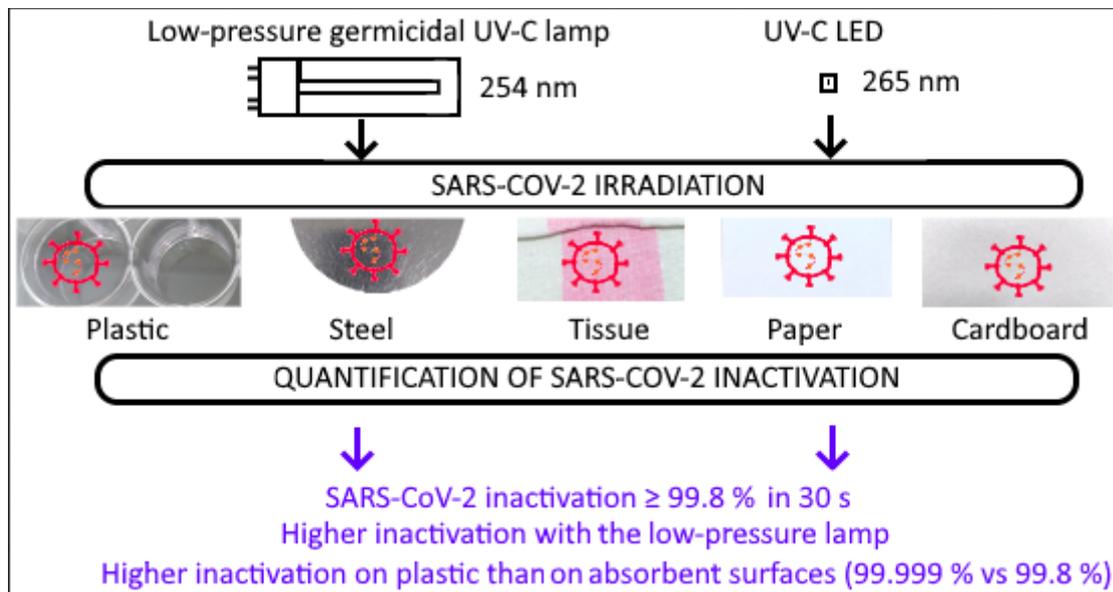
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1 **Rapid SARS-CoV-2 inactivation by mercury and LED UV-C lamps on different surfaces**

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
12 unclear. We demonstrated that efficient SARS-CoV-2 inactivation ($\geq 99.8\%$) can be achieved
13 by both a UV-C mercury lamp and a UV-C LED in less than 30 seconds. Inactivation on a
14 plastic surface was more efficient with the mercury UV-C lamp ($p < 0.0001$). SARS-CoV-2
15 inactivation levels were $> 99.999\%$ on plastic, $\geq 99.8\%$ on steel, tissue, paper and cardboard
16 after irradiation by both lamps at a distance of 3 cm during 30 sec.

17

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

19

20 **MANUSCRIPT (1636 words)**

21 **BACKGROUND**

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

39

40 **METHODS**

41 **Cell culture and virus**

42 Vero cells (ATCC, CCL-81) and Vero-E6 cells (ATCC, CRL-1586) were maintained in
43 DMEM medium supplemented with 10% FCS and 1% penicillin-streptomycin at 37 °C, under
44 an atmosphere containing 5% CO₂. 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 (TCID₅₀) as previously described [9]. A viral stock of 3.10⁵ TCID₅₀/mL
49 was prepared and stored at – 80 °C.

50 **UV-C lamps**

51 The first UV-C lamp was the Puritec HNS-L 2G11 UV-C germicidal mercury lamp (OSRAM,
52 Rosny-sous-bois, France), emitting at 254 nm and 185nm with a nominal wattage of 18 W.
53 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.
55 LED dimensions were 3.5 x 3.5 mm. Irradiations were performed at 3, 5 or 10 cm with the lamp
56 positioned directly above the irradiated well for durations of 5, 15, 30 or 60 seconds. UV-C
57 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
59 and 10 cm were 12.6 mJ/s/cm², 6.6 mJ/s/cm² and 2.6 mJ/s/cm², respectively. UV-C doses (265
60 nm) received by the sample after irradiation with the LED at 3, 5 and 10 cm were 2.6 mJ/s/cm²,
61 1.34 mJ/s/cm² and 0.68 mJ/s/cm², respectively.

62 **Quantification of SARS-CoV-2 inactivation after UV-C irradiation**

63 The SARS-CoV-2 inoculum (200 µL at 3.10⁵ TCID₅₀/ml) was deposited on relevant surfaces
64 in 12-wells plates. Plastic (bottom of the well), steel (1 cm² steel ring), tissue (1 cm² punch of
65 cotton cloth), paper (1 cm² punch of 80 g/m² white paper) and cardboard (1 cm² punch of solid
66 unbleached board) were used in this experiment. After 5 minutes of contact with the surface,
67 viruses were exposed to UV-C irradiation at 3, 5 or 10 cm for 5, 15, 30 or 60 seconds. Viable
68 viruses were collected by a 5 min elution in 200 µL of infection medium and directly deposited
69 on Vero-E6 cells (MOI of 0.1). These cells had previously been plated at 3.10⁵ cells / well in

70 12-wells plates, 24 h before the experiment. After 1 h of infection, the viral suspension was
71 removed, the cells were washed with 500 μ L of PBS and 1ml of infection medium was added
72 to each well. Twenty-four hours later, the supernatant was collected and the viral titer was
73 determined by endpoint dilution and calculation of the TCID₅₀. All measurements were
74 performed in duplicate in three independent experiments. SARS-CoV-2 inactivation (%) was
75 calculated as follow: $\text{Inactivation (\%)} = (1 - \frac{\text{TCID}_{50} \text{ after UV}}{\text{TCID}_{50} \text{ before UV}}) * 100$
76 SARS-CoV-2 inactivation efficiencies were compared between different UV-C sources,
77 different distance and different durations of irradiation, using the Mann-Whitney (two groups)
78 or the two-way ANOVA tests (more than two groups) with the GraphPad 9 software.

79

80 **RESULTS**

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

82 No viable virus was detected after irradiation with the mercury UV-C lamp, even for low UV-
83 C doses (12.6 mJ/cm²) (Figure 1A). This corresponded to a 6-log₁₀ decrease in SARS-CoV-2
84 TCID₅₀. Low doses of UV-C LED light (3.4 to 13 mJ/cm²) were associated with a 4-log₁₀
85 reduction in SARS-CoV-2 TCID₅₀. Increased doses of LED UV-C light were associated with a
86 slower decrease in SARS-CoV-2 TCID₅₀ (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
91 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
93 1B), even when considering similar UV-C doses (3.4 to 156 mJ/cm², p<0.0001) (Figure 1A).

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

95 In light of previous results, SARS-CoV-2 inactivation by the UV-C lamps on steel, tissue, paper
96 and cardboard was quantified after 15, 30 and 60 seconds of irradiation at a single distance of
97 3 cm (Figure 2B). These conditions corresponded to UV-C doses between 189 to 756 mJ/cm²
98 for the mercury lamp and 39 to 156 mJ/cm² for the LED. No viable virus was detected after
99 irradiation with the mercury or the LED UV-C lamp on a steel surface (Figure 2A). In contrast,
100 viable viruses were still detected after irradiation with the mercury or the LED UV-C lamp on
101 absorbent surfaces (tissue, paper or cardboard) (Figure 2A). In contrast with the plastic surface,
102 there were no differences in inactivation rates between both lamps on absorbent surfaces ($p =$
103 0.31, Figure 2A). SARS-CoV-2 inactivation was $\geq 99.8\%$ on steel, tissue, paper and cardboard
104 after irradiation by a UV-C mercury lamp or a UV-C LED, at a distance of 3 cm during 30 sec.
105 This condition corresponded to 378 and 78 mJ/cm² for the mercury and LED UV-C lamps,
106 respectively (Figure 2B). Short irradiation times (15 seconds) were less effective (90-99 %
107 inactivation, $p < 0.05$) on absorbent surfaces (Figure 2B).

108

109 **DISCUSSION**

110 Efficient inactivation of SARS-CoV-2 on contaminated surfaces ($> 99.999\%$ on plastic and \geq
111 99.8 % on steel, tissue, paper and cardboard) can be achieved by both the UV-C mercury lamp
112 and the UV-C LED at a distance of 3 cm during 30 sec. No viable virus was detected after
113 irradiation with the mercury UV-C lamp for a UV-C dose of 12.6 mJ/cm² (10 cm, 5 s), which
114 is in line with most previous studies [8,10,11]. Few studies described lower performances for
115 other mercury lamps [4,12]. This irradiation (12.6 mJ/cm²) corresponded to a 6-log₁₀ decrease
116 in SARS-CoV-2 TCID₅₀, rarely observed with other mercury lamps for comparable UV-C
117 doses (3 to 5.5-log₁₀ decrease) [8,10–12]. These other studies were probably limited more by a
118 lower infectious titer of the viral inoculum than by the performances of the mercury lamps,
119 because no viable viruses were detected after irradiation [8,10,11]

120 A 4- \log_{10} reduction in SARS-CoV-2 TCID₅₀ was observed after irradiation with the UV-C
121 LED light at 3.4 mJ/cm² (10 cm, 5 s). These performances were in line with another study, in
122 which a 3.5- \log_{10} decrease was observed after irradiation with comparable UV-C doses [8].
123 Another study, by Inagaki *et al.*, demonstrated comparable UV-C LED performances with a 3-
124 \log_{10} reduction in SARS-CoV-2 TCID₅₀ after 10 sec of irradiation at 2 cm using a deep UV-C
125 LED (280 nm) [5]. Interestingly, SARS-CoV-2 inactivation on a plastic surface was more
126 efficient with the mercury UV-C lamp than with the LED UV-C lamp, even when considering
127 similar UV-C doses ($p < 0.0001$). This could be due to the germicidal effect of ozone [7],
128 produced after O₂ irradiation at 185 nm with the low-pressure mercury lamp [13].

129

130 This study is one of the first description of viral inactivation on steel and absorbent materials
131 by UV-C lamps. Interestingly, inactivation rates were lower on absorbent materials (tissue,
132 paper and cardboard: ≥ 99.8 %) than on plastic (> 99.999 %). This was probably because a
133 fraction of the inoculum (200 μ L) was absorbed inside the materials and shielded from the UV-
134 C light, which is less likely to happen with respiratory droplets (5 μ L). SARS-CoV-2
135 inactivation rates on steel were above 99.9 % for each lamp. These inactivation rates were lower
136 than on plastic (99.999 %) but were probably underestimated due to a low viability of SARS-
137 CoV-2 on steel (TCID₅₀ $< 4 \log_{10}$ /mL).

138

139 The mercury UV-C lamp demonstrated a higher efficacy than the LED on a plastic surface. For
140 this reason, the mercury UV-C lamp could be more relevant in high-risk settings, such as
141 medical care or research laboratories. In contrast, the UV-C LED demonstrated good efficacy
142 on steel and absorbent surfaces and has several advantages over the mercury lamp, especially a
143 lower power consumption (18 mW vs 70 W) and a smaller size (3.5 vs 31 cm). In addition, its
144 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 or
146 household applications.

147

148 **STATEMENTS & DECLARATIONS**

149 **Funding statement:** This study was funded by BICE45 (Saint-Ay, France), specialized in
150 electronic cards. This society also provided both lamps.

151 **Competing interests:** This research was funded by BICE45 (Saint-Ay, France), which is
152 specialized in electronic cards. This society also provided both lamps. Authors have no other
153 financial interests in BICE45.

154 **Author contributions:** All authors contributed to the study conception and design. Material
155 preparation, data collection and analysis were performed by both authors. Both authors wrote
156 and approved the final manuscript.

157 **Data availability:** Marlet, Julien (2021), “UV-C COVID”, Mendeley Data, V1, doi:
158 10.17632/6gxgfgdfwc.1. <https://data.mendeley.com/datasets/6gxgfgdfwc/1>

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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195

196

197 **Figure 1: SARS-CoV-2 inactivation on plastic by mercury and LED UV-C lamps**

198 A) SARS-CoV-2 TCID₅₀ depending on the dose of UV-C received after irradiation on a plastic
199 surface by the mercury lamp (plain black circles) or the LED (empty red circles). Empty black
200 circles, non-irradiated control sample. Red curve represents the non-linear regression by
201 exponential two phases decay for the LED. B) SARS-CoV-2 inactivation rates by UV-C lamps
202 on a plastic surface depending on the duration (5 to 60 s) and the distance of irradiation.
203 Distance of 10, 5 and 3 cm are represented with white/grey/black bars for the mercury lamp
204 and white/orange/red dotted bars for the LED. Results are represented as mean with standard
205 deviation for both panels. *, $p < 0.05$; **, $p < 0.01$.

206

207 **Figure 2: SARS-CoV-2 inactivation on steel, tissue, paper and cardboard by mercury and**
208 **LED UV-C lamps**

209 A) SARS-CoV-2 TCID₅₀ depending on the dose of UV-C received after irradiation on steel
210 (diamonds), tissue (circles), paper (squares) or cardboards (triangles) surfaces by the mercury
211 lamp (plain symbols) or the LED (empty symbols). B) SARS-CoV-2 inactivation rates by UV-
212 C lamps at 3 cm depending on the irradiated surface (steel, tissue, paper or cardboard) and the
213 duration of irradiation. Durations of 15, 30 and 60 s are represented with white/grey/black bars

214 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. **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

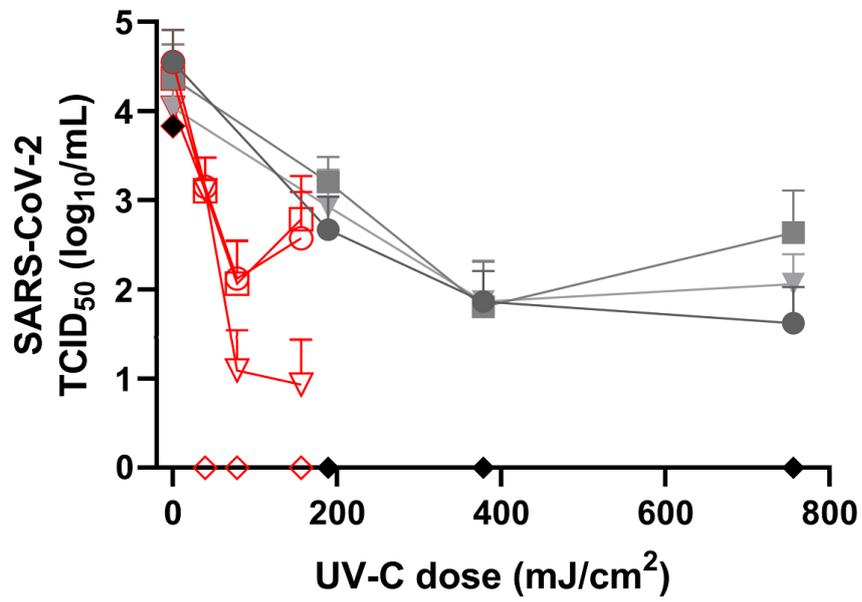


FIG2A

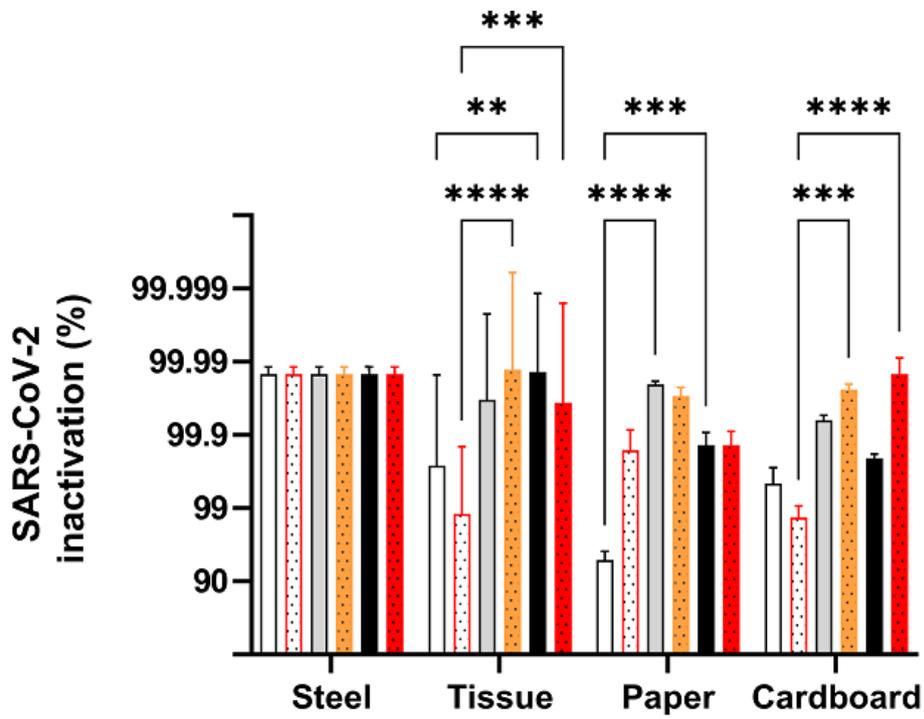


FIG2B