



21 • **ABSTRACT**

22 Effective disinfection technology to combat severe acute respiratory syndrome coronavirus 2  
23 (SARS-CoV-2) can help reduce viral transmissions during the on-going COVID-19 global  
24 pandemic and in the future. Ultraviolet (UV) devices emitting UVC irradiation (200-280 nm)  
25 have proven to be effective for virus disinfection, but limited information is available for SARS-  
26 CoV-2 due to the safety requirements of testing, which is limited to biosafety level (BSL) 3  
27 laboratories. In this study, inactivation of SARS-CoV-2 in thin-film buffered aqueous solution  
28 (pH 7.4) was determined across UVC irradiation wavelengths (222 nm to 282 nm) from krypton  
29 chloride (KrCl\*) excimers, a low-pressure mercury-vapor lamp, and two UVC light emitting  
30 diodes. Our results show that all tested UVC devices can effectively inactivate SARS-CoV-2,  
31 among which the KrCl\* excimer had the best disinfection performance (i.e., highest inactivation  
32 rate). The inactivation rate constants of SARS-CoV-2 across wavelengths are similar to those for  
33 murine hepatitis virus (MHV) from our previous investigation, suggesting that MHV can serve  
34 as a reliable surrogate of SARS-CoV-2 with a lower BSL requirement (BSL-2) during UV  
35 disinfection tests. This study provides fundamental information for UVC action on SARS-CoV-2  
36 and guidance for achieving reliable disinfection performance of UVC devices.

37

38 **IMPORTANCE**

39 UV light is an effective tool to help stem the spread of respiratory viruses and protect public  
40 health in commercial, transportation and healthcare settings. For effective use of UV, there is a  
41 need to determine the efficiency of different UV wavelengths in killing pathogens, specifically  
42 SARS-CoV-2, to support efforts to control the on-going COVID-19 global pandemic and future  
43 coronavirus-caused respiratory virus pandemics. We found that SARS-CoV-2 can be inactivated

44 effectively using a broad range of UVC wavelengths, and 222nm provided the best disinfection  
45 performance. Interestingly, 222 nm irradiation has been found to be safe for human exposure up  
46 to thresholds that are beyond effective for inactivating viruses. Therefore, applying UV light  
47 from KrCl\* excimers in public spaces can effectively help reduce viral aerosol or surface  
48 transmissions.

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50 Keywords: UV disinfection, far UVC, COVID-19, surrogate, human coronavirus 229E, murine  
51 hepatitis virus (MHV), bacteriophage Phi6

52 • **INTRODUCTION**

53 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped, non-  
54 segmented positive-sense RNA virus (1), which is causing the on-going COVID-19 global  
55 pandemic. It is transmitted primarily via respiratory droplets produced while talking, coughing,  
56 and sneezing (2). Indirect routes, such as airborne and surface-mediated transmission, are also  
57 possible, especially considering SARS-CoV-2 can stay viable in aerosols and on surfaces up to  
58 72 hours (3). Effective disinfection procedures can help reduce viral transmission, especially in  
59 high-risk places, such as hospitals, other healthcare facilities, and public transportation systems.

60 Ultraviolet (UV) devices emitting UVC irradiation (200-280 nm) such as low-pressure (LP) UV  
61 lamp and UV light emitting diodes (LEDs) have been widely used for virus disinfection of water,  
62 air, and surfaces since the early 20<sup>th</sup> century (4–8). Compared to other disinfection methods (e.g.,  
63 heating and using chemical oxidants), UVC disinfection has several advantages, including rapid  
64 effectiveness, no chemical residual, and limited to no material degradation (6). One limitation of  
65 conventional UVC devices is that they are not safe for human exposures due to adverse effects  
66 on human skin and eyes (9, 10). Emerging far UVC devices (emitting UVC irradiation in the  
67 wavelength range of 200-225 nm) like the krypton chloride (KrCl\*) excimer, however, have  
68 been proposed to disinfect occupied public spaces as recent studies reported that far UVC light  
69 exposure results in no adverse effects to skin or eyes in mouse studies due to its very limited  
70 penetration into biological materials (11–14).

71 There are only a few studies that document inactivation efficiencies of SARS-CoV-2 using UVC  
72 devices. An average UV fluence of 1.2 mJ/cm<sup>2</sup> to over 60 mJ/cm<sup>2</sup> were required for 1-log  
73 inactivation (90%) of SARS-CoV-2 in aqueous solutions using LP UV lamps, reported in  
74 previous investigations (15–18), whereas 1.6 mJ/cm<sup>2</sup> of UVC irradiation from a KrCl\* excimer

75 with a 222 nm bandpass filter was needed to achieve the same virus reduction (19). A few other  
76 studies also investigated UV inactivation effectiveness against SARS-CoV-2 in virus droplets  
77 and on surfaces using LP UV lamps and KrCl\* excimers (17, 20, 21). Despite these prior works,  
78 information on UVC inactivation of SARS-CoV-2 is still limited across UV wavelengths and in  
79 comparison to surrogate enveloped viruses, primarily due to the safety requirement of testing,  
80 which is limited to biosafety level (BSL) 3 laboratories. Thus, comparative studies including  
81 reliable and accessible surrogates of SARS-CoV-2 with a lower BSL requirement are needed for  
82 extensive assessment of UVC device, source, and wavelength performance.

83 In this study, UVC inactivation of SARS-CoV-2 in thin-film aqueous solution was determined  
84 using five UVC devices with different emission spectra in a bench-scale collimated beam  
85 apparatus. The UV sensitivities of SARS-CoV-2 and its potential testing surrogates classified as  
86 BSL-1 and BSL-2 viruses, including human coronavirus (HCoV) 229E, murine hepatitis virus  
87 (MHV), and bacteriophage Phi6 (22), were compared and recommendations for reliable UV  
88 testing surrogates of SARS-CoV-2 are made.

89

## 90 • MATERIALS AND METHODS

91 **Virus preparation and enumeration.** SARS-CoV-2 (Isolate USA WA1 2020), an enveloped  
92 respiratory virus, was propagated and assayed in the monkey kidney VeroE6 cell line (ATCC  
93 CRL-1586). VeroE6 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with 10%  
94 fetal bovine serum (FBS), 100µg/mL kanamycin sulfate, 200U/mL penicillin, 200µg/mL  
95 streptomycin, and 0.5µg/mL amphotericin B and incubated for 3 days at 37 °C in a 5% CO<sub>2</sub>  
96 atmosphere. The viral stock was then added into VeroE6 cells with fresh medium and incubated  
97 for 2 days at the same conditions, when cytopathogenic effects (CPE) were observed in the

98 monolayer. Infected cells were subjected to three freeze-thaw cycles to release the viruses, and  
99 the cell lysates were centrifuged at 1,000×g for 20 minutes to pellet the cell debris for removal  
100 and discard. The viruses in the supernatant then underwent a polyethylene glycol (12% w/v, MW  
101 8000) precipitation with 0.5 M sodium chloride, and slow mixing overnight at 4°C. After  
102 centrifugation at 10,000 ×g for 60 minutes at 4°C, the pelleted virus was resuspended in 0.01M  
103 phosphate buffered saline (PBS; pH 7.4) to approximately a 10 mL volume. The SARS-CoV-2  
104 stocks were then aliquoted and stored at -80 °C.

105 The viral stocks were enumerated on VeroE6 cells seeded into 96-well cell culture trays using  
106 the TCID<sub>50</sub> (tissue culture infectious dose at the 50% endpoint) technique as described by  
107 Payment and Trudel (23). This technique determines the dilution at which 50% of the wells show  
108 CPE. Serial 10-fold dilutions of SARS-CoV-2 samples were prepared in DMEM without FBS,  
109 followed by plating onto VeroE6 monolayers prepared in 96-well trays in replicates of six per  
110 dilution with 50 µL per well. DMEM containing 2% FBS was then added to bring the volume in  
111 each well up to 180µl. After the plates were further incubated for 7 days at 37 °C in a 5% CO<sub>2</sub>  
112 atmosphere, TCID<sub>50</sub> values were then calculated using the Spearman and Kärber algorithm  
113 (detection limit=6 TCID<sub>50</sub>/mL) (24).

114 **UV exposure experiments.** The UV lamps were set up in a bench-scale collimated beam  
115 apparatus (Fig. 1-A) as described by Bolton and Linden (25). Normalized emission spectra for  
116 these UV lamps as used in the experiments (Fig. 1-B) were measured using a calibrated Maya  
117 2000 Pro spectrometer (Ocean Insight, Dunedin, FL). Five UV sources were used in this  
118 investigation: an unfiltered KrCl\* excimer lamp emitting primarily at 222 nm with a small peak  
119 at 258 nm (USHIO, Cypress, CA, USA), a filtered KrCl\* excimer lamp with a 220 nm bandpass  
120 filter pre-installed (USHIO, Cypress, CA, USA), a conventional LP mercury lamp emitting at

121 254 nm, and two benchtop UV LED systems with peak emission wavelengths of 270 nm and 282  
122 nm (AquiSense Technologies, Earlanger, KY, USA).

123 **Figure 1.** Schematic diagram of bench-scale collimated beam apparatus (A) and relative lamp  
124 emission (RLE) for the UV devices used in this investigation, absorbance of samples used in UV  
125 exposure tests, and normalized absorbance (normalized to maximum value at 200 nm) of nucleic  
126 acid (DNA/RNA) and protein (B). The nucleic acid and protein absorbance data were reproduced  
127 from Ma et al. (22).  
128

129 UV exposure experiments were performed according to a standard protocol by Bolton and  
130 Linden (25). Virus samples (5 mL each) were made by diluting virus stocks 100-fold into sterile  
131 PBS (pH 7.4) and placed in 50 mm × 35 mm (diameter × height) sterile glass dishes (0.38 cm in  
132 depth) with a customized quartz lid (Corning 7980, Corning, NY, USA; Fig 1-A) with UV  
133 transmittance (UVT) greater than 90% at 200-400 nm, according to the information provided by  
134 the manufacturer and confirmed by measuring the UV absorbance of the lid using a UV-Vis  
135 spectrophotometer (DR-6000, Hach Company, Loveland, CO, USA). Absorbance for the  
136 samples was measured using a UV-Vis spectrophotometer. The UV incident irradiance at the  
137 center of sample surface was measured using a calibrated radiometer (ILT-2400, International  
138 Light Technologies, Inc., Peabody, MA, USA) set at the respective peak emission wavelength  
139 for a UVC device (222 nm was used for unfiltered KrCl\* excimer). The radiometer detector was  
140 placed directly under the quartz lid during irradiance measurements at the liquid surface to  
141 include any effects from absorption and reflection of the quartz plate. UV exposure time for each  
142 sample was calculated using the target UV fluences for unweighted emissions between 200 nm  
143 to 300 nm according to a protocol by Linden and Darby (26) and Bolton and Linden (25), where  
144 average UV fluence calculation included corrections for radiometer detector sensitivity  
145 correction across lamp emission spectra (i.e., lamp correction factor), sample absorbance from  
146 200 nm to 300 nm and path length (i.e., Beers law), divergence of light through the sample,

147 reflection factor, and non-uniformity of incident irradiance across the sample surface (i.e., petri  
148 factor). Duplicate control samples (no UV exposure) were collected at the beginning and the end  
149 of UV exposure tests for each UVC device. Eight virus samples were exposed with five different  
150 UV fluences for each UVC device, in which three fluences were tested with duplicate samples  
151 and the other two fluences were tested with one sample. All virus samples after UV exposures  
152 were collected sacrificially so no subsampling was performed in the UV exposure tests. The  
153 infectivity of virus samples without and after UV exposure was measured as described above,  
154 and the infectivity reduction in  $\log_{10}$  scale was determined.

155 **Statistical analysis.** The UV dose-responses using different UVC devices were evaluated based  
156 on a pseudo-first-order inactivation kinetics model in  $\log_{10}$ -scale:

$$\log_{10} I = \log_{10} \left( \frac{N_0}{N} \right) = k \times D$$

157 where  $\log_{10} I$  is infectivity reduction in  $\log_{10}$  scale,  $N_0$  and  $N$  are the virus sample infectivity  
158 before and after UV exposure,  $D$  is UV fluence in  $\text{mJ}/\text{cm}^2$ , and  $k$  is the pseudo-first-order  
159 inactivation rate constant in  $\text{cm}^2/\text{mJ}$  computed from a  $\log_{10}$ -scale kinetic model. The  $\log_{10}$ -scale  
160 inactivation rate constant was used, which made it easy to calculate the log inactivation from the  
161 rate constant.

162 The mean and standard error (SE) of inactivation rate constants were calculated using ‘linear  
163 regression’ function in OriginPro 2021 (intercept was fixed at zero). Samples with infectivity  
164 equal to or less than the detection limits were excluded from the regression analyses. Analysis of  
165 covariance (ANCOVA) was used to determine if there was a statistically significant difference in  
166 the inactivation rate constants.

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168



169

170 • **RESULTS AND DISCUSSION**

171 All UVC devices tested in this study were very effective in inactivating SARS-CoV-2 in aqueous  
172 solution (Fig. 2). Among all tested UVC devices, unfiltered and filtered KrCl\* excimer exhibited  
173 the greatest performance with inactivation rate constants ( $\pm$ S.E.) of  $1.52 \pm 0.17 \text{ cm}^2/\text{mJ}$  and  
174  $1.42 \pm 0.40 \text{ cm}^2/\text{mJ}$ , respectively. These values are much higher than the value reported by  
175 Robinson et al. (19) ( $0.64 \text{ cm}^2/\text{mJ}$ ). One possible explanation for such difference is that sample  
176 absorbance at 222 nm was much higher in the Robinson study ( $>30 \text{ cm}^{-1}$ ) than this study ( $0.05$   
177  $\text{cm}^{-1}$ ; Fig. 1-B), and UV absorption by constituents in the sample matrix (i.e., proteins and other  
178 constituents from the cell culture extracts) may affect the virus sensitivity toward UV irradiation.  
179 Greater performance of KrCl\* excimers compared to other UVC devices were also observed for  
180 non-enveloped viruses (e.g., MS2 coliphage and adenovirus (4, 5, 27), enveloped bacteriophage  
181 Phi6 (22), and coronaviruses (22) in previous studies, suggesting such superior performance may  
182 be universal across virus types. This is likely because KrCl\* excimers were capable of inflicting  
183 greater viral protein and nucleic acid damage compared to other UVC devices due to the higher  
184 protein absorbance at far UVC wavelengths around the 222 nm wavelength emitted by these  
185 devices (Fig. 1-B). The superior performance of KrCl\* excimer is particularly promising because  
186 far UVC device are safe to be applied in occupied public spaces, up to the allowable threshold  
187 limit value of  $25 \text{ mJ}/\text{cm}^2$  at 220 nm, ACGIH 2021 (28) or perhaps beyond, to disinfect viruses in  
188 respiratory secretions and airborne droplets as well as on contaminated surfaces, to limit the  
189 presence and transmission of SARS-CoV-2 or other respiratory viruses. Previous studies on  
190 aerosol and surface UV disinfection (29, 30) suggested that viruses in airborne droplets and on  
191 surfaces tend to be more susceptible to UVC irradiation. Recent work with UV 222 nm

192 inactivation of SARS-CoV-2 on surfaces (17, 20, 21) and of other coronaviruses in air (12),  
193 however, show very similar inactivation compared to this study, suggesting data for inactivation  
194 generated using thin-film aqueous suspensions can represent inactivation of coronaviruses across  
195 various media.

196 An average UV dose of  $1.3 \text{ mJ/cm}^2$  was required for 1-log inactivation of SARS-CoV-2 using  
197 the LP UV lamp, which is similar to the results from several previous studies ( $1.2\text{-}5.0 \text{ mJ/cm}^2$  for  
198 1 log inactivation (15–17)). Another study by Heilingloh et al. (18), however, suggested 1-log  
199 inactivation would require above  $60 \text{ mJ/cm}^2$  using a LP UV source. This divergence from the  
200 UV doses reported in numerous other studies is likely due to the significant difference in the  
201 experimental setup for UV exposures and calculation for UV fluences. The inactivation tests  
202 reported in Heilingloh were performed in cell culture media in 24-well plates with the UV source  
203 placed only 3 cm above the bottom of the plate, which could lead to differences in UV intensity  
204 between each well. Also no information is given on how the UV irradiance was measured, there  
205 is no report of the absorbance of the suspending media, and standardized procedures for UV  
206 fluence calculation (e.g., corrections for sample UV absorbance, depth of sample, UV beam  
207 reflection and divergence, and petri factor) were not followed. Based on the data presented  
208 herein, no statistically significant difference in UV inactivation performance was observed  
209 between the LP UV lamp and the UV LED 270 ( $P=0.16$ ; Fig. 2). Viral genome damage is likely  
210 to be the primary inactivation mechanism for these UVC devices (27), and SARS-CoV-2 should  
211 have similar sensitivities to UV irradiation from these devices due to similar nucleic acid  
212 absorbance at their peak emission wavelengths (i.e., 254 nm and 270 nm, respectively; Fig. 1-B).  
213 UV LED 282 provided the lowest inactivation rate constant among all tested UVC devices. Viral  
214 genomes tends to absorb less UV irradiation in the wavelength range emitted from UV LED 282

215 (4, 31) (Fig. 1-B), which leads to less genome damage. While viral proteins, should be slightly  
216 more sensitive to UV irradiation from around 282 nm wavelength (32), this previous observation  
217 did not appear to enhance the effectiveness for the 282 nm LED in this current study.

218

219 **Figure 2.** Dose-response of SARS-CoV-2 to UV irradiation from all tested UVC devices.  
220 Dashed lines represent linear regression results computed from experimental data. The  $k$  values  
221 (mean  $\pm$  standard error; in  $\text{cm}^2/\text{mJ}$ ) and adjusted  $R^2$  values are listed. Open symbols represent  
222 samples with infectivity equal to or less than the detection limits. Solid symbols with black edge  
223 represent two samples overlapping in the plot with the same UV dose response. Primary  
224 emission wavelengths for UVC devices are listed in each sub-figure legend.  
225

226 The inactivation rate constants of SARS-CoV-2 were compared with the values of potential  
227 enveloped virus surrogates: HCoV 229E, MHV, and bacteriophage Phi6 (Fig. 3). These three  
228 viruses were selected as candidates of SARS-CoV-2 surrogates for UV inactivation tests due to  
229 their molecular similarities (i.e., all are enveloped RNA viruses) and lower biosafety  
230 requirements (BSL-1 for Phi6, and BSL-2 for HCoV 229E and MHV). All virus surrogates were  
231 previously tested in the identical collimated beam apparatus except that the quartz lid was not  
232 applied for non-BSL3 organisms. The inactivation rate constants were also calculated following  
233 the same data analysis method (22). Among the three candidates, MHV exhibited the greatest  
234 similarities in inactivation rate constants across UVC devices compared to SARS-CoV-2. No  
235 statistically significant difference in the rate constant values ( $P>0.05$ ) were observed for all  
236 tested UVC devices except for unfiltered KrCl\* excimer ( $P=0.008$ ), for which the inactivation  
237 rate of MHV was only 26% lower than the value for SARS-CoV-2 (Fig. 3). These results suggest  
238 that MHV can serve as a reliable UV surrogate of SARS-CoV-2 testing across UVC wavelengths  
239 when a lower biosafety requirement is needed. HCoV 229E could also serve as a viable surrogate  
240 of SARS-CoV-2, especially for testing unfiltered KrCl\* excimer (Fig. 3). Considering SARS-

241 CoV-2, MHV, and HCoV 229E are all coronaviruses, evidence suggests that coronaviruses in  
242 general have similar sensitivities to UVC irradiation across wavelengths due to their similar  
243 molecular structures. This is further supported by comparing the UV inactivation rate constants  
244 of other coronaviruses, such as HCoV-OC43. UV inactivation rate constants of 0.77, 0.64, and  
245 0.43 cm<sup>2</sup>/mJ were reported by Gerchman et al. (33) using UV LEDs with peak emission at 267,  
246 279, and 286 nm, respectively, which are similar to the values we observed using UV LED 270  
247 and UV LED 282 (0.93 and 0.53 cm<sup>2</sup>/mJ; Fig. 2). Although significantly lower inactivation rate  
248 constants were observed for bacteriophage Phi6 (P<0.05; Fig. 3), it can still serve as a  
249 conservative virus surrogate where use of coronaviruses is not feasible (e.g., lack of mammalian  
250 cell culture facilities). Compared to non-enveloped viruses, use of enveloped viruses like  
251 bacteriophage Phi6 is particularly desirable in surface and aerosol disinfection tests to best  
252 represent any interactions between the viral envelope and its surrounding environment that may  
253 affect viral sensitivity to UVC irradiation (34–36). Other bacteriophage, such as T1 and T7,  
254 although non-enveloped double stranded DNA phages, exhibit similar sensitivities to SARS-  
255 CoV-2 across UVC wavelengths (31) and could also serve as UV disinfection surrogates.

256 **Figure 3.** UV inactivation rate constants of SARS-CoV-2, two coronaviruses (HCoV 229E and  
257 MHV), and enveloped bacteriophage Phi6 for all tested UVC devices. The mean inactivation rate  
258 values are labeled. The values for Phi6, HCoV 229E and MHV were published by Ma et al. (22).  
259 Asterisk brackets represent two inactivation rate values that were not statistically significantly  
260 different at 95% confidence level (P>0.05).

261

262 This research defines the fundamental inactivation rate constants of SARS-CoV-2 for UVC  
263 devices with peak emission wavelengths of 222 nm to 282 nm. These devices can be used to  
264 effectively inactivate SARS-CoV-2, among which far UVC devices like KrCl\* excimer provided  
265 the best disinfection performance, with the added benefit of limited safety requirements when  
266 applied in occupied spaces. MHV is recommended as a reliable UV testing surrogate of SARS-

267 CoV-2 due to its similar UV sensitivities across UVC wavelengths, but other T-phages could  
268 also serve as surrogates. While these inactivation data align well with previous studies of UV  
269 disinfection of coronaviruses in aerosols and dried on surfaces, future work should continue to  
270 evaluate UV inactivation of SARS-CoV-2 in aqueous and other media relative to surrogates such  
271 as MHV or bacteriophage, and expand these comparisons to other disinfectants important to  
272 minimizing the transmission of respiratory viruses.

273

274 • **ACKNOWLEDGMENTS**

275 Financial support for this work was provided by the National Science Foundation, Grant CBET  
276 2029695.

277 • **DISCLOSURES**

278 The authors declare no competing interest.

279

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