MAJOR ARTICLE

Rapid SARS-CoV-2 Inactivation in a Simulated Hospital Room Using a Mobile and Autonomous Robot Emitting Ultraviolet-C Light

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The spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) since 2019 has made mask-wearing, physical distancing, hygiene, and disinfection complementary measures to control virus transmission. Especially for health facilities, we evaluated the efficacy of an UV-C autonomous robot to inactivate SARS-CoV-2 desiccated on potentially contaminated surfaces. ASSUM (autonomous sanitary sterilization ultraviolet machine) robot was used in an experimental box simulating a hospital intensive care unit room. Desiccated SARS-CoV-2 samples were exposed to UV-C in 2 independent runs of 5, 12, and 20 minutes. Residual virus was eluted from surfaces and viral titration was carried out in Vero E6 cells. ASSUM inactivated SARS-CoV-2 by ≥ 99.91% to ≥ 99.99% titer reduction with 12 minutes or longer of UV-C exposure and onwards and a minimum distance of 100 cm between the device and the SARS-CoV-2 desiccated samples. This study demonstrates that ASSUM UV-C device is able to inactivate SARS-CoV-2 within a few minutes.

Keywords. SARS-CoV-2 inactivation; UV-C radiation; autonomous robots; exponential viral-load reduction; virus inactivation.

The coronavirus disease 2019 (COVID-19) pandemic continues to make necessary the collaboration of researchers, medical professionals, technologists, and industry to find rapid and reliable solutions until we reach herd immunity conferred by globally available vaccines. Up to 7 October 2021, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused more than 235 million confirmed COVID-19 cases and 4.8 million deaths, and over 6000 million vaccine doses have been administered [1]. The rapid spread of SARS-CoV-2 since the end of 2019, and the challenge of world population immunity, make other prevention measures still crucial to control SARS-CoV-2 transmission.

In respiratory diseases, spread occurs primarily through direct patient contact, droplet transmission, or by the airborne route [2]. In the case of SARS-CoV-2, transmission has been mainly reported to be from infective droplets produced by coughing, sneezing, and breathing near to another person or by aerosols. However, the virus in aerosols and droplets deposited on surfaces has been demonstrated to remain infectious for a few days, especially in cold environments, and can be a risk for infection [3–5]. In places such as hospitals and health care facilities, disinfection of surfaces is especially required to avoid SARS-CoV-2 nosocomial transmission [6–8].

SARS-CoV-2 has a lipid-containing envelope with a nonsegmented positive-sense RNA genome [9], and is relatively easy to inactivate by heat, chemical disinfectants, detergents, gamma irradiation, or UV light [10, 11]. UV irradiation works as a virucidal agent by cellular damage (photohydration, photosplitting, photodimerization, and photocross-linking) and thereby inhibiting cellular replication [12]. The disinfection of medical and laboratory objects using UV light is widely used as a germicidal method for certain bacteria, fungi, and viruses without the use of harmful chemicals or heat [13]. UV-C light (100–280 nm wavelength) can be artificially generated from low-pressure mercury lamps or from pulsed xenon lamps [12]. Previous studies reported that high doses of UV-C are effective for inactivating SARS-CoV-2 and SARS-CoV in blood products or in culture medium [10]. Thus, UV disinfection is an environmentally friendly method that does not leave a residue or require ventilation after treatment. Although the efficacy of UV irradiation on the inactivation of pathogens, and particularly SARS-CoV-2, has been previously described [14–16], there is little scientific evidence of similar studies in realistic scenarios.

Hence, the aim of the present work was to explore the efficacy of an autonomous and mobile UV-C robot (ASSUM, autonomous sanitary sterilization ultraviolet machine; MTS Tech), equipped with 4 UV-C lamps of 254 nm wavelength arranged in 360°, for application to high-touch surfaces potentially contaminated with SARS-CoV-2.

METHODS

Ultraviolet-C Light Device

A mobile UV-C robot, ASSUM, was used. This device is equipped with 4 UV-C Phillips lamps of 254 nm arranged in 360° and its dimensions are $600 \times 900 \times 1500$ mm, width, length, and height. ASSUM scans and moves throughout the space always respecting a programmed distance from any obstacle or surface, reaching a maximum UV-C efficiency.

Cells and Virus

Vero E6 cells (Vero C1008; American Type Culture Collection CRL-1586) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS). SARS-CoV-2 (lineage B.1) was isolated from a nasopharyngeal swab collected from an 89-year-old man from the first patient cluster in Catalonia, Spain (Global Initiative on Sharing Avian Influenza Data ID EPI_ISL_510689) as previously described in detail [17]. Working virus stocks with a titer of 10^6 50% tissue culture infectious dose ($TCID_{50}$)/mL were generated by infecting Vero E6 cells at a multiplicity of infection of 0.01 in the presence of 5% FCS. Cell culture supernatants were collected at 72 hours postinfection, clarified for 10 minutes at 3000g, aliquoted, and stored at −75°C until required. For this study, virus was thawed immediately before the experiment and sterile plastic plates (35 mm; SPL, Ref. PLC20035) were used as the surface to desiccate 100 μL/plate of SARS-CoV-2 in a period of 90–100 minutes under airflow in a class 2 biosafety cabinet. All infectious work was carried out using the appropriate personal protective equipment (double gloves, double gown, and powered air-purifying respirator Sundström equipment) at Institut de Recerca i Tecnologia Agroalimentàries-Centre de Recerca en Sanitat Animal High Biocontainment Unit (biosafety level 3 facilities).

Study Design

The study was carried out in and experimental box with floor area 11-m^2 mimicking a hospital room. A total of 42 sterile plastic plates were prepared with 100 μL/plate of SARS-CoV-2 at 10^6 $TCID_{50}$/mL. Thirty-six of the 42 plates with desiccated SARS-CoV-2 were irradiated by the ASSUM UV-C robot for different time periods (5, 12, and 20 minutes). In addition, 2 plates per exposure time were left in the laboratory as a negative control (plate with culture medium) and positive control (plate with desiccated, non–UV-C exposed SARS-CoV-2). Two independent runs for each exposure time were performed, and 6 plates in different

positions were located throughout the room for each run. In each position, next to the plates with desiccated SARS-CoV-2, 2 adhesive chemical indicators were placed to record the actual cumulative UV-C radiation dose received per sample site. One of the indicators ranged from 0 to 200 mJ/cm^2 (ASSUM stickers) and the other from 500 to 1000 mJ/cm^2 (Chemdye ChemDose). Four of the 6 SARS-CoV-2 desiccated plates used for each exposure time were located at 100 cm height on the 4 room walls (1 on each wall). Another plate was located under the closest to ASSUM robot corner of a table and the remaining plate was placed on the floor at one of the room's corners (Figure 1 and Supplementary Video). For all replicates, the ASSUM robot was set to keep a minimum distance of 1 m from the UV-C exposed samples when freely moving around the box.

SARS-CoV-2 Detection and Titration

After each exposure time, all plates were eluted with 1 mL of DMEM (1% FCS) and stored at −75°C until titration. One day before titration, 96-well plates with 1.5×10^4 Vero E6 cells/well (in 100 μL of DMEM-10% FCS) were seeded. Prior to infection, cells were checked under an inverted light microscope. On the day of titration, 2 replicates of five 10-fold serial dilutions of each sample were prepared. A total of 20 μL
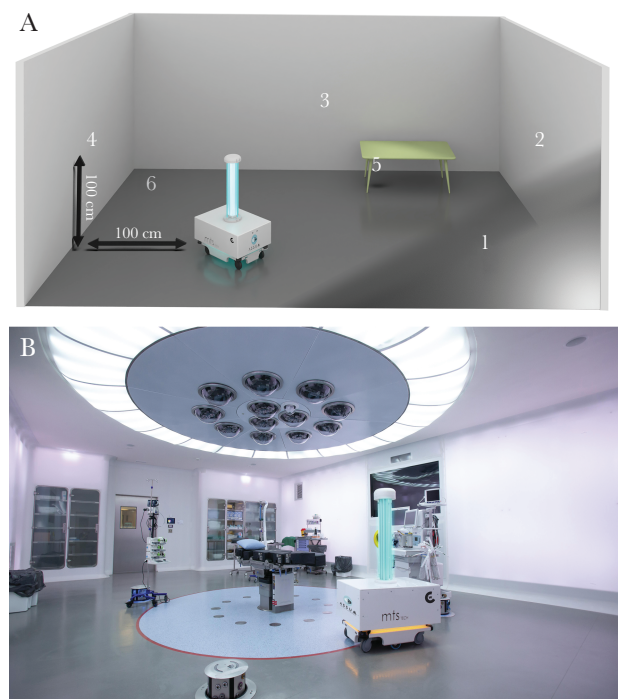

Figure 1. Schematic representation of the autonomous sanitary sterilization ultraviolet machine (ASSUM) UV-C device. *A*, Position of tested desiccated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) samples and chemical indicators in the experimental box. Black numbers indicate positions of samples at 100 cm height on the 4 room walls and sample 5 located under the edge of a table. Sample 6 was located on the floor in a corner of the room. *B*, Simulation of ASSUM running in an operating room of a hospital.

of each diluted sample was added per well. A bulk titration was also performed for each sample. For this purpose, 20 μL of each undiluted sample was added in 24 wells containing Vero E6 cells (a total volume of 480 μL/sample was titrated). All plates were incubated for 1 hour at 37°C in 5% CO_2. After incubation, 130 μL of DMEM-10% FCS was added per well. Plates were incubated at 37°C in 5% CO_2 for 6–7 days and then the SARS-CoV-2–induced cytopathic effect was determined. Viral titers were calculated as 50% tissue culture infectious doses ($TCID_{50}$) according to Spearman-Kärber methods [18, 19].

Statistical Analysis

The 2 runs performed for each exposure time (5, 10, and 20 minutes) were considered as independent runs. To analyze the differences between the different sample position with UV-C exposure and the control not exposed to UV-C, Tukey multiple comparisons test (ANOVA) was used to compare the viral titer means obtained at all positions and P values lower than .05 were accepted as statistically significant. Calculations were done using Graphpad Prism 9.

RESULTS

Results of virus inactivation from the present study are summarized in Table 1. All control samples without desiccated virus were negative for each replicate and exposure time. Control plates not exposed to UV-C showed a mean viral load between 3.49 and 4.19 $\log_{10}$ $TCID_{50}$/mL in 2 consecutive runs, which were analyzed independently. The detection limit of the technique was 0.45 $\log_{10}$ $TCID_{50}$/mL.

The minimum distance between the ASSUM robot and the desiccated samples was set at 1 m and the maximum was registered close to 3 m to the opposed wall. As expected, the highest reductions of infectious SARS-CoV-2 were observed particularly in the positions that were in direct line of sight (samples located at 100 cm height on the walls). Samples located on the walls or on the floor showed statistically significant differences ($P < .01$) compared to the SARS-CoV-2 controls not exposed to UV-C. This was not the case for the samples located under the edge of a table ($P > .05$).

At 20 and 12 minutes of UV-C exposure, samples located on the walls showed reduction ranging between ≥ 99.91% and ≥ 99.99%. At 20 minutes, the sample located on the floor in the corner showed the same reduction percentage. The infectivity reduction of samples located on the floor ranged from 99.47% to 99.93% after 12 minutes of UV-C exposure. Finally, with 5 minutes of UV-C exposure, 2 of the 4 samples on the walls reached a percentage of SARS-CoV-2 reduction comparable to those at 12 and 20 minutes, but the other 2 samples located on the walls showed a 94.11% reduction. At 5 minutes, samples on the floor achieved 97.43%–98.00% infectivity decrease. The samples located under the edge of a table showed the lowest viral reduction (63.7%–98.41%).

Table 1. SARS-CoV-2 Titration Results Expressed as $TCID_{50}$/mL

Sample Location	SARS-CoV-2 Titer, Mean $\log_{10}$ $TCID_{50}$/mL	Titer Reduction Range, logR	Reduction Range, %
UV-C robot working time 20 min			
Wall 1, at 100 cm height	≤0.45	≥3.04 to ≥3.74	≥ 99.91 to ≥ 99.99
Wall 2, at 100 cm height	≤0.45	≥3.04 to ≥3.74	≥ 99.91 to ≥ 99.99
Wall 3, at 100 cm height	≤0.45	≥3.04 to ≥3.74	≥ 99.91 to ≥ 99.99
Wall 4, at 100 cm height	≤0.45	≥3.04 to ≥3.74	≥ 99.91 to ≥ 99.99
On the floor	≤0.45	≥3.04 to ≥3.74	≥ 99.91 to ≥ 99.99
Under the edge of a table	2.69	0.49 to 1.81	67.64 to 98.41
UV-C robot working time 12 min			
Wall 1, at 100 cm height	≤0.45	≥3.04 to ≥3.74	≥ 99.91 to ≥ 99.99
Wall 2, at 100 cm height	≤0.45	≥3.04 to ≥3.74	≥ 99.91 to ≥ 99.99
Wall 3, at 100 cm height	≤0.45	≥3.04 to ≥3.74	≥ 99.91 to ≥ 99.99
Wall 4, at 100 cm height	≤0.45	≥3.04 to ≥3.74	≥ 99.91 to ≥ 99.99
On the floor	1.14	2.27 to 3.14	99.47 to 99.93
Under the edge of a table	2.58	0.84 to 1.69	85.54 to 98.00
UV-C robot working time 5 min			
Wall 1, at 100 cm height	2.27	1.22 to ≥3.74	94.11 to ≥ 99.99
Wall 2, at 100 cm height	≤0.45	≥3.04 to ≥3.74	≥ 99.91 to ≥ 99.99
Wall 3, at 100 cm height	≤0.45	≥3.04 to ≥3.74	≥ 99.91 to ≥ 99.99
Wall 4, at 100 cm height	0.45	3.04 to ≥3.74	99.91 to ≥ 99.99
On the floor	2.20	1.59 to 1.69	97.43 to 98.00
Under the edge of a table	3.37	0.44 to 0.51	63.70 to 69.80

SARS-CoV-2 infectious titer was calculated from serial dilutions and bulk titration mean ($\log_{10}$ $TCID_{50}$/mL). Titer reduction range (logR) is shown compared to the SARS-CoV-2 desiccated stocks (3.49 and 4.19 $\log_{10}$ $TCID_{50}$/mL) and the percentage of viral reduction range was obtained from both independent experimental runs.

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; $TCID_{50}$, 50% tissue culture infectious dose.

After allowing the robot to freely move around the experimental box, the UV-C radiation dose at the different positions ranged between 50 mJ/cm^2 and 500 mJ/cm^2, according to the UV-C dosimeters (stickers placed next to the sample). After both 12 and 20 minutes of UV-C exposure, samples located at 100 cm height on the walls showed an accumulative UV-C radiation dose of 500 mJ/cm^2 approximately, for both runs. For these 2 exposure times, samples located on the floor and under the table received a UV-C radiation dose of 150 mJ/cm^2 and 50 mJ/cm^2, respectively.

After 5 minutes of UV-C exposure, samples at 100 cm height on the walls received an accumulated UV-C radiation dose between 200 and 500 mJ/cm^2, while samples underneath the table and on the floor received around 50 mJ/cm^2. None of the positive or negative controls received UV-C radiation.

DISCUSSION

With herd immunity as a huge challenge in the current COVID-19 pandemic, all preventive measures are still crucial to minimize SARS-CoV-2 spread. This study provides useful baseline data towards securing a safer environment where surfaces are potentially contaminated with this and other pathogens sensitive to UV-C radiation. This is especially focused on health care facilities where viral loads can be high. Here, we demonstrated for the first time under comparable hospital intensive care unit (ICU) room conditions (not within a biosafety cabinet) that SARS-CoV-2 load can be drastically reduced within a few minutes using UV-C irradiated by the ASSUM autonomous robot. A preliminary assay was carried out to set the optimal timing and distance conditions for the UV-C mobile ASSUM robot. Results from this preliminary assay showed that 20 minutes were enough to inactivate desiccated SARS-CoV-2 from surfaces at a distance between 1 and 2 m from the ASSUM device (data not shown). The results of the present assay showed infectious virus reduction ≥ 99.91% after 12 minutes of UV-C exposure with a minimum distance of 100 cm from the UV-C–exposed samples placed at 100 cm height and a cumulative UV-C radiation dose between 50 and 500 mJ/cm^2. As expected, in shadowed positions, viral reduction was less evident, and the lowest viral reduction was observed for the samples located under a table; however, this location is also less likely to be contaminated with SARS-CoV-2.

Considering that the main route of SARS-CoV-2 transmission occurs from infectious droplets, which are very diverse in size, and produced by coughing, sneezing, and breathing near another person [20], SARS-CoV-2 nosocomial transmission has been reported over longer distances, principally due to high air fluxes inside health care facilities [21]. So far as it is known, human coronaviruses, including SARS-CoV and SARS-CoV-2, can remain infectious in aerosols for 3 to 16 hours and can survive on surfaces at room temperature and relative humidity of 65% for a few days [3].

The risk of COVID-19 transmission by fomites has been questioned, with the argument that most of the published studies have been carried out with high viral titers compared to those in natural aerosols or droplets from infected patients. As a consequence, it is believed that the risk of transmission by fomites is lower compared to aerosol or droplet transmission [22]. However, the number of viral particles present in aerosols or contaminated surfaces is not well known and the risk of infection from fomites cannot be ruled out. Therefore, stringent preventive measures should be taken, especially in high-risk locations such as health care settings, to avoid this possible route of transmission [6–8].

There have been no previous studies on the use of autonomous UV-C robots, which can freely move around ICU and other health care rooms where COVID-19 patients have been treated, to inactivate SARS-CoV-2 on contaminated surfaces. Our work simulates an actual hospital scenario, including room obstacles, and the robot was set to maintain a minimum distance of 100 cm to all objects. Previous reports with this and other coronaviruses are in line with the results of our study and showed viral reduction mediated by UV-C irradiation in a laboratory enclosed within biosafety cabinet conditions. However, most of the published studies were performed with shorter exposure distances (a few cm) between the UV source and SARS-CoV-2 samples [14, 16, 23, 24]. Darnell and colleagues [23] showed that after 15 minutes of UV-C irradiation at 3 cm from the virus plate and a UV-C dose emitted of 4016 µW/cm^2, SARS-CoV was inactivated below the detection limit (≤1.0 log$_{10}$ TCID$_{50}$/mL). Moreover, Heilingloh and colleagues [14] also showed complete inactivation of SARS-CoV-2 after 9 minutes of irradiation and with a UV-C dose emitted of 1048 mJ/cm^2 at 3 cm. In both cases, the total inactivation implied about a 5-log reduction. These data on SARS-CoV and SARS-CoV-2 indicting total viral inactivation were obtained under ideal conditions with short UV exposure distances, which differ considerably from our attempt to model a more realistic setting for testing SARS-CoV-2 UV-C inactivation.

Besides its efficiency, an advantage of the UV-C robot is its self-autonomy and capability to freely move and maintain a desired distance from room obstacles. This UV-C device may be especially useful to avoid excessive exposure of cleaning or health care personnel to SARS-CoV-2 contaminated surfaces in hospital facilities, where high numbers of COVID-19 patients may shed large amounts of virus. Moreover, the ASSUM UV-C robot may inactivate other pathogens even more environmentally resistant than SARS-CoV-2.

Taken together, the present study demonstrated that UV-C irradiation is a highly effective method to inactivate high titers of infectious SARS-CoV-2 from surrogate surfaces, mimicking a natural health care center environment and high touch surfaces. This technology would be reliable not only for disinfection purposes in health care rooms but also for other applications and

equipment or spaces with high risk of transmission, such as public transport facilities. Further studies regarding the efficacy of UV-C irradiation in other situations and other viruses will provide useful information regarding prevention for this and future pandemics.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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