JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Bactericidal efficacy of mobile ultraviolet-C disinfection devices in reducing contamination in biosafety laboratories

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Abstract

Introduction: Biosafety research requires a wide range of microorganisms and thorough disinfection to prevent laboratory infection is often required. Ultraviolet-C (UV-C) exposure reduces bacterial and viral concentrations. Therefore, in this study, we aimed to evaluate the efficacy of a mobile UV-C device as a non-contact disinfection strategy.

Methodology: The bactericidal efficacy of the UV-C device was determined based on $\log_{10}$ decreases in the relative abundances of bacterial indicators, including *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus albus*, and *Pseudomonas aeruginosa* at 0.5 and 1.0 m after irradiation for 30, 60, and 90 min. Next, the reduction of natural bacteria in air and on surface as a result of the UV-C device exposure in the laboratory were determined.

Results: Exposure to the UV-C disinfection device resulted in mean $\log_{10}$ decreases in microbial contamination of 3.55 and 5.85 following irradiation for 30 and 90 min, respectively, at a distance of 0.5 m. Further, *P. aeruginosa* and *E. coli* were the most and least sensitive to UV-C exposure, respectively. The bacterial load in air decreased by 65.53% after 60 min of irradiation, while those on surfaces decreased by 44.19% and 78.23% after 30 and 60 min of irradiation, respectively.

Conclusions: The UV-C device effectively reduced bacterial load after irradiation for over 60 min. Further studies are encouraged to determine the effectiveness of the UV-C disinfection device in frequently occupied institutions, such as primary medical, health, and nursery, and its efficiency in infection control.

Key words: ultraviolet; irradiation; disinfection; bactericidal efficacy; air disinfection.

J Infect Dev Ctries 2023; 17(11):1574-1580. doi:10.3855/jidc.18091

(Received 16 February 2023 – Accepted 11 April 2023)

Introduction

Air disinfection, which reduces the concentration of microorganisms in air [1], is important for preventing and controlling the spread of airborne infectious diseases. Common strategies for the prevention of such diseases in healthcare settings include the use of personal protective equipment (PPE), air purification, and disinfection [2]. Given that ultraviolet-C (UV-C) irradiation does not require room ventilation, is associated with reduced residue and is characterized by a flexible operation that is disinfectant-free and easy to operate, UV-C germicidal irradiation is the most frequently used disinfection strategy in primary medical and health institutions, biosafety laboratories, nursing institutions, and schools.

The UV-C wavelength band (100–280 nm) directly overlaps with the absorption peak of the DNA and RNA of microorganisms (approximately 260 nm). Further, after UV-C absorption, the pyrimidines in the RNA or DNA of these microorganisms are converted into pyrimidine (6–4), pyrimidone photoproducts, and cyclobutane pyrimidine dimers [3,4]. This results in the formation of pyrimidine dimers, which inhibit DNA replication and consequently prevent microbial reproduction [5]. Therefore, UV-C is basically used universally as a disinfection strategy for almost all bacteria [6]. With advances in no-touch disinfection technology, novel UV-C disinfection devices are becoming increasingly common as auxiliary environmental decontamination methods. Furthermore, disinfection products, such as UV disinfection robots and UV disinfection systems [7-10], provide practical solutions for the daily disinfection of medical and health institutions.

The effectiveness of the UV-C disinfection technology largely depends on environmental factors, including temperature, humidity, distance from the radiation source, UV-C intensity, residence time,

placement of objects in the room, microorganism sensitivity, and ability of the UV-C light to reach microorganisms in folds, crevices, and under surfaces [11]. Reducing infection is primordial in healthcare institutions. Therefore, it is necessary to evaluate the effect of UV disinfection devices to ensure disinfection efficiency. In this regard, the purpose of this study was to investigate the disinfection efficiency and irradiation intensity of a mobile ultraviolet disinfection device equipped with two tubular UV-C lights (30 W).

Methodology

UV-C device

The study was conducted according to the Standard for Field Disinfection Evaluation employed during the COVID-19 Epidemic (WS/T774-2021) [12] and the Standard for Evaluating the Efficacy of Disinfection on Site (WS/T 797-2022) [13] with minor adjustments.

The mobile UV-C disinfection device (Shenxing, Jiangsu, China) was composed of two low-pressure mercury lights (30 W), a base, a box, and a light arm. The light arm was 90 cm long and could rotate 180°. Further, the working ambient temperature was 5–40 ℃, and the working relative humidity (RH) was less than 80%, according to the product instruction manual. The experiments were conducted at 22 ± 2 ℃ and 60% ± 5% RH.

Preparation of biological indicators

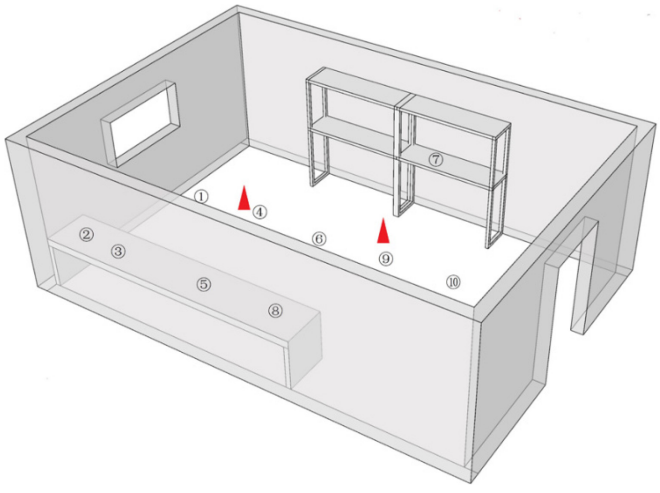
The disinfection efficacy of the UV-C irradiation was quantitatively evaluated using indicator cultures, including *Escherichia coli* (8099, *E. coli*), *Staphylococcus aureus* (ATCC 6538, *S. aureus*), *Staphylococcus albus* (8032, *S. albus*), and *Pseudomonas aeruginosa* (ATCC 15442, *P. aeruginosa*). *E. coli* and *S. aureus* were used as Gram-negative and Gram-positive bacterial indicators, respectively. Additionally, *E. coli* served as an indicator of enteric infection, while *S. aureus* served as an indicator of suppurative infection. Further, *S. albus* and *P. aeruginosa* were used as indicators of airborne and nosocomial infections, respectively.

The biological indicators were prepared in accordance with our previous report with some modifications [14]. In brief, the bacteria were obtained from freeze-dried cultures in vials from the Chinese General Microbiological Culture Collection Centre (Beijing, China). This was followed by culturing and staining on smooth sterilized stainless-steel discs with a diameter of 1 cm. Thereafter, the bacteria were sub-cultured until their 5th to 7th generations were obtained and adjusted to a concentration of 1.0×10^8 colony forming units (CFU)/mL. Subsequently, 10 μL of the bacterial suspension was introduced on sterilized stainless-steel discs to obtain an inoculum of approximately 5.0×10^5 – 5.0×10^6 CFU/disc. Phosphate-buffered saline (PBS) and tryptic soy broth (TSB: tryptone, 1.5%; soybean peptone, 0.5%; and sodium chloride, 0.5%) were used to suspend the bacteria, simulating clean and polluted conditions, respectively. The stained discs were dried in an incubator at 37 ℃ for 30 min. Finally, the dried test discs were placed in a 90-mm Petri dish and transferred into the test enclosure for irradiation disinfection.

Evaluation of bactericidal efficacy

The evaluation of bactericidal efficacy was conducted in two directions; one was at the centre point 0.5 and 1.0 m vertically below the lights, and the other was 0.5 m to the right side of the two lights. PBS and TSB were used to suspend the bacteria for bacterial indicator preparation, simulating a clean and polluted state, respectively. After irradiation for 30, 60, and 90 min, the bacterial growth indicator discs were transferred into sterilized glass tubes containing 5 mL of PBS, wherein the discs were immersed for 30 min and thereafter, vortexed for 20 s to dislodge the surviving bacteria. Next, the extract was serially diluted 10-fold, and 1.0 mL of the suspension was placed on plates in duplicates. To each plate, 15–20 mL of nutrient agar (45 ℃) was added, followed by mixing via gentle rotational swirling. The plates were then incubated at 37 ℃ for 48 h prior to the counting of bacterial colonies.

Figure 1. Schematic presentation of selected locations in a 69 m^3 laboratory.



Locations ①, ④, ⑥, ⑨, and ⑩ were on the floor, while locations ②, ③, ⑤, and ⑧ were on a table, ⑦ was on a shelf. All the locations were approximately 0.7 m above the floor. △ represents the locations of the UV-C disinfection device.

Evaluation of field disinfection

The effectiveness of field disinfection, including the effectiveness of the device in disinfecting air and object surfaces, was evaluated in a 69 m^3 biosafety level-1 laboratory using two sets of UV-C disinfection devices. The doors and windows were closed, and nobody stayed in the room. The locations for air and surface sampling are shown in Figure 1.

Air disinfection evaluation: The plate exposure method was used to measure the depositing bacterial concentration. Nutrient agar plates (diameter 90 mm) were placed at each sampling site 0.8–1.5 m above the ground prior to irradiation. The plates were exposed for 15 min, and after irradiation, another set of plates were placed at the same position and exposed for the same duration were applied for sampling before disinfection. Simultaneously, two plates without any exposure were used as negative controls. The pre- and post-irradiation plates were incubated at 37 °C for 48 h, and the number of colonies was counted. The mean natural bacteria-killing rate of the treatment was then calculated.

Surface disinfection evaluation: Natural bacteria were collected from each surface before and after disinfection. Specifically, a sterilized cotton swab that had been immersed in a neutralizing solution was used to wipe each sample surface (100 cm^2) within a defined square (5 × 5 cm). The cotton swab was wiped back and forth vertically and horizontally five times with rotation. Subsequently, the swab was vortexed in 10 mL of the neutralizing solution in PBS. In the next step, 1 mL of this mixture was used to inoculate a nutrient agar plate in duplicates. The number of bacterial colonies (CFU/cm^2) was determined after incubation at 37 °C for 48 h.

Detection of UV-C irradiation intensity

The irradiation intensity was detected at 254 nm using a UV-C irradiation meter (Beijing Shida Photoelectric Technology Co. Ltd, Beijing, China) after turning on the lights for 5 min. The light frame was rotated parallel to the ground, and the irradiation intensity was measured at the centre point, 0.5 and 1.0 m vertically below the lights, and 0.5, 1.0, and 1.5 m vertically to the right side of the two lights.

Statistical analysis

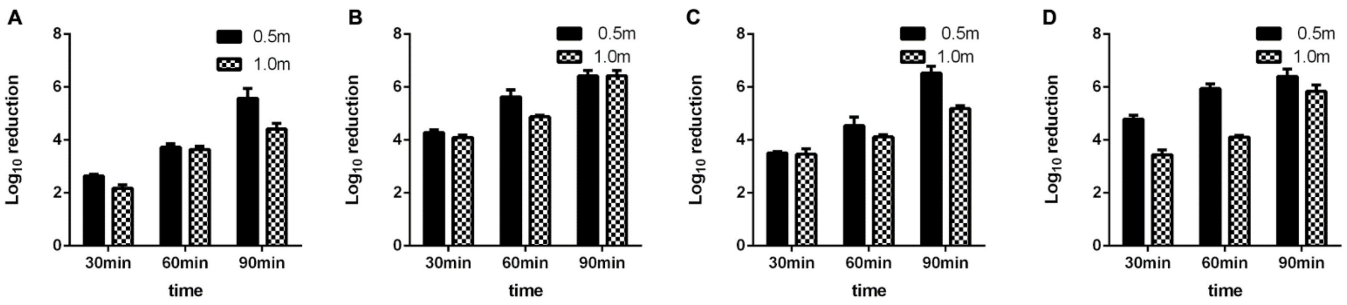
The collected data were entered into Microsoft Excel (Microsoft Corp, Redmond, WA, USA), GraphPad Prism software v6.0 (GraphPad, San Diego, CA, USA), and SPSS 18.0 (IBM, New York, USA), and analyzed by performing the paired t-test as well as Kruskal–Wallis and Wilcoxon tests. Statistical significance was set at $p < 0.05$.

Results

Bactericidal efficacy

The bactericidal efficacy evaluation was conducted at 0.5 m (UV-C 285 μW/cm^2) and 1.0 m (UV-C 100 μW/cm^2) vertically below the light sources, and the irradiation was performed for 30, 60, and 90 min. The mean log_{10} decreases in bacterial load were 3.55, 4.57, and 5.85 after irradiation at 0.5 m for 30, 60, and 90 min, respectively. Further, the log_{10} decrease in the load of each type of bacterium was > 5.00 after irradiation for 90 min (Figure 2). As shown in Figure 2, the log_{10} decrease in bacterial load observed at 0.5 m was slightly higher than that observed at 1 m for each type of bacterium, and the lowest decrease among the four bacteria was observed for *E. coli* (Figure 2A), while the highest was observed for *P. aeruginosa* (Figure 2D). Our results also indicated that distance influenced bactericidal efficacy, and *P. aeruginosa* and *E. coli* showed the highest and lowest sensitivities to UV-C, respectively.

Figure 2. Log10 decreases in bacteria contamination at 0.5 and 1.0 m following irradiation for 30, 60, and 90 min.



A: E. coli; **B:** *S. aureus*; **C:** *S. albus*; and **D:** *P. aeruginosa*.

The analysis of $\log_{10}$ decreases in bacterial load via the Kruskal–Wallis test indicated significant differences among the 30-, 60-, and 90-min irradiation groups ($p < 0.05$). Multiple comparisons also showed that the $\log_{10}$ decrease for the 90-min irradiation group was significantly higher than that observed for the 30-min irradiation group ($p < 0.05$). However, there was no significant difference between the 30- and 60-min irradiation groups ($p = 0.2313$) and between the 60- and 90-min irradiation groups ($p = 0.1432$). Additionally, the Kruskal–Wallis test revealed no significant differences between the 0.5 and 1.0-m groups for the different bacteria ($p < 0.05$), even though the $\log_{10}$ decrease at 0.5 m was slightly higher than that observed at 1 m. The above analysis indicated that irradiation time had a significant effect on disinfection efficacy.

Effects of organic interfering substances

The effect of organic interfering substances was measured at the centre point 0.5 m vertically below the lights (UV-C 285 μW/cm^2) for *E. coli* and *S. aureus*, and at the centre point 0.5 m vertically at the right side of the two lights (UV-C 588 μW/cm^2) for *S. albus* and *P. aeruginosa*. The $\log_{10}$ decrease in bacterial load in the PBS groups was slightly higher than that observed for the TSB groups for *S. aureus* (Figure 3B), *S. albus* (Figure 3C), and *P. aeruginosa* (Figure 3D), while no significant differences were observed for the *E. coli* groups (Figure 3A). Further, the $\log_{10}$ decreases in bacterial contamination for each type of bacterium between the PBS and TSB groups were individually analyzed via Wilcoxon nonparametric test. Thus, no significant differences were observed for all the groups ($p > 0.05$).

Field disinfection of air

The total number of bacterial CFUs in air pre- and post-disinfection are shown in Figure 4A. The mean killing rate for natural air bacteria was 65.53% (40.48–96.30%) following irradiation for 60 min. Further, the total number of bacterial CFUs pre-disinfection was higher than that post-disinfection as analyzed via paired t-test ($p < 0.05$). These results showed that the UV-C disinfection device has a notable air decontamination effect following irradiation for 60 min.

Field disinfection of surfaces

The mean killing rate of the natural bacteria on object surfaces was 44.19% and 78.23% following irradiation for 30 and 60 min, respectively (Figure 4B). A significant difference was also observed between the two mean killing rates based on analysis via the paired t-test ($p < 0.05$). The total number of natural bacteria before irradiation, 30 min post-irradiation, and 60 min post-irradiation was analyzed via the Kruskal–Wallis test. Thus, significant differences were observed among the groups ($p < 0.05$). Specifically, multiple comparisons indicated a significant difference between

Figure 4. A: Bacterial colony forming units (CFUs) in air pre- and post-disinfection; **B:** Mean natural bacteria killing rate after irradiation for 30 and 60 min.

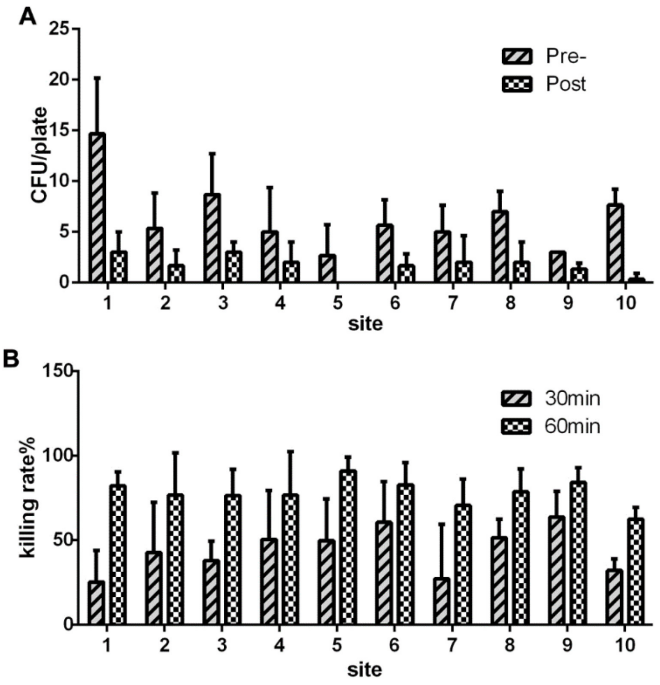
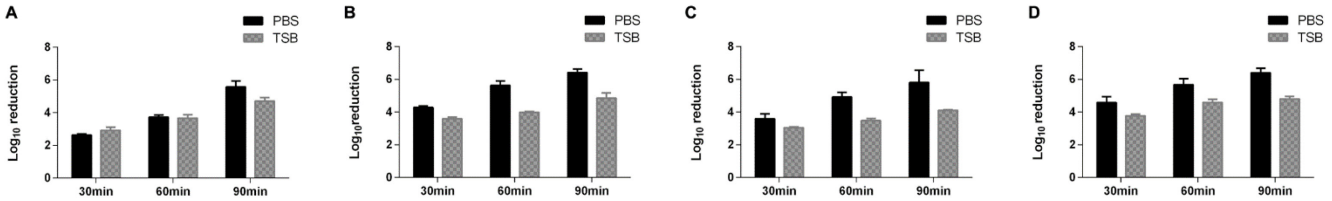


Figure 3. Log10 decreases in bacterial load with and without organic interfering substance.



A: *E. coli*; **B:** *S. aureus*; **C:** *S. albus*; **D:** *P. aeruginosa*. PBS: phosphate-buffered saline; TSB: tryptic soy broth.

the 60 min irradiation group and the group before disinfection ($p < 0.05$); however, no significant differences were observed between the 30 min irradiation group and the group before disinfection ($p = 0.858$), and between the 30 and 60 min irradiation groups for 60 min ($p = 0.329$). Therefore, the evaluation of surface disinfection indicated that a minimum of 60 min was required to achieve a satisfactory disinfection effect.

Discussion

The susceptibility of microorganisms to UV-C varies based on several factors, including the variation of the biological structure, environmental conditions, and degree of environmental cleanliness [15]. In this study, experiments involving mobile two-light UV-C disinfection devices were conducted, and UV-C intensity in different orientations was analyzed. Additionally, the effect of the UV-C light was analyzed using four different bacteria: two Gram-negative bacilli, *E. coli* and *P. aeruginosa*, and two Gram-positive cocci, *S. aureus* and *S. albus*. Our results indicated a higher UV-C sensitivity for *P. aeruginosa* than for *E. coli*, *S. aureus*, and *S. albus*, consistent with the findings of Chang *et al*. [16], who showed that *P. aeruginosa* is more susceptible to UV-C than *Legionella pneumophila* and *S. aureus*. Furthermore, we found that *E. coli* was the most resistant to UV-C. This observation differs from that obtained in another study, which indicated that the Gram-negative species, *E. coli* and *S. marcescens*, show a significant decay rate and sensitivity to UV-C, while the Gram-positive species show a reduced decay rate and sensitivity [17]. As *E. coli* is a common pathogen that causes enteric tract infection, we recommend that evaluation be intensified at medical and nursery institutions, where UV-C is used for daily decontamination to guarantee disinfection efficacy.

The efficacy of UV-C-based decontamination technologies is promising but depends on numerous environmental, physical, and technical factors. The mean killing rate of natural bacteria in the air was 65.53% (40.48–96.30%) following irradiation for 60 min. This is similar to the values reported by Xu *et al*. [18], who reported a killing rate of 46–98%, and was also consistent with the results of a study conducted in hospital wards (42%) [19]. It has been suggested that UV-C technologies should not be used in isolation but be considered as an adjunct to protocol-driven standard operating procedures for cleaning and disinfection, hand hygiene practices, and appropriate PPE use [20].

The effectiveness of UV-C light treatment depends on the UV-C dose, i.e., the irradiation intensity and time, and the characteristics of each microorganism. During our experiments, the mean UV-C intensity at the centre point and at 0.5 and 1.0 m vertically below the lights were 285 and 100 μW/cm^2, respectively. Further, the mean UV-C intensities 0.5, 1.0, and 1.5 m vertically to the right side of the two lights were 588, 176, and 89 μW/cm^2, respectively. Reportedly, different orientations result in different UV-C irradiation intensities [21]. The radiation intensity at the right side of the two lights was approximately two-fold that below the lights. Further, the $\log_{10}$ decreases in bacterial contamination below the lights were slightly reduced compared with the observations made on the right side of the lights. However, the difference was not statistically significant. These findings indicated the varying effect of UV-C light under varying intensities and conditions.

Over the past few decades, UV-C light technology has been increasingly used in healthcare settings to prevent infection in environments, including object surfaces, water, and air. Recent studies have demonstrated the effectiveness of this technology in this regard and in reducing contamination by multidrug-resistant organisms [22,23]. However, the UV-C light delivery method is associated with several limitations as it can only be used in unoccupied hospital wards. Additionally, it only provides one-time disinfection. Thus, the environment becomes contaminated again when the room is occupied. During our experiment, we tested for natural bacteria in the air and on surfaces the following day and observed that the bacterial concentration returned to the pre-disinfection level. It has also been reported that laboratory irradiation is only effective for 5 h before microbial resurrection [24].

Based on the results of our UV-C irradiation intensity monitoring experiments, we identified some issues affecting the disinfection efficacy of the UV-C device. For example, one of the commonly observed issues was that the UV-C lights in the room were not properly installed; one was hanging too high from the ceiling. Thus, the distance between it and the object surfaces was more than 1.5 m, resulting in the irradiation intensity being too weak to reach the target. Reportedly, UV-C light irradiates for up to 30 min, and based on most standards, the minimum required UV-C intensity is 70 μW/cm^2. In this study, we examined the effect of UV-C on air disinfection based on irradiation for 30 min. The results thus obtained showed limited microbial inactivation efficacy. Therefore, we suggested that prolonging the irradiation time for

hanging UV-C lights could enhance their efficacy. Another identified issue was that when the irradiation time was too long, and the UV-C irradiation intensity weakened by time. Therefore, we recommend that users monitor the UV-C radiation intensity regularly and keep the light tubes clean to ensure effective disinfection.

Conclusions

In this study, the mobile UV-C device showed efficacy in reducing bacterial concentration. The disinfection efficacy of the device was also found to be affected by the irradiation distance, organic interfering substances, and the relative orientation of the light with respect to the surface to be disinfected. In the future, it would be necessary to determine the effectiveness of infection control based on the UV-C disinfection device in frequently occupied institutions, such as primary medical, health, and nursery institutions.

Authors' contributions

Chunai Tao designed the study, carried out experiments, and wrote the manuscript. Xiaolan Tang, Jiayi Luo and, Xinbi Zhang participated in conducting experiments and contributed in revising the manuscript. All the authors agreed on the final version of the manuscript.

Funding

This research was supported by a self-funded project of the Health Commission of the Guangxi Zhuang Autonomous Region(Z-A20230417).

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Conflict of interests: No conflict of interests is declared.