

## Investigating the Effect of Different Wavelengths of UV Radiation in Disinfection of Airborne and Surface SARS-Cov-2

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### ABSTRACT

**Introduction:** COVID-19 pandemic which is caused by SARS-CoV-2 has caused focus on reliable and effective disinfection methods such as ultraviolet radiation. There are significant gaps in the literature regarding the effectiveness of various UV wavelengths and its performance on different surfaces for viral RNA destruction.

**Materials and Methods:** This study evaluates the efficacy of UVA, UVB, and UVC radiation in inactivating SARS-CoV-2 in contaminated air streams on various surfaces. The experiment measured cycle threshold (Ct) values of viral RNA under different UV exposure times and airflow rates.

**Results:** UVC radiation achieved complete viral RNA destruction after 5 minutes at an airflow rate of 1 L/min, significantly outperforming UVA and UVB. Higher airflow rates reduced the efficacy of UVA and UVB, but UVC remained highly effective, showing significant viral reduction. On surfaces, UVC exposure increased Ct values over time, indicating reduced viral RNA, with rapid effects on paper and glass, and longer times needed for cloth and iron.

**Conclusion:** The findings emphasize the importance of selecting the appropriate UV wavelength and optimizing exposure conditions for effective disinfection. UVC, due to its high energy and shorter wavelength, is ideal for rapid and thorough viral inactivation, making it suitable for air and surface disinfection in healthcare and public spaces. In conclusion, UVC radiation is the most effective UV wavelength for SARS-CoV-2 inactivation, offering significant advantages in both air and surface disinfection. Future strategies should leverage UVC's high efficacy and optimize exposure conditions to maximize viral inactivation.

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### Introduction

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has profoundly impacted global health, economies, and daily life since its emergence in late 2019. The virus swiftly spread across continents, leading to unprecedented disruptions in daily activities, healthcare systems and economic

stability. Lockdowns, social distancing measures, and the closure of businesses and educational institutions became commonplace as governments and health authorities grappled with controlling the virus's transmission<sup>1,2</sup>.

The rapid spread of the virus underscored the urgent need for effective methods to mitigate transmission, particularly in indoor environments

where people spend a significant portion of their time indoors. Indoor settings, such as homes, workplaces, schools, healthcare facilities, and public transportation systems, pose a heightened risk for airborne and surface contamination. The ability of the virus to linger in the air and on surfaces for extended periods makes these environments critical points of focus for disinfection and infection control strategies<sup>3,4</sup>.

As scientists and public health experts raced to understand and combat the virus, numerous research efforts and innovations emerged. Among these, ultraviolet (UV) radiation is a promising tool for disinfection. The use of UV radiation for sterilization is not a new concept; it has been employed for decades in various applications, including water purification, air disinfection, and surface decontamination<sup>5</sup>. However, the COVID-19 pandemic has brought renewed attention to its potential efficacy against SARS-CoV-2.

UV radiation, which is part of the electromagnetic spectrum, is categorized into three types based on wavelength: UVA (320-400 nm), UVB (280-320 nm), and UVC (100-280 nm). Among these, UVC is known for its potent germicidal properties and can inactivate a wide range of pathogens, including bacteria, viruses, and fungi. Historically, UVC has been employed in various settings, such as healthcare facilities, water treatment plants, and air purification systems, to reduce microbial loads and prevent the spread of infectious diseases<sup>6</sup>.

The mechanism by which UVC radiation inactivates microorganisms involves the absorption of UVC photons by nucleic acids (DNA and RNA), leading to the formation of pyrimidine dimers and other photoproducts. These molecular changes disrupt the genetic material of pathogens, rendering them unable to replicate and infect host cells. This well-documented mode of action underpins the use of UVC as a disinfection strategy, particularly in the context of airborne and surface contamination<sup>7,8</sup>.

Given the pressing need to control the spread of SARS-CoV-2, researchers have investigated the effectiveness of UVC radiation in deactivating this

virus. Preliminary studies have shown that SARS-CoV-2 is susceptible to UVC exposure, suggesting that UVC irradiation could be a viable approach for disinfecting air and surfaces in environments where the virus may be present. However, the efficacy of UV disinfection can be influenced by several factors, including the wavelength of UV radiation, exposure time, and physical properties of the contaminated surfaces<sup>9</sup>.

This study aimed to investigate the effects of different wavelengths of UV radiation on the removal of SARS-CoV-2 from contaminated air streams and surfaces. By systematically exploring the germicidal effectiveness of UVA, UVB, and UVC radiation, this study aimed to identify optimal disinfection protocols that can be applied in various settings to reduce the risk of COVID-19 transmission.

To achieve this, the research was divided into several key components. The study will initially assess the inactivation kinetics of SARS-CoV-2 when exposed to various wavelengths and intensities of ultraviolet (UV) radiation. This assessment will involve conducting controlled laboratory experiments to establish the dose-response relationship and to identify the minimum effective dose required for viral inactivation. Subsequent research will evaluate the practical implementation of ultraviolet (UV) radiation in real-world contexts, specifically focusing on surfaces commonly encountered in public and healthcare environments. Accordingly, this study aims to provide valuable insights into the use of UV radiation as a disinfection tool against SARS-CoV-2, with the potential to enhance public health measures and reduce the spread of COVID-19.

## Materials and Methods

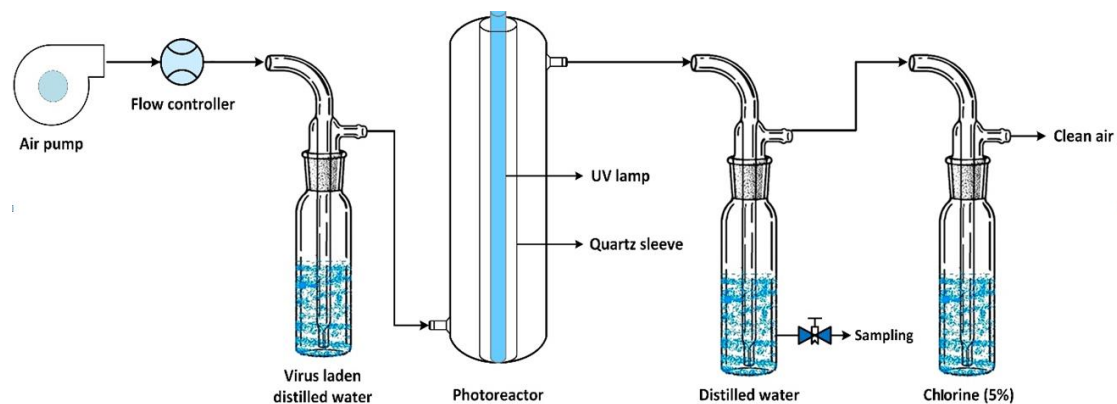
### Exposure experiments

#### 2.1.1. SARS-CoV-2 removal from contaminated air stream

Figure 1 illustrates a schematic of the experimental setup designed to assess the removal of the Coronavirus-2 from an air stream contaminated with the virus. To achieve this, a virus-infected liquid medium was prepared by

adding a specific amount of virus to 100 mL of distilled water. The Coronavirus-2 used in this study was freshly isolated from positive samples

obtained from the cell-molecular laboratory dedicated to diagnosing Coronavirus-2 in Maragheh.



**Figure 1:** Schematic of the pilot designed to assess the elimination of the Coronavirus-2 from contaminated air using UV treatment

Following the preparation of the contaminated medium in the impinger, airflow was introduced to facilitate the transfer of the virus from the liquid phase to the air phase. It is important to note that the successful transmission of the virus from the liquid phase to the air phase was verified by inspecting the impinger output for the presence of the virus.

Subsequently, the contaminated air stream was directed into a UV reactor that employed UVA or UVC lamps. The polluted airflow underwent UV treatment before entering another impinger containing distilled water. To assess the potential of the UV reactor for virus removal, samples were collected at 30, 60, and 120-second intervals from the impinger following the UV reactor. At each sampling run, 200  $\mu\text{L}$  of the samples were obtained, their characteristics were documented, and they were sent to the laboratory for virus isolation and quantification.

It is worth noting that to prevent any potential release of the virus into the surrounding air, the air flow exiting the sampling impinger was directed into another impinger containing a 5% chlorine solution. Owing to technical constraints at the experimental stage, it was not possible to directly measure the irradiance ( $\text{mW}/\text{cm}^2$ ) of the UV lamps deployed in this study. Consequently, the precise cumulative UV dose ( $\text{mJ}/\text{cm}^2$ ) imparted to

the samples was not included in the study. To overcome this limitation, the results are scenario-specific, that is, exposure times and lamps used in the experimental conditions are described.

#### *Coronavirus-2 removal from surfaces*

To investigate the effectiveness of different wavelengths of UV radiation in Coronavirus-2 removal from various surfaces, experiments were conducted on iron, glass, plastic, and paper. Initially, these surfaces were intentionally contaminated with a known quantity of virus. Subsequently, the contaminated surfaces were exposed to UVA, UVB, and UVC light sources, with the UV lamp positioned 1 cm from the target surface.

Following UV light exposure, sampling was conducted at intervals of 30, 60, and 120 s. Moreover, sampling was carried out using a sterile swab, and the collected samples were transferred to physiological serum. Subsequently, the samples were transported to the laboratory for virus extraction and detection.

It is important to emphasize that throughout the entirety of the experiment, stringent measures were taken to prevent virus contamination, including the use of specialized protective gear such as clothing designed to protect against the virus, masks, gloves, and protective eye goggles.

### **Analysis of samples**

#### **Preparation and processing of samples**

Upon reaching the laboratory, all samples underwent a concentration step before Coronavirus-2 extraction and detection. In summary, 200  $\mu$ L of each sample taken from the test aimed at assessing the removal of the Coronavirus-2 from the virus-infected air stream was subjected to concentration process. The aluminum hydroxide adsorption-precipitation method was utilized, which is outlined in the reference criteria to amplify the concentration of Coronavirus-2 within the samples<sup>1,10,11</sup>. Following this concentration step, water samples were preserved at -80 °C until they were ready for RNA extraction.

For the samples collected during the virus removal tests from different surfaces (swab inside physiological serum), the following procedure was employed: initially, these samples were vortexed for 5 min to ensure that the possible viruses were transferred into the liquid phase. Subsequently, 200  $\mu$ L of each prepared sample was extracted, and the concentration and storage processes were carried out following the method outlined above<sup>12</sup>.

#### **Coronavirus-2 extraction**

Coronavirus-2 RNA extraction and one-step reverse transcription quantitative polymerase chain reaction (RT-qPCR) were performed at the Maragheh Cellular-Molecular Diagnostics Laboratory, a specialized facility dedicated to diagnosing Coronavirus-2. The concentrated samples were subjected to RNA extraction using the RNJia Virus Kit (ROJETechnologies, Yazd, Iran), following the manufacturer's protocol<sup>13</sup>.

As a precaution against potential cross-contamination during the viral RNA extraction process, a negative control was included in the procedure. The negative control was prepared using nuclease-free deionized water.

#### **Coronavirus-2 detection**

The isolated RNA underwent RT-qPCR analysis to detect the presence of Coronavirus-2. The authors used the COVID-19 ONE-STEP RT-PCR kit (Pishtaz Teb Diagnostics, Tehran, Iran)

following the manufacturer's instructions. This kit was designed to target two different regions of the Coronavirus-2 genome, specifically the RdRp and N genes, using a dual-target gene method. To improve sensitivity and prevent false-negative results, the kit contained a solution with a probe and an internal control primer (RNase P). Additionally, it included positive and negative controls for PCR, with the negative control using diethylpyrocarbonate-treated water. In each PCR run, 10  $\mu$ L of the sample was combined with 10  $\mu$ L of the master mix and primer-probe mixture. The thermal cycling conditions for the RT-qPCR assay were as follows: reverse transcription at 50 °C for 20 min, initial denaturation of cDNA at 95 °C for 3 min, followed by 45 cycles of denaturation at 94 °C for 10 s, primer annealing and extension reaction at 55 °C for 40 s, and finally cooling at 25 °C for 10 s.

#### **Quality control**

In this study, additional Coronavirus-2 detection kits were employed to enhance precision and double-check the samples. These kits included the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit from Sansure Biotech in China, which targets the ORF-1ab and N genes, and the Novel Coronavirus (2019-nCoV) Real-Time Multiplex RT-PCR Kit from Liferiver Bio-Tech in the US, which targets the ORF1ab, E, and N genes. Each sample was tested three times. The interpretation of the RT-qPCR results was as follows: A positive result for the gene target (RdRp, N), was considered when the Ct value was equal to or lower than 40. If at least two of the three replicates showed a positive result, the corresponding sample was classified as positive for Coronavirus-2. To quantify Coronavirus-2, standard curves were generated using 10-fold dilutions of the Coronavirus-2 positive control from the reference kit, following the manufacturer's instructions. It's worth noting that RNA extraction and RT-qPCR preparation were conducted in separate laboratories to prevent any potential cross-contamination.

In the experimental setup, baseline (non-

irradiated) samples were included as internal references to validate the effectiveness of each UV-exposure scenario.

**Results**

**The Effect of UV radiation wavelength**

Ultraviolet radiation, encompassing UVA, UVB, and UVC wavelengths, is instrumental in the inactivation of viruses in various environmental matrices. Among these, UVC radiation, which spans wavelengths between 200 and 280 nanometers, is particularly effective in disrupting the DNA and RNA of viruses, thereby rendering them non-infectious. This high efficacy is attributed to UVC’s shorter wavelength and higher energy of UVC, which can penetrate and damage the nucleic acids of microorganisms <sup>14</sup>.

UVB radiation, with wavelengths ranging from 280 to 315 nanometers, also contributes to viral inactivation, although it is less effective than UVC. UVB's lower energy of UVB means that it requires longer exposure times or higher intensities to achieve the same level of disinfection. Nevertheless, it plays a significant role in reducing viral loads in various applications <sup>15</sup>.

UVA radiation, which spans 315–400 nm, has

the least impact on viruses because of its longer wavelength and significantly lower energy. As a result, UVA is often less effective at inactivating viruses and requires much longer exposure times to achieve disinfection compared to UVB and UVC <sup>14</sup>.

The efficiency of UV radiation for disinfection is influenced by several factors, including exposure time, intensity of UV light, and specific type of virus being targeted. These parameters are crucial for determining the overall effectiveness of UV radiation in various disinfection applications <sup>16</sup>.

In the experiments aimed at assessing the removal of the COVID-19 virus from contaminated air streams, specifically the cycle threshold (Ct) values were measured for different UV types (Table 1). The Ct value is an important metric in virology, indicating the point at which the genetic material of the virus is detectable. For UV type A, the Ct values for the RdRp and N molecular targets were both 31, suggesting a moderate viral load in the sample. UV type B yielded Ct values of 26 for RdRp and 27 for N, indicating a higher viral load than that of type A.

**Table 1:** Mean amplification cycles of SARS-CoV-2 RNA exposed to various UV wavelengths

UV- type	Molecular target	Ct value
A	RdRp	31
	N	31
B	RdRp	26
	N	27
C	RdRp	N.D
	N	N.D

Ct scale:	20	21	22	23	24	25	26	27	28	29	30
	31	32	33	34	35	36	37	38	39	40	

Time = 5 min, air flow rate= 1 L/ min

**SARS-CoV-2 removal from contaminated air stream**

The airflow rate plays a significant role in the efficacy of UV light in reducing viral loads. As the air flow rate increases, the residence time of the virus particles in the UV exposure zone decreases, which can impact the effectiveness of viral inactivation <sup>17</sup>. Table 2 provides data for airflow

rates ranging from 2 to 6 L/min. As shown in the table, Both RdRp and N molecular targets showed similar Ct values of 22-25 for UVA and UVB at an air flow rate of 2 L/min, indicating moderate viral loads with little difference between UVA and UVB efficacy. In contrast, UVC radiation completely destroyed the viral RNA at the lowest investigated airflow rate.

**Table 2:** Mean amplification cycles of SARS-CoV-2 RNA exposed to UV lights at various air flow rate

Air flow rate	Molecular target	UV A	UV B	UV C
2	RdRp	22	22	N.D
	N	24	25	N.D
3	RdRp	26	26	37
	N	25	25	N.D
4	RdRp	27	27	27
	N	26	26	26
5	RdRp	25	25	28
	N	23	23	27
6	RdRp	30	30	25
	N	30	30	24

Ct scale:	20	21	22	23	24	25	26	27	28	29	30
	31	32	33	34	35	36	37	38	39	40	

Time= 5 min

### **SARS-CoV-2 removal from various contaminated surfaces**

Table 3 shows the mean amplification cycles (Ct values) for SARS-CoV-2 RNA on different surfaces (paper, plastic, cloth, iron, and glass) after exposure to UVC light for varying durations (30 s, 60 s, and 120 s). The table differentiates between

two molecular targets, RdRp and N, which are specific to the SARS-CoV-2 genome. The Ct values provided indicate the number of cycles needed for SARS-CoV-2 RNA to be detected by PCR, with higher Ct values suggesting lower amounts of viral RNA present <sup>1</sup>.

**Table 3:** Mean amplification cycles of SARS-CoV-2 RNA on various surfaces exposed to UVC

Surfaces	Molecular target	UV C		
		T = 30 s	T = 60 s	T = 120 s
Paper	RdRp	36	N.D	N.D
	N	33	N.D	N.D
Plastic	RdRp	33	N.D	N.D
	N	32	N.D	N.D
Cloth	RdRp	33	33	33
	N	31	32	32
Iron	RdRp	32	35	N.D
	N	31	36	N.D
Glass	RdRp	N.D	N.D	N.D
	N	N.D	N.D	N.D

Ct scale:	20	21	22	23	24	25	26	27	28	29	30
	31	32	33	34	35	36	37	38	39	40	

## Discussion

### **Radiation wavelength**

The experimental results verified that UVC radiation is far superior to UVA and UVB in inactivating the detectable RNA of SARS-CoV-2 under the tested conditions. Its better performance is due to the shorter wavelength (200–280 nm) and higher photon energy of UVC, which are more

effective at causing photochemical damage to viral nucleic acids, especially the generation of pyrimidine dimers that interfere with RNA replication<sup>18</sup>. The experimental conditions involved a 5-minute exposure time and an airflow rate of 1 L/min, under which UVC radiation achieved complete viral destruction.

These results underscore the importance of

selecting an appropriate UV wavelength and optimizing the conditions for effective disinfection. Although UVA and UVB can contribute to viral inactivation, UVC is markedly more efficient and effective under the tested conditions. UVA and UVB also contribute to viral RNA degradation, and their lower energy levels result in a markedly reduced disinfection effect, which is consistent with their limited ability to break molecular bonds in RNA. These observations align with prior research demonstrating UVC's unique ability of UVC to cause direct photolytic inactivation, whereas UVA and UVB act more slowly or through indirect mechanisms (e.g., generation of reactive oxygen species). This study highlights that the specific characteristics of UV radiation, including wavelength, exposure time, and intensity, are critical determinants of its disinfection efficacy.

In conclusion, the effectiveness of UV radiation in viral inactivation varies significantly across the UVA, UVB, and UVC wavelengths. UVC radiation, with its shorter wavelength and higher energy, is the most potent in disrupting viral DNA and RNA, making it highly effective for disinfection. UVB also contributes to viral inactivation, albeit less effectively, whereas UVA's impact of UVA is minimal because of its lower energy. Understanding these differences is essential for optimizing UV-based disinfection strategies, particularly in mitigating airborne viral transmission, such as that of COVID-19.

#### *Air stream disinfection*

As shown in Table 2, increasing the air flow rate to 3 L/min resulted in almost similar results for UVA and UVB radiation. The Ct values remained in the range of 25-26 for RdRp and N genes, showing modest viral inactivation. Under UVC radiation, a significant increase in the Ct value to 37 for RdRp indicated substantial viral inactivation. This high Ct value reflects a low viral load, demonstrating UVC's superior efficacy of UVC at this flow rate. Going forward to the air flow rate of 4 L/min in the UVA process shows that Ct values for RdRp and N are 27 and 26,

respectively, showing a slight decrease in viral load compared to lower flow rates. The results were similar to those of the UVB process, with Ct values of 27 and 26. However, in the UVC, Ct values were also 27 for both RdRp and N, showing significant viral inactivation comparable to that of UVA and UVB at this flow rate. At the higher air flow rate of 5 L/min in the UVA and UVB processes, the Ct values for RdRp and N targets remain around 26-28, indicating that UVA and UVB are slightly less effective as the air flow rate increases. The results of the UVC process showed a distinct advantage with Ct values of 25 for RdRp and 27 for N, indicating better viral reduction compared to UVA and UVB. However, a slight reduction in the removal efficiency was observed at higher air flow rates. To better clarify the reduction in removal efficiency, an air flow rate of 6 L/min was selected for the investigated processes. The Ct values for RdRp and N were both 30 (for both UVA and UVB radiations), indicating the highest viral load among all the tested airflow rates, which suggested the limited effectiveness of UVA at this rate. UVC process also exhibited superior performance, with Ct values of 25 for RdRp and 24 for N, demonstrating significant viral inactivation even at the highest air flow rate compared to that of UVA and UVB.

According to the obtained results across all airflow rates, UVC consistently demonstrated superior viral inactivation, particularly at 3 L/min, where it showed the highest Ct value (lowest viral load). As the airflow rate increased, the effectiveness of UVA and UVB decreased, as evidenced by lower Ct values (higher viral loads). This indicates that higher flow rates reduce the residence time of the virus in the UV exposure zone, thereby diminishing its inactivation efficacy.

This analysis underscores the importance of selecting appropriate UV wavelengths and optimizing operational conditions to achieve effective disinfection in air-treatment systems. This highlights an important consideration for practical UV air disinfection systems: a balance must be struck between airflow efficiency and disinfection efficacy. Optimizing this trade-off is crucial for

designing effective systems in real-world environments, particularly in ventilation and recirculation units. Finally, UVC proved to be highly effective even at higher airflow rates, making it the preferred choice for air disinfection systems targeting SARS-CoV-2.

### Surface disinfection

The results showed that UVC exposure impacts the detectability of SARS-CoV-2 RNA on different surfaces, with the effect varying based on surface type and duration of exposure. For instance, cloth and iron exhibited a noticeable increase in Ct values over time, suggesting that longer UVC exposure reduces viral RNA more effectively on these surfaces. Paper and glass showed high initial Ct values, indicating that UVC light rapidly reduced detectable SARS-CoV-2 RNA on these materials. Other studies have also examined the effectiveness of UVC light in deactivating SARS-CoV-2 on various surfaces. For example, a study by Heilingloh and Aufderhorst<sup>19</sup> found that UVC irradiation effectively inactivated SARS-CoV-2 on different materials, with complete destruction achieved within 9 min for most surfaces. Similarly, Inagaki, Saito<sup>20</sup> demonstrated that UVC light could significantly reduce SARS-CoV-2 infectivity on surfaces such as plastic and stainless steel within a few seconds to minutes. These findings align with the current data in Table 3, where increased exposure times correspond to higher Ct values, indicating reduced viral RNA. However, there were differences in the efficiency and time required for inactivation, which could be due to variations in UVC intensity, environmental conditions, the initial viral load, or the specific type of UVC device used.

UVC light inactivates viruses by damaging their nucleic acid. The energy from UVC photons causes the formation of pyrimidine dimers in the viral RNA, leading to replication errors or complete inhibition of replication. This mechanism is consistent across different studies, where UVC exposure results in the degradation of viral RNA, as reflected by increased Ct values in PCR tests<sup>17,21</sup>.

The application of UVC light for practical disinfection in healthcare and public environments can be guided by these findings. Surfaces such as cloth and metal might require extended exposure durations for successful viral inactivation, whereas others, such as paper and glass, could be disinfected at faster rates. This difference highlights the importance of specific disinfection strategies based on the surface type and application scenario of UVC.

It is of particular interest to know the effect of UVC light on the reduction of SARS-CoV-2 RNA with respect to various surfaces. Based on these results, UVC light had a significant effect on viral detectability, and variation occurred as a function of surface type and exposure time.

Correlating such results with similar studies reinforces the understanding of the antiviral properties of UVC and highlights the need for optimized disinfection processes to ensure efficient viral inactivation in different settings.

### Conclusion

This study investigated the efficacy of UV radiation, specifically UVA, UVB, and UVC, in inactivating SARS-CoV-2 in various contexts, including contaminated air streams and surfaces. The results conclusively demonstrated that UVC radiation, with its shorter wavelength and higher energy, is the most potent in disrupting viral RNA, making it highly effective for disinfection. Following are the key findings of the study

- UVC radiation achieved complete destruction of SARS-CoV-2 RNA in contaminated air streams under specific conditions, surpassing the efficacy of both UVA and UVB. The highest Ct values, indicating the lowest viral loads, were observed after UVC exposure, even at varying airflow rates.
- On surfaces, UVC exposure led to significant increases in Ct values over time, particularly on cloth and iron, demonstrating its superior ability to reduce viral RNA.
- The efficacy of UVC radiation in reducing viral loads was evident across different airflow rates, with the best performance observed at a flow rate

of 3 L/min.

- Higher airflow rates reduced the residence time of viruses in the UV exposure zone, diminishing the inactivation efficiency of UVA and UVB, but UVC remained highly effective in inactivating viruses.
- UVC light was effective in reducing SARS-CoV-2 RNA on various surfaces, with rapid reductions observed on paper and glass, and longer exposure times required for cloth and metal.

These findings highlight the critical importance of selecting appropriate UV wavelengths and optimizing exposure conditions for effective disinfection. UVC radiation, owing to its high energy and short wavelength, is particularly suitable for rapid and thorough viral inactivation in both air and surface disinfection applications. Understanding the nuances of UV radiation efficacy across different environmental matrices is essential for developing effective disinfection protocols, especially in settings such as healthcare facilities and public spaces, where mitigating airborne transmission of viruses such as SARS-CoV-2 is crucial.

In summary, UVC radiation is the most effective UV wavelength for inactivating SARS-CoV-2, offering significant advantages for air and surface disinfection. Future disinfection strategies should leverage the high efficacy of UVC, ensuring optimized exposure times and conditions to maximize viral inactivation and enhance public health.

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### Conflict of Interest

The authors declare no conflicts of interest.

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### Ethical Considerations

The study protocol was reviewed and approved by the Ethics Committee of the Maragheh University of Medical Sciences.

### Code of Ethics

The ethic code of current study is IR.MARAGHEHPHC.REC.1399.010

### Authors' contributions

Ali Behnami did the investigation, validation, data curation, visualization conducted experiments, and wrote the original draft.

Ehsan Aghayani was involved in methodology, supervision, and writing - reviewing and editing

Ali Abdolahnejad was involved in methodology, supervision, and writing - reviewing and editing

Saber Raeghi conducted the experiments.

Mojtaba Pourakbar engaged in investigation, conducting experiments, validation, data curation, visualization, and writing—original draft preparation.

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