IEEE Access
Multidisciplinary | Rapid Review | Open Access Journal

Date of publication xxxx 00, 0000, date of current version xxxx 00, 0000.

Digital Object Identifier 10.1109/ACCESS.2017.Doi Number

Comparative Study of Blue Light with Ultraviolet (UVC) Radiations on SARS-CoV-2 Virus

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ABSTRACT The ongoing coronavirus pandemic requires more effective disinfection methods. The disinfection using ultraviolet light (UV), especially longer UVCs such as 254 nm, 270/280 nm have been proved to have virucidal properties, but its adverse effects on human skin and eyes limit its use to enclosed unoccupied spaces. Several studies conducted the in past have shown the effectiveness of blue light (405nm) against bacteria and fungi, but the virucidal property of 405nm has largely been unexplored. Based on previous studies, visible light mediates inactivation by absorbing the porphyrins and reacting with oxygen to produce reactive oxygen species (ROS). This causes oxidative damage to biomolecules such as protein, lipids, and nucleic acids, essential constituents of any virus. The virucidal potential of visible light has been speculated because the virus lacks porphyrins. This study demonstrated porphyrin independent viral inactivation and a comparative analysis of the effectiveness of 405nm against other UVC wavelengths. The beta coronavirus 1 (strain OC43) was treated against 405nm, 270/280nm, 254nm, and 222nm, and its efficacy was determined using median tissue culture infectious dose, i.e., TCID50. The results support the disinfection potential of visible light technology by providing a quantitative effect that can serve as a basic groundwork for future visible light inactivation technologies. In the future, blue light technology usage can be widened to hospitals, public places, aircraft cabins, and/or infectious laboratories to inactivate SARS-CoV-2.

INDEX TERMS Visible light, 405nm, Virucidal, Inactivation, SARS-CoV-2, Irradiation, Far-UVC, 222nm

I. INTRODUCTION

The severe acute coronavirus 2 (SARS-CoV-2) pandemic has been an ongoing concern since its major outbreak in 2019, affecting the health and lives of millions of people across the world. While several unprecedented measures have been taken to control its spread such as vaccinating people against the virus and strict sanitization measures have been quite effective in combatting the ill effects of the virus. As of February 2022, more than 10 billion doses of vaccines have been administered across the globe [1]. Rigorous sanitization, which uses 60 to 95% ethyl alcohol, has well-established benefits and has thus been adopted by most households to clean surfaces and hands. However, despite strict sanitization, the virus is still spreading and affecting millions of people globally, which points out the need for more effective disinfection technologies.

Ultraviolet (UV) is an invisible part of the electromagnetic spectrum which ranges from 100 to 400 nm. The UV range is mainly categorized into three sections based on wavelength. The range from 100 to 280nm comes under - UVC; the range from 280nm to 320nm as UVB; and 320 to 400nm as UVA [2]. The lower range of UVC wavelengths between 200 to 222 nm is termed as Far-UVC. UVC is absorbed by the ozone layer but due to its antimicrobial properties it has been artificially created and used for disinfection purposes for the past few centuries. The most used UVC wavelength has been 254nm and it has well-established efficacy against bacteria, fungi, and viruses. Other UVC wavelengths such as 260-280nm have also shown the potential to inactivate the virus.

II. BACKGROUND

A study [3] conducted in past used 260nm, a combination of 260/280nm and 280nm to treat viruses. The required fluences (UV doses) were approximately 8 mJ/cm^2 for coxsackievirus A10 and poliovirus 1, 10 mJ/cm^2 for enterovirus 70, and 13 mJ/cm^2 for echovirus 30 [3]. Amongst these three, 260nm was more effective for enteroviruses and 280nm showed relatively higher efficacy for human

adenovirus (a DNA virus) [3]. Though UVCs have proven inactivation potential, they can cause severe burns on the skin, eye injuries like photokeratitis on human eyes, skin [4]. Additionally, the effect of UVC radiations on materials raises concerns about compromising the structural integrity or the premature aging of the products [5].

In the last decade, far-UVC has gained huge popularity for its higher irradiation power, resulting in low dose requirements for inactivation of bacteria, fungi, and viruses and also its lower penetration in human live tissue compared to 254nm, making it safer for human exposure [6]. Former studies on the antimicrobial property of far-UVC have demonstrated its capacity to inactivate the influenzas and coronaviruses in the air at a dose that does not damage the human cells [7] [8]. Prior research has a dearth of data on the long-term effects of far-UVC and its exposure to human eyes.

The way UVC light attacks pathogens are different for different wavelengths. The structure of SARS-CoV-2 contains structural proteins i.e., spikes(S), envelope(E), membrane(m), and nucleocapsid(N) proteins [9]. The SARS-CoV-2 uses its spikes protein to attach to the host cell. Once it's inside the host cell, it multiplies and kills the cells [10]. The two ways UVC attacks the virus are either by viral genome damage (DNA/RNA) or viral protein damage. For viral genome damage, the UVC range from 200 to 280nm is absorbed by the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) through the formation of pyrimidine dimers [11]. The 254nm causes genome damage which prevents the virus from replicating. However, the DNA can repair itself in the presence of blue light through the process called photo-reactivation [12]. The 222nm, however, can cause both genome damage and protein damage because 222nm is absorbed more by structural proteins in contrast to 254nm [13]. Among shorter wavelengths (200 to 230nm) range, 222nm has been more effective than 210nm and 230nm [13].

The visible blue light ranges from 400-470nm, and in the current study, we have used 405nm for the inactivation of the SARS-CoV-2 virus. Unlike UVC, visible light is considered safe for human skin and eyes [14]. Furthermore, a study [15] conducted showed the comparative analysis of the degrative effects of UVC and 405nm on endoscope tube material. The UVC caused substantial photodegradation damage whereas 405 had no notable detrimental effect [15]. Lower irradiation blue light dose had shown inactivation for bacteria like Clostridium spp and Listeria spp [16] [17], fungal species such as Saccharomyces spp and Candida spp [18], and a higher dose on viruses like feline calcivirus (FCV)30 [19], and viral hemorrhagic septicemia virus (VHSV) [20]. The mechanism of inactivation by blue light is by absorbing the light via photosensitizers such as porphyrins and reacting with oxygen or any other cell components [21] [22]. This produces a reactive oxygen species (ROS) which causes oxidative damage [21] [22]. The non-selective nature of ROS can cause direct damage to biomolecules such as protein, lipids, and nucleic acids which are essential constituents of bacteria, fungi, and viruses [21] [22]. As the virus lacks porphyrins, the virucidal property of blue light is highly speculated. Most of the research conducted used porphyrins to inactivate the virus in the media. In this study, the media had no porphyrins, and the test was conducted without the use of external photosensitizers. A recent study also showed the inactivation of SARS-CoV-2 and influenza A H1N1 without the use of photosensitizer [23]. Nevertheless, mechanisms by which blue light causes nonselective damage to the virus is still unknown. A future investigation is required to understand the mechanism through which blue light causes inactivation.

The dose requirement varies with wavelengths and pathogens. The wavelengths below 240nm can more readily generate ozone [24] thus should be a deciding factor for dose determination. The dose of 222nm used was 3mJ/cm^2 for this study. Whereas for 254nm and 270/280nm, a slightly higher dose of 10mJ/cm^2 was used. For 405nm measured a higher dose of 17280mJ/cm^2 was used.

The goal of this study is to investigate blue light (405nm) inactivation efficacy against the virus and compare it with far-UVC (222nm), standard mercury bulbs(254nm), and LED UVC (270/280nm). In what follows, a comparative study for the blue light with the three UVC lights and described their efficacy of Covid-19 virus disinfection.

III. METHODOLOGY

A. LIGHT SOURCES

The 405nm LED (Fig. 1) light manufacturer used in this study was DIEHL Aviation [25]. The LED prototype uses the same mechanical component as the regular mini spotlight. The light has the peak wavelength at 405nm with measured spectral power distribution. The LED light has a single mode with constant power wattage.

The 222nm light used was from manufacturer Ushio Care222, krypton-chloride (Kr-Cl) excimer lamp module. The typical excimer lamp emits peak irradiation at 222nm alongside a longer UVC wavelength whereas, Ushio uses a special short-pass filter to block other UVC wavelengths that are above 230nm [26] (Fig. 2).

The Ushio germicidal low-pressure mercury arc lamp emits peak radiation of 253.7nm (Fig. 3).

The 270/280nm was from manufacturer GMKJ. Each led module shown in Fig. 4A was connected in a three-corner arrangement. Each module has the same peak wavelength and same irradiance power. The specifications of UVC led used are shown in Table I. DC voltage from the main power supply is applied to each UV-led module in accordance with the available current.

Figure 1. DIEHL 405nm Light

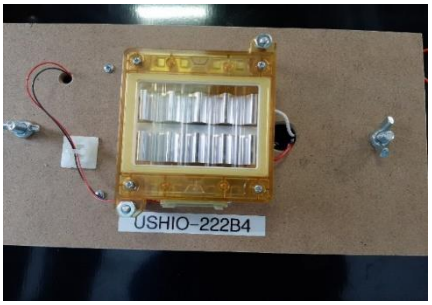


Figure 2. Ushio Excimer Lamp

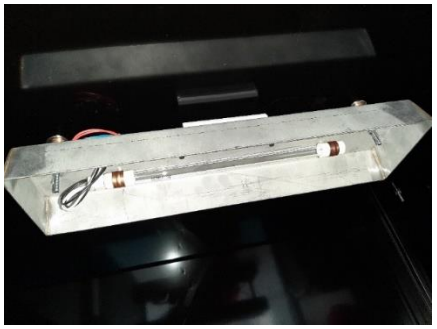


Figure 3. Low Pressure Mercury Bulb

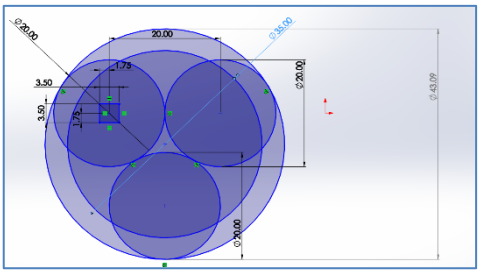


Figure 4. a) Three corner arrangement for UVC led

Figure 4. b) Final UVC PCB Board with a toggle switch

Table I
270/280nm LED Specifications

Unit Name	Model Name	Wavelength	Voltage (DC)	Current (mA)	Radiant power (mW)	Beam angle
Star 1.0W Board	GG-3535UV610	270/280nm	5-8	100-150	6-10	120°

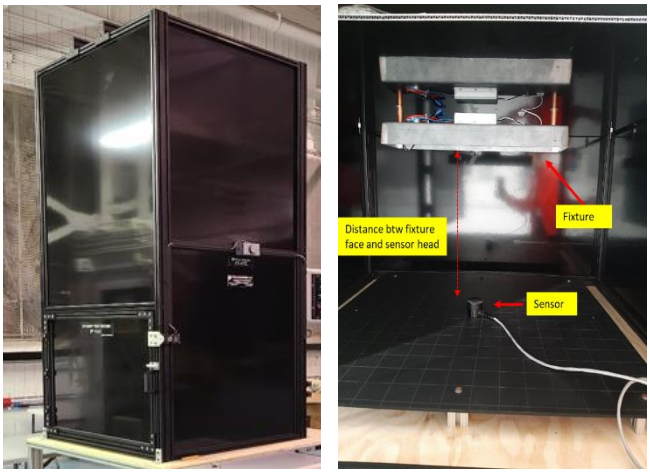
B. EXPERIMENTAL SETUP

For testing, a vertical test rig was designed (Fig. 5a). The rig was shaped like a vertical tower with a platform to keep virus samples in a petri dish and a fixture to install the light. The rig was designed in a way that the distance between the light source and the viral sample could be adjusted. An ILT2500-UVGI-X UVC Flash Meter [27] was used to measure the irradiance in mW/cm^2. It had the sensor with the peak calibration at 280nm and a range of 200nm ~ 450nm. This was used for measuring irradiance of mercury bulb (254nm) and UVC LED (270/280nm) light source. As the sensor had a peak at 280nm, to make the irradiance accurate, the correction calibration factor was used which was provided by the manufacturer. The built-in sensor was replaced by SED240/FUVC/W and XSD140A sensor to measure the irradiance of Ushio Care222 (222nm) and DIEHL blue LED (405nm) respectively.

For full examination, the irradiance was measured at varying distances from 10cm to 50cm. The distance between the fixture face and the head of the sensor was changed by raising the fixture platform manually. For the viral testing, the distance between the viral sample and the face of the fixture was fixed at 10cm. Based on the measured irradiance (mW/cm^2) and required dose(mJ/cm^2) which is described previously, the exposure time was calculated using equation (1).

$$\text{Dose}(mJ/cm^2) = \text{Irradiance}(mW/cm^2) \times \text{Exposure time}(\text{seconds}) \quad \textbf{(1)}$$

The viral sample in the petri dish was exposed to the irradiance of 1.80E-03 W/cm^2, 9.18E-04 W/cm^2, 3.50E-03 W/cm^2, and 3.47E-05 W/cm^2 from the light sources 405nm, 222nm, 254nm, and 270/280nm respectively.



a b

Figure 2. a) Test Rig b) Inside test rig showing the fixture to install the light source and the sensor to measure the irradiance (mW/cm^2)

C. CELLS AND VIRUS PREPARATION

The HCT8 cells (CCL244) purchased from ATCC were cultured according to the manufacturer's protocol. The cells were grown in a complete medium prepared by supplementing RPMI 1640 medium with 10% horse serum (Gibco; 26050070) and 1% penicillin-streptomycin (Gibco; 15 140122). The cells culture flask was placed in the incubator maintained at 37°C with 5% CO2. Beta coronavirus 1 strain OC43 (VR-1558) purchased from ATCC is propagated using host cell HCT8. All the experiments involving OC43 were conducted within the biosafety level 2 safety cabinet at the Toronto St. Michaels Hospital facility. Viral stocks were prepared by infecting the confluent monolayers of HCT8 cells with OC43. Initial viral absorption is allowed for 1-2 hours with continuous rocking, and then the cells were supplied with an infection medium consisting of RPMI1640 supplemented with 2% horse serum. The virus-infected cells were incubated at 37°C with 5% CO2 and monitored for 4-6 days or until achieving an 80% cytopathic effect. The produced viral stocks were stored at -80°C for further use.

D. TCID50

The commonly used Polymerase Chain Reaction (PCR) test does not apply to our test as it measures virus infection. Instead, TCID50 assay was used to measure the infectivity of the virus, i.e., virus inactivation. TCID50 represents a dilution of virus that makes 50% of the test wells showing cell detachment. The infectivity titer is expressed as TCID50/ml. To perform TCID50, host cells (HCT-8) (1.5 x 104 cells in 100 µl) in complete medium were plated in a 96-well plate (Cryo Vial Holder) (Fig. 6a). Incubate the plates for 16-24 h at 37°C in a 5% CO2 incubator to achieve 80% confluency of the cells. To prepare the virus for infection, dilute 100 µl of virus from stock to 900 ul of serum-free media (10-1 dilution) and make subsequent 10-fold serial dilutions of the virus (10-2 to 10-10 dilution). On day 1, remove the culture medium from each well. Inoculate 100 µl of the virus solution to each well. Aliquots of the same sample are inoculated in multiples of 4 wells. Incubate the plates for 1 h at 37 °C in a 5% CO2 incubator. After incubation, remove the inoculum and add an overlay infection medium containing 2% horse serum. Incubate the plates at 37°C in a 5% CO^2 incubator and observe the cytopathic effect (CPE) for 6-12 days. Using an inverted microscope (Fig. 6 b), count the number of wells with or without CPE. TCID50/ml is then calculated using the Reed-Muench method [28].

Figure 3. a) TCID50 (with virus samples)

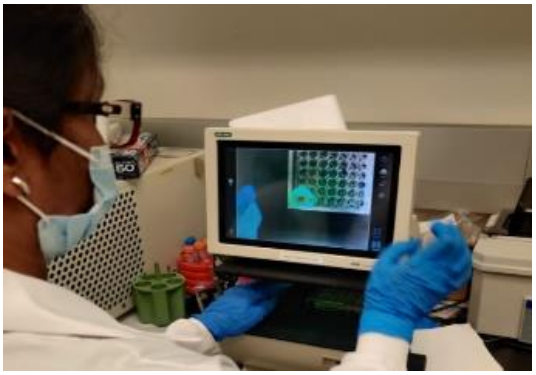


Figure 6. b) Cryo Vial Holder with virus sample under a microscope

IV. RESULT

To visually investigate the efficiency of ultraviolet rays, a randomly chosen 270/280nm source was used to treat the virus sample with a dose of 10mJ/cm^2, and basic infectivity results are shown in Fig. 7. Fig. 7a shows the control sample (no UV more conclusive and quantitative results, TCID50 was conducted.

The irradiance(mW/cm^2) for each light source was measured at 10cm distance which is shown in Table II below and based on measured irradiance and required dose, the exposure time was calculated using equation (1). The inactivation efficiency based on dose and exposure time was calculated using the TCID50. For 405nm the virus was exposed to 1720mJ/cm^2 for

2hours 40 minutes and a reduction of log10 2.84 was observed i.e., is 99.85%. For 222nm the virus sample was exposed to 3mJ/cm^2 for 3.27seconds which resulted in log10 2.50 reduction i.e., 99.68%. For 254nm and 270/280nm ultraviolet rays, the virus samples were exposed to 10mJ/cm^2 for 3.26 seconds and 288.18seconds respectively, which resulted in log10 3.17, i.e., is 99.93% and log10 3.37 i.e., is 99.95% reduction. After each test, the test rig was cleaned with ethyl alcohol, and the treated samples were stored in the dark at 4°C. 405nm required the highest dose and maximum exposure time to reach 2.84 log reduction. The summarized results are shown in Table III.

Table II
Shows irradiance(W/cm2) for different UVCs and blue light at different distances

Distance btw the virus sample and fixture(cm)	Irradiance(W/cm^2)			
	405nm	222nm	254nm	270/280nm
10	1.80E-03	9.18E-04	3.50E-03	3.47E-05
20	4.46E-04	3.17E-04	1.03E-03	7.33E-06
30	1.98E-04	1.32E-04	5.29E-04	2.83E-06
40	1.15E-04	8.09E-05	3.19E-04	1.43E-06
50	7.36E-05	5.08E-05	2.12E-04	8.51E-07

Table III
Showing TCID50 result for treated virus sample, the dose used, and exposure time for different UVCs and blue light

Light Source	Distance (cm)	Irradiance (W/cm^2)	Exposure Time (s)	Dose (mJ/cm^2)	TCID50	
					Log Reduction	%
405	10	1.80E-03	9600	17280	2.84	99.85 %
222	10	9.18E-04	3.27	3	2.50	99.68 %
254	10	3.50E-03	3.26	10	3.17	99.93 %
270/280	10	3.47E-05	288.18	10	3.34	99.95 %

V. DISCUSSION

The ongoing pandemic is in dire need of uninterrupted inactivation technology. Visible blue light inactivation has indicated the log reduction of 2.84 (that is a 99.85% reduction), which is a significant reduction. The irradiation used in this study was 1.8mW/cm^2 and the exposure time was 2 hours and 40 minutes.

The mechanism by which visible blue light attacks the virus is still unknown and in past studies, external photosensitizers were used in the medium. In this research, 405nm (Fig. 8 shows the visible blue light in our setup) caused inactivation to virus without the presence of any external photosensitizers. Before using visible light for continuous inactivation, its photobiological hazards to humans should be studied. The IEC 62471 [14] standards have taken into consideration the skin, cornea, and retinal hazard. These hazards depend on wavelengths, physiological sensitivity to various wavelengths, angular subtense, and exposure time.

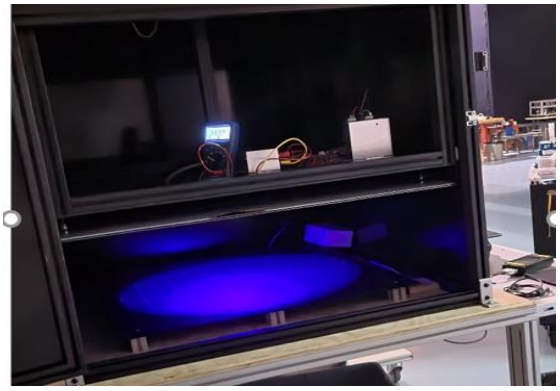


Figure 8. Demo of DIEHL 405nm Light

The brief mention of eyes anatomy is pertinent for understanding the angular subtense. The basic parts of the eyes are the retina, cornea, pupil, lens, and conjunctiva. The pupil is the small opening that allows the light into the eye and behind the pupil is the lens that focuses the light energy density on the retina. The superficial part of the eye is the cornea and conjunctiva. The amount of light that enters the eye depends on how big the pupil is. The pupil's average maximum constriction and dilation are about the diameter of 3mm and 7mm, respectively. This diameter is a limiting factor to correspond to the field of view (FOV). It is assumed that the pupil remains constricted to a diameter of 3mm for visible light, whereas, for wavelengths outside the visible range, the pupil is dilated. Exposure time also determines the field of view. For disinfection purposes, the exposure time is more than 10000seconds. The exposed area of the retina will be around a FOV angle of 0.1 radians which is over 5 degrees.

By IEC 62471 [14], the skin or cornea hazard by blue light is denoted by E_B. The equation provided by the IEC standard for E_B is for small sources, i.e., the aperture less than 0.011 [14]. The skin and cornea hazard (E_B) ranges from low risk at 1.0 W.m^{-2} and moderate risk at 400 W.m^{-2}. LB denotes retinal hazard, and the value is based on radiance (W.m^{-2}.sr^1). The LB limit ranges from low risk at 10000 W.m^{-2}.sr^{1}and moderate risk at 4000000 W.m^{-2}.sr^1.

The human exposed to a light source with radiance lower than 100 W.m^{-2}.sr^{1}will receive a dose of greater than 10^6 J.m^{-2}.sr^1 in 10000seconds exposure time (2hours and 45 minutes), and this is classified as no risk [29]. If exposed to a light source of radiance higher than 100W.m^{-2}.sr^1 but less than W.m^{-2}.sr^1, the person will receive the dose greater than 10^6 J.m^{-2}.sr^1 within 100seconds exposure time; and this is classified as low risk [29]. The light source whose radiance is higher than 10,000 W.m^{-2}.sr^1 is classified as medium to high risk, and that light source is prohibited from domestic general lighting [29]. There are many commercial lights like vital vio [30] that have been tested for IEC 62471 [14] and have been placed in Exempt Group (RG O) i.e. it poses no optical hazard for continuous, unrestricted exposure to humans. The skin and cornea hazard is determined in irradiance, whereas retinal hazard is considered radiance. Assuming that only domestic general visible blue light is used for disinfection which means

retinal hazard is already considered and only skin and cornea hazard must be calculated for uninterrupted disinfection purposes.

The inverse square law can be used to draw irradiance and the distance dependence assuming the constant angular position (θ= 0°). From Fig. 9, the curve equation can be used to calculate the intermittent irradiance value at different distances for the same blue light source used in this study, which can be further used to calculate the dose (mJ/cm^2).

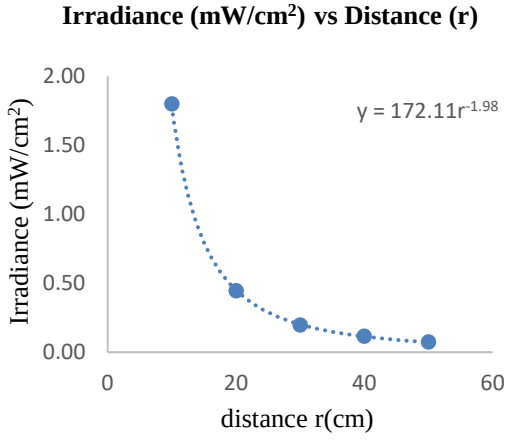


Figure 4. Illustration of inverse square law for calculating irradiance for DIEHL blue light source

VI. CONCLUSION

The 405nm exposure resulted in a log reduction of 2.84 in 2 hours and 40 minutes. For safety reasons, the beta coronavirus was used and not the SARS-CoV-2, as there have been many reported accidental cases of usage of SARS-CoV-2 [31]. Based on the results and discussed advantages of using 405nm over UVC, the 405nm deployment in public arenas seems pragmatic and doable. Therefore, 405nm can potentially inactivate the coronavirus, and its limitation of slower inactivation rate in comparison to UVCs can be resolved by using multiple light sources with irradiance being within the acceptable range provided by IEC 62471 [14]. Ergo, blue light technology can be the first of its kind for uninterrupted disinfection.

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