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Efficacy of Ultraviolet Radiations against Coronavirus, Bacteria, Fungi, Fungal Spores and Biofilm

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Abstract: Ultra-violet (UV) C (200–280 wavelength) light has long been known for its antimicrobial and disinfecting efficacy. It damages DNA by causing the dimerization of pyrimidines. A newly designed technology (MUVi-UVC; Mobile UV Innovations Pty Ltd., Melbourne, VIC, Australia) that emits UVC at 240 nm is composed of an enclosed booth with three UVC light stands each with four bulbs, and has been developed for disinfecting mobile medical equipment. The aim of this project was to examine the spectrum of antimicrobial activity of this device. The experiments were designed following ASTM E1052-20, EN14561, BSEN14476-2005, BSEN14562-2006 and AOAC-Official-Method-966.04 standards for surface disinfection after drying microbes on surfaces. The disinfection was analyzed using *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (6294), *Candida auris* (CBS 12373), spores of *Aspergillus niger* (ATCC 16404), coronavirus (SARS-CoV-2 surrogate ATCC VR-261) as well as a methicillin-resistant *Staphylococcus aureus* (SA31), a carbapenem- and polymyxin-resistant *Pseudomonas aeruginosa* (PA219), *Escherichia coli* K12 (ATCC 10798) and *Salmonella typhi* (ATCC 700730). The parameters of time, the number of lights and direction of the sample facing the lights were examined. The MUVi-UVC was able to kill 99.999% of all of the tested bacteria, fungi, coronavirus and bacteria in the biofilms if used for 5 min using all three lights in the setup with the glass slides in a vertical position. However, for fungal spores, 30 min were required to achieve 99.999% killing. There was a small but insignificant effect of having the surface horizontally or vertically aligned to the UV lights. Therefore, this UVC device is an effective technology to disinfect medical devices.

Keywords: UVC radiation; biofilms; coronavirus; bacteria; fungi; fungal spores; disinfection

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1. Introduction

Ultraviolet light (UV) has been used for many years to disinfect contaminated surfaces, as well as for treating infections. Ultraviolet radiation has a shorter wavelength than visible light, but is longer than x-rays [1]. UV radiation is divisible into four spectra, based on its wavelengths, and these are UV (100–200 nm), UVC (200–280 nm), UVB (280–315 nm) and UVA (315–400 nm) [1,2]. UVC is the most effective at killing different types of microorganisms [3].

UVC inactivates microorganisms by damaging their genetic material [4,5]. The UVC range, particularly between 250–270 nm, is absorbed by nucleic acids, with 262 nm being the peak germicidal wavelength [3]. The DNA or RNA of microorganisms is damaged by the dimerization of the nucleic acid bases, particularly pyrimidines, which prevents microbial replication and reduces viability [1,5]. Compared to routine disinfection, UVC has several advantages such as killing a broader range of microorganisms, taking less time to kill vegetative bacteria, being eco-friendly, generally safe to use (provided appropriate protective clothing and equipment are used), having relatively low costs, and the associated technology being generally easy to operate [6,7]. However, as it is light, it does have a disadvantage of shielding or shadowing [8], whereby the places not in the direct line of sight of the UVC source do not obtain adequate disinfection. This can be overcome

by adding several sources of UVC, so that the shielding or shadowing is minimized or removed altogether.

Infections can spread via contaminated surfaces [9,10]. Therefore, cleaning and disinfection strategies are important tools to avoid the spread of infection [9]. However, the use of suboptimal disinfectants, reduced exposure time of disinfectants, the improper dilution of disinfectants sometimes as the result of ignoring the manufacturers' recommendations and non-compliance with hygiene standards, can led to increased cases of infection with microbes, such as methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals [11,12]. The efficacy of chemical disinfectants can be affected by different parameters, including the target microorganism, the surface features of the materials on which the microbes are present, the composition of the disinfectants, the concentration of the disinfectants and the cleaning and pre-cleaning protocols [13]. The microbes are able to escape two cycles of cleaning by forming biofilms [14]. Biofilms are aggregations of microbes, often multiple types, encased in a self-made polymer matrix. The consumables used on the patients in hospitals can carry multi-drug resistant (MDR) microorganisms in biofilms [15]. The presence of biofilms in the hospitals may help MDR bacteria to survive and spread in the hospital environment [16]. If the biofilms dry onto surfaces, forming so-called dry biofilms, they can be very difficult to remove, even after 50 wiping actions [17], and the bacteria in these dry biofilms can be transferred to other surfaces [18]. Even extensive room disinfection can leave 50% or more of the surfaces contaminated [12]. Due to these limitations, there is a need to develop improved disinfectants and ways of applying these to machines and surfaces in hospitals to prevent the spread of microbes and infections.

In comparison to liquid disinfectants, UVC has some advantages as it can be used automatically and remotely, and can be applied to liquids and solids, and to decontaminate air in different types of rooms. UVC has been used to aid in manual disinfection in hospitals [19], because of its germicidal efficacy. In addition, UVC has been used to control outbreaks of tuberculosis [20] and the H1N1 influenza virus [21]. UV radiation has had a wide application in sterilizing both critical and non-critical medical devices [22,23] Non-critical patient care items disinfected using UVC showed a reduction in the spores of *Clostridium difficile* and Adenovirus DNA to below detectable levels [24]. UVC may be a more reliable way to disinfect surfaces, as it can be used remotely and automatically operated with set parameters. The current research focused on the ability of UVC to disinfect surfaces contaminated with various microbes, including a coronavirus surrogate of SARS-CoV-2, using standard protocols.

2. Materials and Methods

2.1. MUVI-UVC Disinfection System Setup

A newly designed technology, MUVi-UVC (Mobile UV Innovations Pty Ltd., Melbourne, VIC, Australia), which is composed of an enclosed booth with three UVC light stands, each with four bulbs (Figure 1), was developed for disinfecting hospital equipment. MUVi-UVC operates at a wavelength of 254 nm. In the current study, the UVC lights were set up in the corners of the booth and a sample-holding stand was set up in its center. All of the three light racks were operated with separate remote controls, so that different numbers of light racks could be used. The specifications of the setup are given in Table 1.

Table 1. Specifications of MUVi-UVC booth disinfection system.

Specification	Details
Dimensions (m)	120 × 120 × 2000
Voltage	240 V ± 10% 50 Hz
UVC light frequency (Nm)	253.7
UV lamp	40 W × 4 lights = 160 W per set × 3
UV output (uW/cm^2)	1620 at 1 m (12 × 135 uW/cm^2)

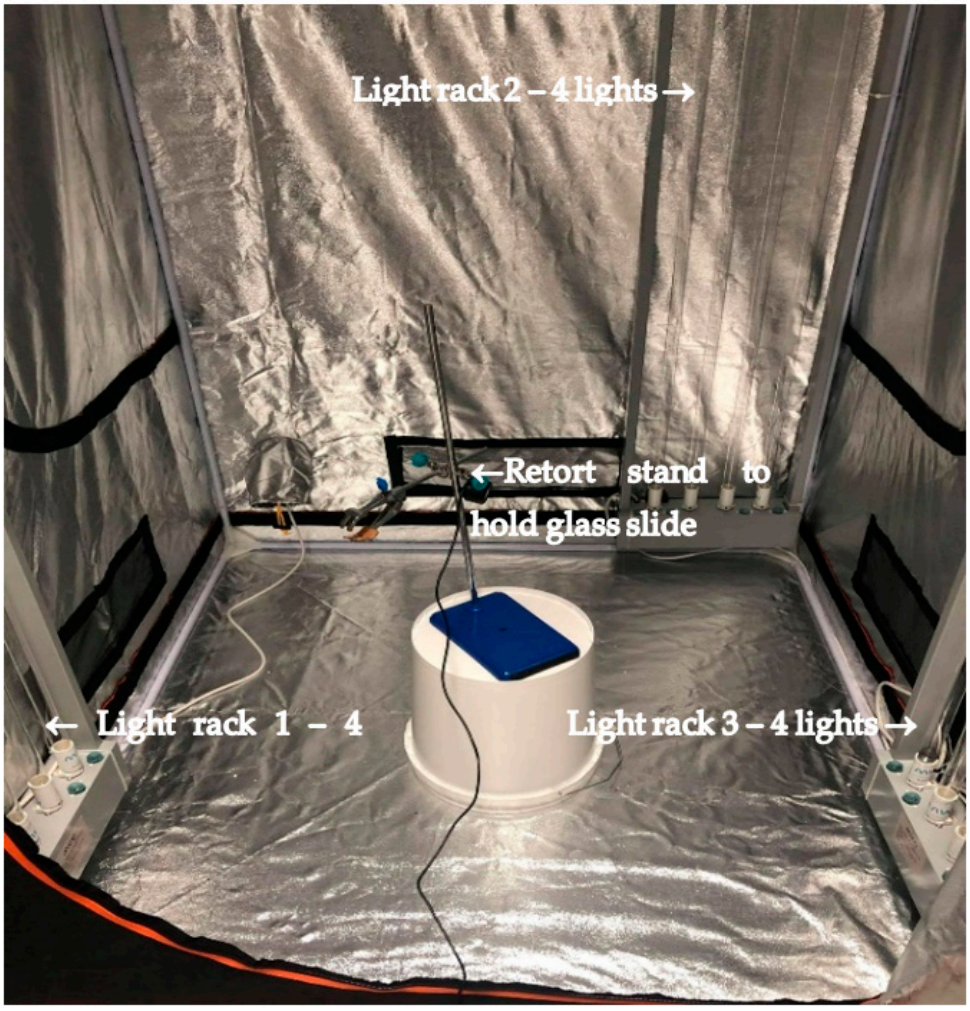

Figure 1. The MUVi-UVC set-up: within a 120 m wide tent, three 1.5 m tall light racks were placed in three corners. Each light rack contained four bulbs. There is a central retort stand to hold the inoculated surfaces. Each light rack could be operated separately by remote control.

2.2. Microorganisms Studied

The activity of UVC was analyzed using *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* 6294, *Candia auris* CBS 12373, spores of *Aspergillus niger* ATCC 16404, coronavirus MHV-1 (a SARS-CoV-2 surrogate) ATCC VR-261, *Escherichia coli* K12 (ATCC 10798) and *Salmonella typhi* ATCC 700730, as well as clinical isolates of methicillin-resistant *Staphylococcus aureus* SA31 [25] and carbapenem- and polymyxin-resistant *Pseudomonas aeruginosa* PA219 [26]. *Staphylococcus aureus* SA38 (microbial keratitis isolate; known biofilm former) [27] was used for the biofilm assay. The strains labelled ATCC were obtained from the American Type Culture Collection (Manassas, VA, USA) and the others were retrieved from the microbial culture collection of the School of Optometry and Vision Science UNSW, Sydney, Australia.

2.3. Microbial Growth, UV Exposure and Microbial Recovery

The MUVi-UVC was tested for its efficacy against all of the different bacteria, yeast, fungal spores and the virus when dried on glass. Only the *S. aureus* was tested on vinyl (chlorine, ethylene), Formica (@60% paper and 30–40% cured phenol-formaldehyde resin), stainless steel 304 (chromium 18–20%, nickel 8–10.5%), PVC (ethylene and chloride) and ceramic surfaces. The bacteria, yeast, fungal spores and the coronavirus were processed using different standards: BSEN14476-2005 and ASTM E1053-11 for the viral testing, EN14561 for the bacterial and yeast testing and AOAC-Official-Method-966.04 for the

fungal spores. Briefly, the bacteria and vegetative cells of the fungi were grown overnight in tryptic soy broth (TSB; Becton Dickinson, Heidelberg, Germany) at 37 °C. After 24 h growth, 1×10^6 colony forming units (CFU) were spread on the glass or other surfaces. The fungal spores were grown on Sabouraud's dextrose agar (SDA; Becton Dickinson, Heidelberg, Germany) for 10 days and collected and stored in the sterile water. The stored spores of 1×10^6 CFU were dried on the glass surface. The coronavirus was grown on mouse fibroblasts cells A9 (ATCC/CCL 1.4) and collected in Dulbecco's modified Eagle medium (DMEM; Remel, KS, USA). The plaque-forming units (PFU) of coronavirus was adjusted to 1×10^6 and dried on the glass surfaces. The surfaces were dried for 30 min and then exposed to UVC.

2.4. Biofilm Formation

Wet biofilms were grown on glass slides as previously described [28]. Briefly, a 100 µL of 1×10^6 CFU of SA38 overnight culture was added onto the slide, spread on the slide, then put in a petri dish containing wet tissue towels to provide a moist environment for the bacteria to develop into biofilms. The bacteria were incubated for 48 h at 37 °C. For the dry biofilms, SA38 was grown as described previously [29]. Briefly, the biofilms were grown on the glass slides by inoculating with 100 µL of 1×10^8 CFU of SA38 from an overnight culture. The slides were kept moist by placing in chambers containing sterilized water on tissues. The slides were incubated for 12 days in alternate wet and dry conditions [29]. The incubation regimen was: 48 h for 35 °C with media on slides; 48 h room temperature after media had been drained off; three cycles of rehydration for 6 h in media; three dehydration phases of 66, 42 and 66 h.

2.5. UVC Exposure

The MUVi-UVC setup consisted of three light racks, each 1.5 m tall and containing four light bulbs, and the racks were placed in up to three corners of the booth (Figure 1). The inoculated slides were held by a retort stand placed in the center, in such a way that the slides were facing one rack at a distance of 43 cm, the second rack at a distance of 53 cm and the third rack at a distance of 73 cm. The parameters that were tested included the number of light racks, the distance of the sample surface from the light racks, time of exposure and the position of the sample surface, either horizontal or vertical. Later, based on the initial trial tests, the parameter of distance was not included. After the biofilm formation, the slides were either placed in the UVC chamber and exposed to UVC light (from two light racks) for 2, 5 or 10 min, or left without exposure for the same time points (un-exposed controls).

2.6. Microbial Recovery

No neutralizing agents were used during the microbial collection, as UVC does not leave any actives present once the light has been switched off. The bacterial and fungal strains and fungal spores were recovered in TSB by placing the UVC-exposed slides in 50 mL centrifuge tubes containing TSB and vortexing for 1–2 min. After the recovery of the supernatants and serial dilution, the bacteria were incubated at 37 °C for 18–24 h on TSA plates. The fungi and fungal spores were incubated on Sabouraud's dextrose agar (SDA; Becton Dickinson, Heidelberg, Germany) for 30 °C for 48 h. After incubation, the number of colonies forming units were calculated. The controls of the surfaces were also used to determine the number of surviving microbes on surfaces without exposure to UVC.

The amount of biofilms was analyzed in separate experiments by staining with 125 µL 0.1% crystal violet staining for 10–15 min. After washing in tap water, the bound crystal violet was removed, using 30% acetic acid. The aliquots of the released crystal violet were added to 96 well microtiter plates, and the amount analyzed in a spectrophotometer at a wavelength of 550 nm, using 125 µL of 30% acetic acid as a control [30]. After either wet or dry biofilm formation and treatment, the slides were washed three times in phosphate-buffered saline to remove the non-adherent bacteria, then placed with a magnetic stirring

bar and vortexed for 2 min at maximum speed to detach the adherent cells [31]. The detached cells were then diluted in phosphate-buffered saline and grown overnight at 37 °C on tryptic soy agar.

The coronavirus, at 1×10^6 plaque-forming units (PFU), was dried on glass and exposed to UVC. The cells were recovered in 50 mL centrifuge tubes containing filtered bovine serum albumin (20% *w*/*v*; BSA, Sigma Aldrich, MO, USA) by vortexing and then diluted up to four times. The mouse fibroblasts cells were grown in 12-well plates, using Dulbecco's modified Eagle medium (DMEM; Remel, KS, USA) containing 10% fetal bovine serum (FBS) and antibiotics (streptomycin sulphate and penicillin G). The UVC-treated or non-treated virus, or sterile filtered BSA were added to 12-well plates covering with agarose. These 12-well plates were inoculated for up to 3–4 days at 37 °C, then fixed with paraformaldehyde for 2–3 h and then stained with 0.1% (*w*/*v*) crystal violet staining to count the PFUs of the virus.

A steam disinfection system (manufactured by Duplex Cleaning Machines Australia, Northcote, VIC, Australia) supplied by the MUVi, was used as a comparison in this study. The steam disinfection system operates at the temperature of 161 °C and is used as a commercial steam cleaner for different surfaces. The steam was sprayed onto the virus-inoculated glass slides using the narrow head of the steam system. During the application of steam for 30 s and 1 min, the glass slides were placed in the sterile petri plates to avoid the loss of any of the virus. The whole procedure of steam application and virus recovery from the slides was performed in a biosafety cabinet. The viral recovery from the glass slides was performed as described above.

3. Results

3.1. Efficacy of UVC against Different Bacterial Species

According to the European standard test, EN14561-2:2006, used in the study, a 5 $\log_{10}$ reduction in the number of viable bacteria cells is needed to pass the test. The results indicated a 5 $\log_{10}$ reduction could be achieved on the vertical and horizontal glass surfaces when three light racks were used, and exposure was for five minutes (Table 2). When one light rack was used, it took 10 min to produce a 5 $\log_{10}$ reduction for all of the bacterial strains, whether the glass slides were set up horizontally or vertically.

Table 2. Efficacy UVC against different bacterial species dried on glass.

Microorganisms	Position of Sample	No. of Light Racks	Time of Exposure (min)/Killing %			
			2	2.5	5	10
S. aureus ATCC 6538	Vertical	1 light	99.9	99.9	99.99	99.999
		3 lights	99.9	99.9	99.999	99.999
	Horizontal	1 light	99.9	99.9	99.99	99.999
		3 lights	99.9	99.9	99.999	99.999
S. aureus SA31 *	Vertical	1 light	99.9	99.9	99.9	99.999
		3 lights	99.9	99.9	99.999	99.999
	Horizontal	1 light	99.9	99.9	99.99	99.999
		3 lights	99.9	99.9	99.999	99.999
P. aeruginosa 6294	Vertical	1 light	ND	85	98	99.999
		3 lights	ND	95	99.999	99.999
	Horizontal	1 light	ND	99	99.99	99.999
		3 lights	ND	99.9	99.999	99.999
P. aeruginosa PA219 #	Horizontal	1 light	ND	ND	99.99	99.999
		3 lights	ND	ND	99.999	99.999

Table 2. *Cont.*

Microorganisms	Position of Sample	No. of Light Racks	Time of Exposure (min)/Killing %			
			2	2.5	5	10
Escherichia coli K12 (ATCC 10798)	Vertical	1 light	ND	99	99.99	99.999
		3 lights	ND	99.9	99.999	99.999
	Horizontal	1 light	ND	90	99.99	99.999
		3 lights	ND	99	99.999	99.999
Salmonella typhi ATCC 700730	Vertical	1 light	ND	95	99.99	99.999
		3 lights	ND	99.9	99.999	99.999
	Horizontal	1 light	ND	95	99.99	99.999
		3 lights	ND	99.9	99.999	99.999

*, methicillin-resistant; #, carbapenem- and polymyxin-resistant.

3.2. Efficacy of UVC against Bacterial Biofilms

As measured using crystal violet staining, SA38 formed the same amount of wet or dry surface biofilm before UVC treatment (Figure 2), that contained the same number of viable cells (Table 3). After UVC treatment, the amount of biofilm reduced to two-thirds of the untreated biofilm. The UVC was able to reduce the amount of the live SA38 cells to the same extent (99.99%) in either the wet or dry biofilms on the glass surface (Table 3) when exposed to two light racks.

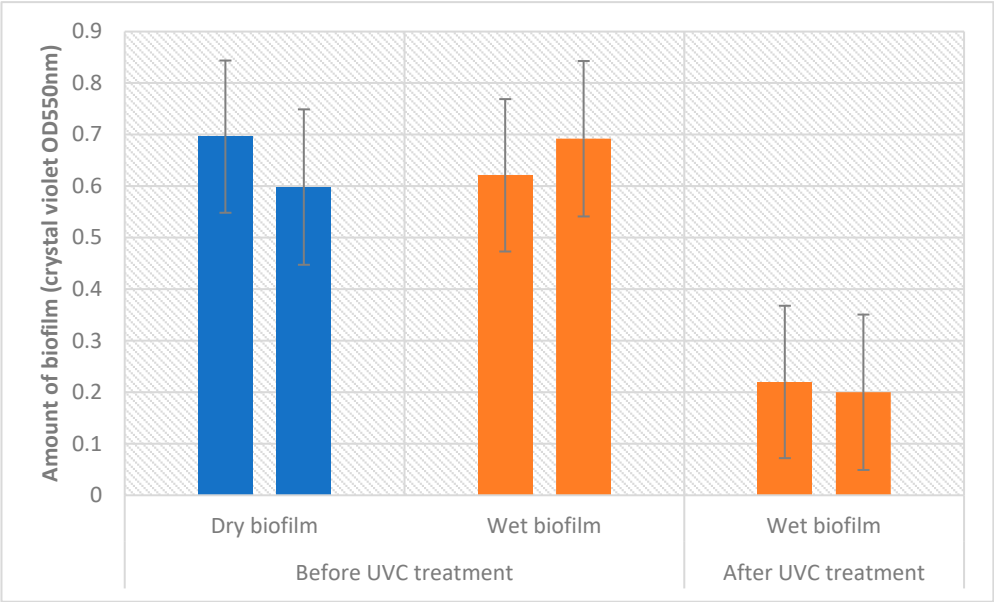

Figure 2. Quantification of wet and dry surface biofilms before and after UVC treatment.

Table 3. Efficacy of UVC against SA38 in biofilms.

Microorganisms	Materials	No. of Light Racks	Time of Exposure (min)/Killing %			Viable Bacteria Recovered from the Untreated Biofilm
			2	5	10	
Wet surface biofilm	Glass	2	92	99.99	99.99	46.6×10^6
Dry surface biofilm			ND	99.99	99.99	43.1×10^6

3.3. Efficacy of UVC against Coronavirus Fungus and Fungal Spores

UVC reduced the numbers of viable yeast cells and coronavirus by 5 $\log_{10}$ (99.999%) after 5 min exposure when three light racks were used. The coronavirus was also reduced by 5 $\log_{10}$ when one light rack was used for 5 min. However, the spores of the *Aspergillus niger* needed longer, 30 min, and then reached a 4 $\log_{10}$ reduction (Table 4), whether one or three light racks were used.

Table 4. Efficacy of UVC against fungi, fungal spores and coronavirus when dried on glass.

Microorganisms	Position of Sample	No. of Light Racks	Time of Exposure (min)/Killing %				
			1	**5**	**10**	**20**	**30**
Candida auris CBS 12373	**Horizontal**	1 light	ND	99.9	99.99	ND	ND
		3 lights	ND	99.999	99.999	ND	ND
Aspergillus niger (spores) ATCC 16404		1 light	ND	≤1	88	99.9	99.99
		3 lights	ND	≤1	90	99.9	99.99
Coronavirus (SARS-CoV-2 surrogate; MHV-I; ATCC VR-261)		1 light	99.9	99.999	ND	ND	ND
		3 lights	99.9	99.999	ND	ND	ND

ND = not determined.

As a comparator, a steam disinfection system was used, and it was tested for 30 s and 1 min against the coronavirus surrogate. The steam system produced a 60% and 90% reduction in the numbers of the infectious virus, respectively.

3.4. Efficacy of UVC against S. aureus on Different Surfaces

After analyzing the efficacy of UVC against all of the microorganisms on the glass, *S. aureus* ATCC 6538 was taken as a representative microbe and tested on different materials. Additionally, the time of exposure was standardized to 5 min and 10 min and the samples only placed horizontally. The number of viable *S. aureus* was reduced by 5 $\log_{10}$ CFU (99.999%) on most of the materials within 5 min when three light racks were used. The exception was steel, where 10 min was needed to achieve 5 $\log_{10}$ killing (Table 5). When exposed to one light rack for 10 min, the number of *S. aureus* ATCC 6538 was reduced by 5 $\log_{10}$.

Table 5. Efficacy of UVC against S. aureus on different surfaces.

Microorganisms	Testing Surface	Position	No. of Lights	Time of Exposure (min)/Killing %	
				5	**10**
S. aureus ATCC 6538	Vinyl	Horizontal	1	89	99.999
			3	99.999	99.999
	Ceramic		1	99.99	99.999
			3	99.999	99.999
	Formica		1	99.99	99.999
			3	99.999	99.999
	Steel		1	83	99.999
			3	96	99.999
	Plastic		1	99.99	99.999
			3	99.999	99.999

4. Discussion

The current study investigated the efficacy of a new UVC set-up against several microorganisms, including a coronavirus. The results from the present study demonstrated

that MUVi-UVC was an effective disinfection system meeting the disinfection criteria of the standards used. These disinfection criteria were the reduction in viable microbes by at least 4 $\log_{10}$ for both of the British standards and 5 $\log_{10}$ for the European standard.

The Therapeutic Goods Administration of Australia identifies the MHV as a suitable surrogate of SARS-CoV-2 for testing the disinfection activity of different solutions (https://www.tga.gov.au/surrogate-viruses-use-disinfectant-efficacy-tests-justify-claims-against-covid-19; accessed on 2 February 2021). MHV is very similar in its mechanism of infection to SARS-Cov-2 and can produce the acute respiratory disease in mice. Together with SARS-Cov-2, SARS-CoV-1 and Middle Eastern Respiratory Syndrome virus (MERS), MHV falls in the group 2 coronaviruses [32]. Previous studies have shown that different chemical-based disinfecting regimes are effective against MHV, including 70% alcohol giving a 4 $\log_{10}$ reduction on stainless steel within a minute [33,34]. In the current study, UVC gave a 5 $\log_{10}$ reduction of MHV on glass after 5 min exposure. Other studies have shown that UVC can be used for disinfection against MHV [35–42]. UVC halts DNA or RNA replication by dimerizing their pyrimidine bases after absorption of UVC, and this leads to the inactivation of viral replication [39,43]. SARS-CoV-2 can survive on glass, stainless steel and paper for up to 28 days at 20 °C [44] and touching contaminated surfaces can initiate infection. Therefore, a technology that can reduce the numbers of culturable coronaviruses is likely to be effective at preventing the spread of the disease.

The finding is important that all of the different bacteria, including the MDR strains, could be reduced by a 5 $\log_{10}$ after exposure to UVC from one light rack for 10 min or three light racks for 5 min. *S. aureus* ATCC 6538 is a standard strain used to test the efficacy and resistance of disinfectants [45,46]. This *S. aureus* strain together with *P. aeruginosa, Proteus vulgaris* and *E. coli* are required to be tested in order to qualify for the hospital or household disinfection testing, according to TGA guidelines https://www.tga.gov.au/resource/tga-instructions-disinfectant-testing (accessed on 20 March 2021) *P. aeruginosa* is a common cause of healthcare-acquired infections, where both of the bacteria can form biofilms on the high-touch surfaces in hospitals [47,48]. Antibiotic-resistant isolates are of rising concern, and many are spread through contact with contaminated surfaces. The multidrug-resistant (MDR) bacteria cause nosocomial-, or healthcare-acquired infections. MRSA is disseminated globally and is a leading cause of both community- and hospital-acquired infections [49]. MDR *P. aeruginosa* has been isolated from multiple hospital outbreaks [50], which increases the global health burden and the cost of treatment due to the prolonged hospitalization [51,52]. Together with antibiotic resistance, MDR isolates may also be resistant to the different disinfectants used in the normal cleaning regimens of hospitals [53,54]. The MDR strains of *E. coli*, *S. aureus* and *Salmonella typhi* can be isolated from community-acquired and healthcare-acquired infections [55–57].

Various technologies have been used in hospitals for providing disinfection, including both chemical- and UV-based technologies [58–60]. However, different bacteria may escape the effects of disinfectants, such as chlorine and peracetic acid, by developing tolerance or resistance [61,62]. The current study verified that UVC was effective against the standard strains of *S. aureus*, *P. aeruginosa*, *E. coli* and *Salmonella typhi* along with carbapenem-resistant *P. aeruginosa*, and it has also been shown to be effective against MRSA [26]. This efficacy of UVC at a wavelength of 254 nm has been shown to be germicidal against influenza A viruses, H1N1 and H3N2, but was less efficient against *Mycobacterium tuberculosis*, requiring 20 min to produce a 3 $\log_{10}$ reduction [63]. However, the light set-up was different to that in the current study, and it would be of interest to examine the ability of the MUVI-UVC system to kill *Mycobacterium tuberculosis.*

Other than the development of resistance to disinfectants by mutation or acquisition of genes, biofilm formation can also protect bacteria [64]. For example, sodium hypochlorite was not able to eradicate bacteria from biofilms [29]. In the current study, the SA38 grown in biofilms was more resistant to UVC than when simply dried on glass. However, the UVC treatment for 5 min was able to reduce the numbers of viable cells in the biofilm by 4 $\log_{10}$ CFU (99.99%). The biofilm itself was reduced to two-thirds by UVC treatment,

indicating that some form of abrasive treatment is likely to be required after UVC treatment to more thoroughly remove the traces of the biofilm. Dead bacteria remaining in biofilms on surfaces may act as a food source for new bacterial growth and biofilm formation [65]. Even so, a study, examining the effect of dead biofilms on the formation of new biofilms of *P. aeruginosa*, found that the dead biofilms exerted an effect on the new biofilm formation, with the new biofilms being formed more slowly and were softer (perhaps indicating that they would be easier to remove) [66].

C. auris has been isolated when found in the hospital environment and infections, and responds poorly to antifungals [67]. The Centers for Disease Control and Prevention (CDC) have recommended it be used to test the efficacy of disinfectants (https://www.cdc.gov/fungal/candida-auris/index.html; accessed on 22 August 2021). *Aspergillus* is an opportunistic human pathogen that can cause nosocomial invasive aspergillosis [68]. *Aspergillus* can escape many sterilization techniques by producing resistant spores, and it is recommended to be considered in order to test the efficacy of UVC [69]. Previously, studies have found that the fungal spores of this genera can be resistant [70] or susceptible to UVC treatment [71,72]. In addition, the intense use of different chemical disinfectants have been needed to kill fungal spores, and these disinfectants may have significant toxic effects on human and pollute the environment [73]. The finding that the UVC disinfecting system used in the current study was active against the vegetative cells of *Candida auris* and the spores of *Aspergillus niger* is therefore of importance, especially as these are often difficult to kill with other chemical or UV disinfection systems [74].

The use of current UVC technology was also tested against *S. aureus* dried to plastic, vinyl, ceramics, Formica and stainless steel. The application of UVC on the different surfaces was previously shown to be effective in combination with other cleaning methods including ethanol wipes [75] against bacteria, including a 17% reduction in MRSA, VRE, *Acinetobacter* spp., carbapenem-resistant Enterobacteriaceae and viruses including SARS-CoV-2 [75].

In conclusion, the current study demonstrated that the UVC system could produce a 5 $\log_{10}$ reduction of *S. aureus* ATCC 6538 when dried onto different surfaces (glass, vinyl, Formica, ceramic, steel, plastic), was able to similarly reduce the numbers of other bacteria including MDR strains on glass, as well as a coronavirus and the yeast *C. albicans*, when three light racks were used for 5 min. The spores of *A. niger* were more resistant to the UVC system, requiring 10 min and three light racks to produce a 4 $\log_{10}$ reduction. These reductions in the numbers of microbes on these surfaces, which may be present in hospital wards and as part of medical devices, indicates that the UVC system has the possibility of reducing the transmission of microbes around hospitals. This should be tested in follow-up experiments.

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