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Experimental analysis to quantify inactivation of microorganisms by Far-UVC irradiation in indoor environments.

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Abstract

Far-UVC irradiation at a 222 nm wavelength is a promising technology for inactivating microorganisms in indoor environments to mitigate transmission of infection. Here we report experimental measurements in a room-scale chamber to evaluate the performance of filtered Krypton-Chloride (KrCl) lamps in reducing the steady-state concentration of *Staphylococcus aureus* and *Pseudomonas aeruginosa* under different ventilation rates in indoor environments. The results showed a mean 95.5% lowering of *S. aureus* load and 94.9% of *P. aeruginosa* load at 3 air changes per hour (ACH) using one Far-UVC lamp and 97.8% and >97.5% using five lamps. At 1.5 ACH, the mean microbial reduction for *S. aureus* was >94.6% and >99.5% and at 9 ACH, it was 66.3% and 91.9% for 1 lamp and 5 lamps, respectively. Initial results at a shorter distance between the microbial source and collection sampling show a reduced but still substantial effect of the Far-UVC. The findings indicate that within these experimental conditions, Far-UVC can be effective at room-scale inactivation of a range of pathogens in a range of ventilation scenarios and also show promise at short-range inactivation. This research paves the way for future work to explore efficacy in real-world scenarios and to quantify usability and acceptability.

Highlights

- Far-UVC effectively inactivated multiple airborne pathogens in a room-sized chamber
- Inactivation was good at all mechanical ventilation regimes
- Inactivation was consistent across ventilation regimes
- At a short distance, Far-UVC inactivation was reduced but remained significant

1. Introduction

Managing exposure to microorganisms in the air and on surfaces is critical for controlling transmission of infection, particularly in high-risk environments such as healthcare. Data from 2016-17 suggests that hospital acquired infections cost the NHS in the UK over £2.7 billion per year and affected over 834,000 people [1]. COVID-19 has substantially raised this figure with around 20% of patients in hospitals acquiring infection in the UK during the first wave of the pandemic [2]. Airborne transmission of infection is already recognised for many diseases including Tuberculosis, Measles, SARS, Influenza, and COVID-19 [3, 4], and airborne dispersion including deposition onto surfaces has been indicated for several other pathogens including methicillin-resistant *S. aureus* (MRSA), *Clostridium difficile*, *Pseudomonas* and *Norovirus* [5, 6].

Ventilation is well recognised as a key strategy for controlling airborne exposure. It is embedded in UK healthcare guidance [7] and there is also evidence that effective ventilation and air cleaning can reduce contamination of surfaces [5, 8]. However, ventilation in a large proportion of buildings within the healthcare sector and in other settings does not meet current standards and improving ventilation can be costly and practically difficult to achieve [9]. Air cleaning strategies are increasingly being recommended to enhance infection control without the complexity of installing new ventilation equipment [10]. Some of these technologies, including HEPA filters and 254nm ultraviolet (UVC), have significant evidence base whereas other emerging technologies, including Far-UVC, show promise. However, the evidence for emerging technologies is largely based on small-scale experiments and many lack data to support full-scale application.

Krypton-Chloride excimer lamps produce germicidal UVC across a broad spectrum with a peak wavelength around 222 nm. This is known as Far-UVC with current evidence suggesting that 222nm does not cause the acute effects of other UVC wavelengths, with no evidence to date that it harms skin or eyes when used within current guidance exposure values [11, 12]. Research continues to explore the effects of Far-UVC on tissue [13]. Far-UVC exposure limits in the UK and EU follow the International Commission on Non-Ionizing Radiation Protection (ICNIRP) [14] guidelines which recommend an 8-hour exposure limit of 23 mJ/cm^2 at 222 nm. In the US, higher 8-hour threshold limit values of 160 mJ/cm^2 for eyes

and 478 mJ/cm^2 for skin (when eyes are protected) at 222 nm are recommended by the American Conference of Governmental Industrial Hygienists [15]. With the use of appropriate filters to significantly reduce emissions at more hazardous UVC (and UVB) wavelengths, Krypton-Chloride based lamps can be used as an effective source of Far-UVC light.

Evidence from small-scale laboratory studies indicates the potential for Far-UVC to inactivate a wide range of microorganisms in air and on surfaces including SARS-CoV-2, influenza and several bacterial pathogens [16, 17]. Our previous work carried out the first full room scale experimental chamber study and showed over 90% inactivation of *Staphylococcus aureus* under steady contamination conditions with lamps that generated a UV field meeting the ICNIRP exposure guidelines [18]. A chamber study measuring aerosolised *E. coli* under decay conditions indicates rapid inactivation due to Far-UVC and better relative performance at lower ventilation rates [19]. Experimental comparison of Far-UVC with conventional 254nm UVC against a range of bacteria in a duct suggests the technology can be effective when installed in an HVAC system, but highlights the performance depends on the ventilation rate [20]. A real-world study carried out in a meeting room showed an average 66% reduction in total bacterial count after 1 hour of Far-UVC operation, but a smaller impact on fungal concentrations [21]. Buonanno et al. demonstrated a 99.8% reduction of infectious airborne murine norovirus in a mouse-cage cleaning room [22]. A study applying a 222nm lamp in a bathroom environment indicated an overall 64% reduction in colony forming units for aerobic bacteria on surfaces, indicating there is also a potential benefit for mitigating surface concentration of bacteria [23, 21] . Far-UVC light has been shown to inactivate bioaerosols in a variety of settings, including common office areas, air-conditioned rooms, and upper-room systems [24, 25]. This has been shown by both experimental protocols and computer models. Research shows that the proper optimization of lamp intensity and positioning creates safe opportunities for maximizing disinfection effects. This paper builds on our previous room-scale bioaerosol chamber studies to compare the performance of Far-UVC in reducing the concentration for two bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa* which are both representative of hospital pathogens, and to explore the effectiveness under a range of different ventilation rates and ventilation regimes. We consider the impact of Far-UVC on both air and surface microbial contamination under steady-state contamination conditions and carry out preliminary experiments to explore the influence of distance from the microbial source.

2. Experimental Methodology

Bioaerosol chamber and Far-UVC lamps

The experimental approach was similar to our previous study [18]. Experiments were conducted in a controlled bioaerosol chamber at the University of Leeds; the dimensions are comparable to a single-bed hospital room (32.25 m^3): 4.26m (L) x 3.36m (W) x 2.26m (H). The ventilation air was HEPA filtered at the supply and the extract to provide contaminant-free inlet air and ensure safe discharge, and all experiments were carried out with no occupants in the room and with the ventilation operating under negative pressure for safety. Air was supplied through either high- or low-level mounted wall grilles and extracted through similar grilles mounted on the diagonally opposite wall (**Figure 1**). Throughout the experiments, the temperature and relative humidity were maintained at 22°C ± 1°C and 48% ± 2%, respectively.

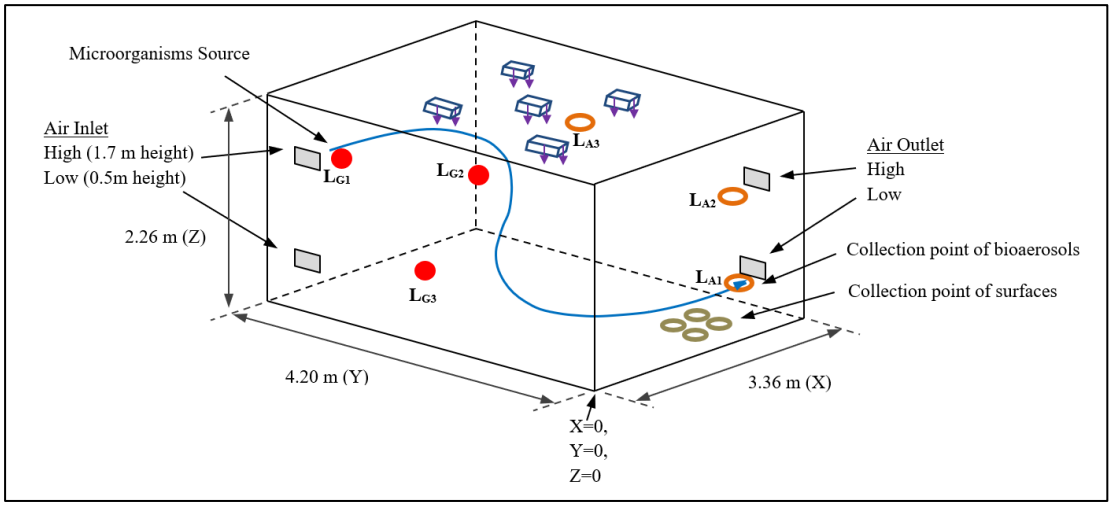


Figure 1: Bioaerosol chamber dimensions, ventilation regime and microorganism release and air and surface sample locations used in the majority of experiments. Ozone measurements were made close to the low air outlet.

Five Krypton Chloride excimer lamps (U3, Ushio Inc., Tokyo, Japan) were mounted close to the ceiling of the chamber in a quincunx formation as described in Eadie et al [18]. The lamps each had a luminous flux of 86 mW with thin PTFE diffuser and operated continuously during all experiments. The thin PTFE diffuser was used to maximise the UVC irradiation volume in the room by spreading the angle of the lamps' emissions. Experiments were carried out with one or five lamps operating, equivalent to room average fluence rates of 0.56 μWcm^{-2} and 2.54 μWcm^{-2} respectively.' [14, 15, 18]. As this study represents whole-room irradiation, rather than upper-room irradiation, it is most appropriate to calculate the average fluence rate by the entire volume.

Generation and sampling of microorganisms

Laboratory strains of *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (NCIMB 10848) culture were prepared by transferring a loopful of bacteria into a 100ml of sterilised nutrient broth (Oxoid Ltd, UK) and incubating at 37°C for 48 hours. The concentration of the strain in the culture broth was determined through serial dilution to be ~1 x10^8 cfu/ml. A Collison 6-jet nebuliser (BGI, USA) was used to generate the aerosolised microorganisms in the range of 0.3-10 µm diameter [8]. The suspension fluid inside the nebuliser vessel was created by adding 1ml from the culture broth to 99 ml distilled water to achieve a concentration of ~1 x10^6 cfu/ml. The nebuliser operated at 12 L.min^{-1} and was located outside the chamber with microorganisms injected into the room and released at one of three locations at coordinates (X, Y, Z)**.**

- L_{G1}: Through a tube and near the high-level supply of fresh air (0.5 m, 3.55 m, 1.7 m).
- L_{G2}: Through a tube and near the middle Far-UVC lamp (0.68 m, 2.1 m, 1.7 m).
- L_{G3}: Through a hole in the wall directly to the centre of the long wall of the chamber (0 m, 2.1 m, 1.2 m).

The location of the source point L_{G1} is as in Eadie et al [18] and was selected for the majority of experiments as it was not located directly under a Far-UVC source. Location L_{G2} was used to explore the influence of distance and was located 2 m away from the sample point with a single Far-UVC lamp directly between the two locations. Location L_{G3} was used to release *Pseudomonas aeruginosa* as it was challenging to create sufficient aerosol in the room (extremely low generation) and this location prevented losses in tubing that are present with other release locations.

Tryptone Soya Agar (TSA) (Oxoid Ltd, UK) was used to prepare 90 mm and 55 mm petri dishes for air and surface sampling respectively. Microorganisms sampled from the air were collected using an Anderson 6-stage impactor air sampler that was operated at a flow-rate of 28 l.min^{-1}; only the sixth stage is used as the focus is on total concentration rather than size distribution of the aerosol [8]. Positive hole correction was applied for the air samples to correct for potential over-counting under higher bioaerosol concentrations [26]. The sampler was located externally to the chamber in the ante-room, and air samples were taken using tubes via a sampling port at one of these three locations at coordinates (X, Y, Z)**:**

- L_{A1}: Near the low air extract (2.85 m, 0.65 m, 0.5 m).
- L_{A2}: Near the high air extract (2.85 m, 0.65 m, 1.7 m).
- L_{A3}: Through a tube and near the middle Far-UVC lamp (2.68 m, 2.1 m, 1.7 m).

The location of the collection points (L_{A1} and L_{A2}) is representative of the average bioaerosol concentration of the whole chamber [27]. Location L_{A3} was chosen to present a social

161 distance of 2 m away from the source of infection (L_{G2}) with the Far-UVC lamp in between
162 L_{G2} and L_{A3}.

163 Airborne pathogens were directly deposited onto agar plates contained within custom
164 Automated Multiplate Passive Air Sampling (AMPAS) device located on the floor of the room
165 [8]. The device comprises a series of 6 Petri dishes arranged in a circle, covered by a
166 rotating tray controlled by a stepper motor. The device is programmed to expose five of the
167 agar plates at pre-determined times and for pre-programmed periods before covering them,
168 without human intervention, to ensure they are no longer exposed to air. Four AMPAS
169 devices were put close together in front of the low grid outlet **Figure 1**. This location was
170 selected as previous experiments and simulations suggest that concentrations in air at this
171 location are most representative of average room conditions and the surface analysis here
172 focuses on providing an initial indication of the impact of Far UVC on airborne
173 microorganism deposition and was not chosen to explore surfaces with high-touch potential.

174 **Microbial experimental process**

175 In each experiment, the nebuliser and ventilation operated continuously over 180 and 210
176 minutes respectively (**Figure 2**); this replicates a realistic scenario in an indoor setting where
177 an infectious person continuously releases a pathogen over a long period. The first 60
178 minutes of each experiment were used to let the room achieve steady-state conditions, then
179 ten replicate air samples were taken over the next 50 minutes with the Far-UVC device(s)
180 off. The device(s) were then turned on and left for 20 minutes before taking ten more
181 replicate samples over a 50-minute period. A small number of air samples were also taken
182 during the 20 min transition period between the two conditions. These are not used in the
183 experimental analysis which focuses on steady-state conditions**Error! Reference source**
184 **not found.**. For air sampling, the duration time of sampling was 1-5 minutes (according to
185 the type of experiment), and for surface sampling, it was in 10-minute cycles and was
186 repeated five times (ten plates with Far-UVC device off and ten plates with Far-UVC device
187 on). Following sampling, the nebuliser and Far-UVC devices were switched off, and the
188 room ventilation rate was increased to 12 ACH for 30 minutes to flush any remaining
189 airborne microorganisms from the room. Following the experiment, the agar plates were
190 incubated at 37 °C for 24 hours. Most experiments were carried out using *S. aureus*.

Ventilation rate comparison experiments were carried out at airflow rates of 0.013 m^3s^{-1}, 0.027 m^3s^{-1}, 0.054 m^3s^{-1} and 0.081 m^3s^{-1} equivalent to 1.5, 3, 6 and 9 air changes per hour (ACH), respectively with the ventilation regime high grid inlet- low grid outlet (**Table 1**).

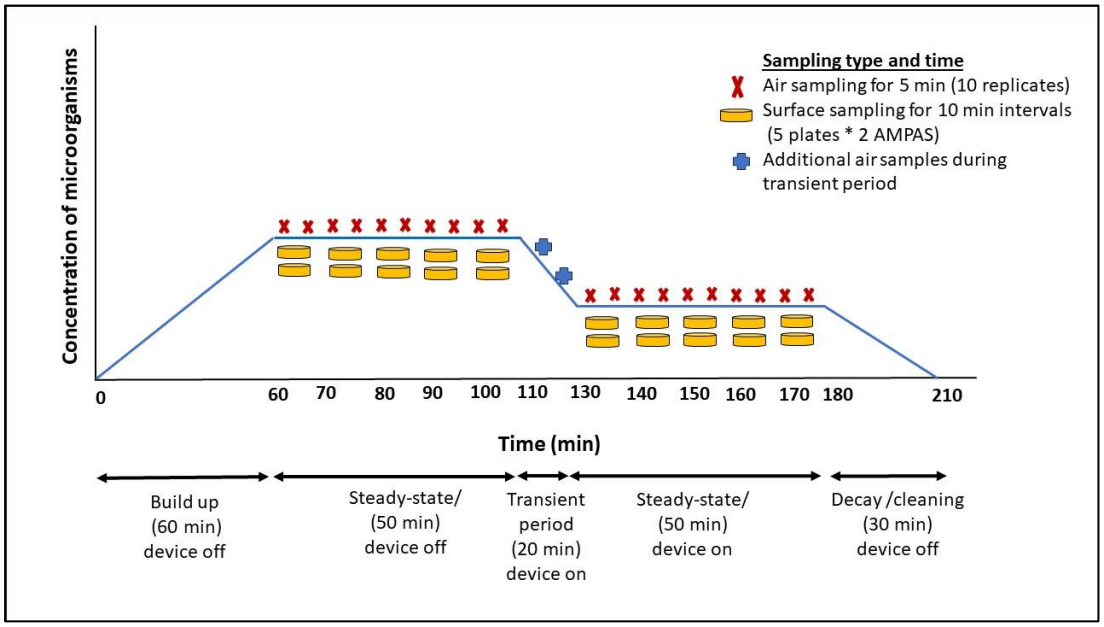


Figure 2: Graph of experimental procedure with samples and timing.

The location of generation sources was $L_{G1,}$ and the collection point of air sampling was L_{A1}. Ventilation regime comparison experiments were carried out at high grid inlet- low grid outlet and low grid inlet- high grid outlet at a constant ventilation rate of 3 ACH. The location of generation sources was L_{G1} and the collection points of air sampling were L_{A1} and L_{A2}. Spatial comparison experiments were carried out at high grid inlet- low grid outlet at 3 ACH. The location of the generation source was $L_{G2,}$ and the collection points of air sampling were L_{A1} and L_{A3}. Microbial species comparison experiments were carried out with *S. aureus* and *P. aeruginosa* at 3 ACH with high grid inlet- low grid outlet and source locations were L_{G1} and L_{G3} and the collection points of air sampling was L_{A1}.

Table 1: Summary of Comparison of Experimental Factors in Far-UVC irradiation at 222 nm.

Experiment factors	Comparison Experiments			
	Ventilation rate	Ventilation regime	short-range inactivation	Microbial species
No. of Far-UVC light (222 nm)	1 & 5	1 & 5	1	1 & 5

Ventilation rate (ACH)	1.5, 3, 6 & 9	3	3	3
ventilation regime	high grid inlet- low grid outlet	high grid inlet- low grid outlet & low grid inlet - high grid outlet	high grid inlet- low grid outlet	high grid inlet- low grid outlet
Microorganisms	*S. aureus*	*S. aureus*	*S. aureus*	*S. aureus* & *P. aeruginosa*
Generation source	L_{G1} (near the high-level supply)	L_{G1}	L_{G2} (near the middle Far-UVC lamp)	L_{G1} & L_{G3} (centre of the wall)
Collection point of air sampling	L_{A1} (Near the low air extract)	L_{A1} & L_{A2} (Near the high air extract)	L_{A1} & L_{A3} (near the middle Far-UVC lamp)	L_{A1}
Collection point of surface sampling	close together in front of the low grid outlet	–	–	–
Temperature	22°C ± 1°C	22°C ± 1°C	22°C ± 1°C	22°C ± 1°C
Relative Humidity	48% ± 2%	48% ± 2%	48% ± 2%	48% ± 2%
Total experiments	8	4	1	1

206

207 **Statistical analysis**

208 Appendix B - 400 Hole Count Correction Table was used to apply positive hole correction for
209 the sampler [28] to correct for potential over-counting under higher bioaerosol
210 concentrations. The reduction percentage represents the proportion of microorganisms
211 inactivated by the Far-UVC treatment and is calculated as:

212 Reduction Percentage = [(Initial Concentration – Post Exposure Concentration) / Initial
213 Concentration] × 100

The remaining percentage indicates the proportion of microorganisms left after exposure to Far-UVC and is calculated as:

Remaining Percentage = (Post Exposure Concentration / Initial Concentration) × 100

Experiment resolution is the limitations of detection of the experiment and is defined as 1 divided by the average number of CFU/plate in the steady state / device off collection period. R version 4.2.0 was used to process the data and generate the graphs. A t-test with Welch correction for groups with unequal variances was performed to assess the separate hypothesis that sampling location and ventilation regime were similar. The significance level used throughout was 0.05.

3. Results

Far-UVC and mechanical ventilation

Figure 3: The performance of Far-UVC (222 nm) irradiation in reducing the concentration of *S. aureus* in the air under steady state contamination at different ventilation rates (The box represents the interquartile range (middle 50% of results), the line inside indicates the median, and the whiskers extend to show variability).**Figure 3** and **Table 2** show the impact of Far-UVC on reducing the airborne steady state concentration (i.e. with continuous contamination) of *S. aureus* under four ventilation rates with either one or five UVC lamps switched on. Results illustrate that the Far-UVC devices significantly impact on steady state reduction of microorganisms across a wide range of ventilation rates in the chamber.

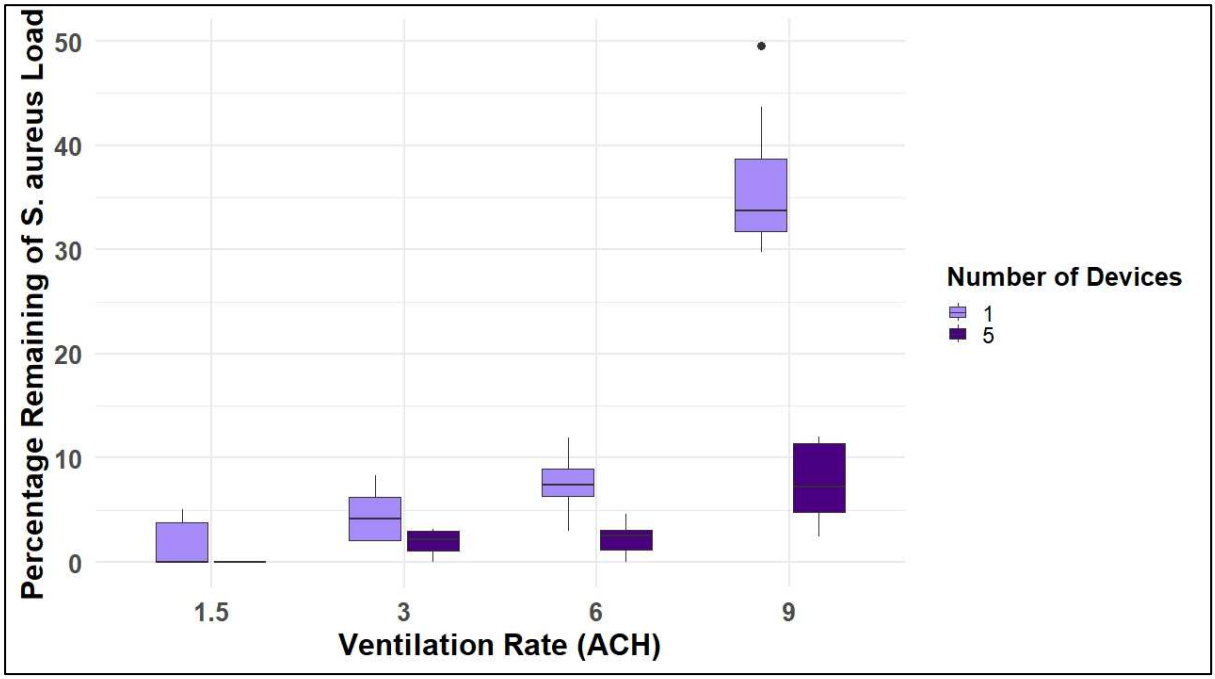


Figure 3: The performance of Far-UVC (222 nm) irradiation in reducing the concentration of *S. aureus* in the air under steady state contamination at different ventilation rates (The box represents the interquartile range (middle 50% of results), the line inside indicates the median, and the whiskers extend to show variability).

As expected, the relative benefit of the Far-UVC is greater at a lower ventilation rate and with a greater number of devices. At a high ventilation rate, there is more removal of microorganisms by the ventilation air, and hence the additional benefit measured by the experiments is relatively less than at a low ventilation rate. This concurs with the findings of Xia et al [19]. In addition, at a higher ventilation rate, the airflow in the room is at a higher velocity and therefore the aerosolised bacteria will have a lower residence time within the UVC field. The relative reduction is in line with data previously reported at 3 ACH (Eadie et al. 2022), confirming consistency of findings between the two studies. At the lowest ventilation rate of 1.5 ACH, the measured data reaches the limit of detection with all five devices operational with no colonies of S. aureus cultured from any of the air samples in the period after the UVC lamps had been turned on. Although this is reported in the table as 100% reduction, this value should be treated with caution as it is relative to the concentration in the room with no lamps operational. The experiments were conducted on different days, which leads to variations in concentration and the absolute values are less significant than the relative values.

Table 3 shows the impact of the Far-UVC on the deposition rate of microorganisms on the AMPAS sampler located on the floor of the chamber under different ventilation rates.

Table 2: Far-UVC impact on airborne microorganisms at various ventilation rates.

No. of devices	Far-UVC light (222 nm)	Ventilation rate (ACH)	Bioaerosols load (cfu/m3), Mean ± SD (Min-Max)	% Reduction		Experiment Resolution
				Median	IQR	
1	Off	1.5	711 ± 162 (536 – 1071)			
		3	1711 ± 391 (1286 – 2393)			
		6	800 ± 180 (583 – 1000)			
		9	1800 ± 313 (1357 – 2286)			
	On	1.5	11 ± 17 (0 – 36)	100%	-	5.4%
		3	75 ± 32 (36 – 143)	95.5%	93.3% - 97.8%	2.2%
		6	58 ± 21 (24 – 95)	92.8%	91.4%-93.9%	1.3%
		9	650 ± 124 (536 – 893)	66.3%	61.4% - 68.3%	2.0%
5	Off	1.5	2456 ± 388 (1702 – 2845)			
		3	3339 ± 424 (2714 – 4000)			
		6	1167 ± 99 (1036 – 1357)			
		9	1486 ± 479 (893 – 2250)			
	On	1.5	0 ± 0 (0 – 0)	100%		0.5%

		3	64 ± 38 (0 – 107)	97.8%	97.0% - 98.9%	1.1%
		6	27 ± 14 (0 – 54)	97.4%	96.8% - 98.8%	1.0%
		9	114 ± 54 (36 – 179)	91.9%	87.2% - 94.6%	2.7%

Table 3: Far-UVC impact on floor-deposited microorganism concentrations at various ventilation rates.

No. of device	Far-UVC light (222 nm)	Ventilation rate (ACH)	Deposited microorganisms' concentration (cfu/plate*), Mean ± SD (Min-Max)	% Reduction		Experiment Resolution
				Median	IQR	
1	Off	1.5	0.30 ± 0.48 (0 – 1)			
		3	1.30 ± 1.16 (0 – 3)			
		6	0.20 ± 0.42 (0 – 1)			
		9	2.00 ± 1.25 (1 – 5)			
	On	1.5	0 ± 0 (0 – 0)	100.0%	-	-
		3	0 ± 0 (0 – 0)	100.0%	-	-
		6	0 ± 0 (0 – 0)	100.0%	-	-
		9	0.60 ± 0.97 (0 – 3)	100.0%	0.00% - 50%	50.0%
5	Off	1.5	0.50 ± 0.71 (0 – 2)			
		3	3.10 ± 2.02 (1 – 8)			
		6	1.40 ± 1.07 (0 – 3)			
		9	1.30 ± 1.25 (0 – 4)			
	On	1.5	0 ± 0 (0 – 0)	100.0%	-	-
		3	0.30 ± 0.48 (0 – 1)	100.0%	0.00% - 25%	33.3%
		6	0 ± 0 (0 – 0)	100.0%	-	50.0%
		9	0.20 ± 0.63 (0 – 2)	100.0%	-	100.0%

The impact of Far-UVC on reducing the load appears to be significant, with the majority of samples detecting no deposited colonies after the lamps were switched on. However, the concentration of deposited microorganisms was low even when the Far-UV light was off as experiments were carried out with small diameter aerosols with a relatively low deposition rate. Furthermore, it should be pointed out that concentration levels differed across different experimental days, implying that the relative reduction in microbial load is more informative than the absolute concentration values.

Far-UVC and changes to air flow (Ventilation regime)

Table 4 and **Figure 4** show the impact of changing the ventilation supply and extract locations and the sampling location on the inactivation of *S.aureus* in air at a ventilation rate of 3 ACH. Under a high-low ventilation regime for a single lamp setup, bioaerosol reduction was observed at 95.5% at location L_{A1} (near the low air extract). While with low to high, a

reduction of 90.3% was recorded at the L_{A1} location and 93.1% at L_{A2} (near the high air extract).

Table 4: Far-UVC performance in reducing airborne microorganisms under varied ventilation regimes (L_{A1}: Near the low air extract and L_{A2}: Near the high air extract).

No. of devices	Far-UVC light (222 nm)	Ventilation regime	Sampling point	Bioaerosols load (cfu/m3), Mean ± SD (Min-Max)	% Reduction			Experiment Resolution
					Median	LOG	IQR	
1	Off	High-Low	L_{A1}	1711 ± 391 (1286 – 2393)				
		Low-High	L_{A1}	2885 ± 573 (1928 – 3803)				
		Low-High	L_{A2}	6138 ± 997 (4601 – 7500)				
	On	High-Low	L_{A1}	75 ± 32 (36 – 143)	95.50%	1.35	93.3% - 97.8%	2.20%
		Low-High	L_{A1}	282 ± 110 (125– 446)	90.30%	1.01	87.3% - 92.0%	0.60%
		Low-High	L_{A2}	413 ± 98 (197 – 482)	93.10%	1.16	92.8%-93.7%	0.30%
5	Off	High-Low	L_{A1}	3339 ± 424 (2714 – 4000)				
		Low-High	L_{A1}	1291 ± 428 (607 – 1964)				
		Low-High	L_{A2}	5930 ± 1465 (3289– 8142)				
	On	High-Low	L_{A1}	64 ± 38 (0 – 107)	97.80%	1.67	97.0% - 98.9%	1.10%
		Low-High	L_{A1}	14 ± 8.2 (1 – 17)	98.60%	1.85	-	1.40%
		Low-High	L_{A2}	179 ± 48 (107 – 250)	97.10%	1.53	96.5% - 97.8%	0.30%

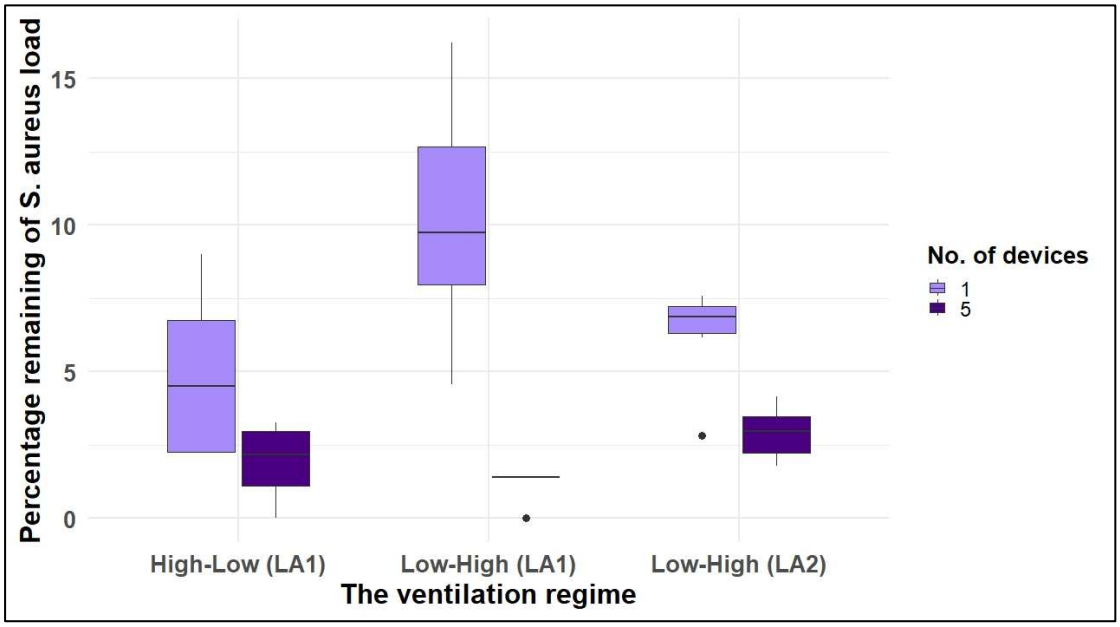


Figure 4: The performance of Far-UVC (222 nm) irradiation in reducing the concentration of *S. aureus* in the air at 3 ACH under different ventilation regimes (L_{A1}: Near the low air extract and L_{A2}: Near the high air extract).

The percentage reduction difference is more noticeable in cases with only one lamp. On the other hand, with five lamps, the percentage reduction is more consistent in varying ventilation regimes and sampling locations. The Far-UVC appears to be slightly more effective when the ventilation air is supplied from a high-level diffuser and extracted at low level with one lamp. However, there is not a clear pattern between ventilation regime and sample location seen in the results, and it is likely that in a reasonably well-mixed room, the ventilation rate is a more important parameter.

Far-UVC and short-range inactivation

The influence of Far-UVC on inactivation based on the distance from the microorganism source of infection was investigated as illustrated in **Figure 5**. Here the Far-UVC lamp was located centrally between the source and sampling points, with a distance of 2m between the two locations. The source and sampling location were selected to represent a typical head height distance between people which was recommended as a mitigation measure by a number of countries including the UK during the pandemic. This is compared to results with the same lamp but with the release and sample locations as in the scenarios above where the separation is 2.87m. Results indicate that in the closer proximity case where the exposure time will be lower, the inactivation during steady-state contamination conditions due to Far-UVC is reduced from 88.5% to 57.4% (**Figure 6** and **Table S1**).

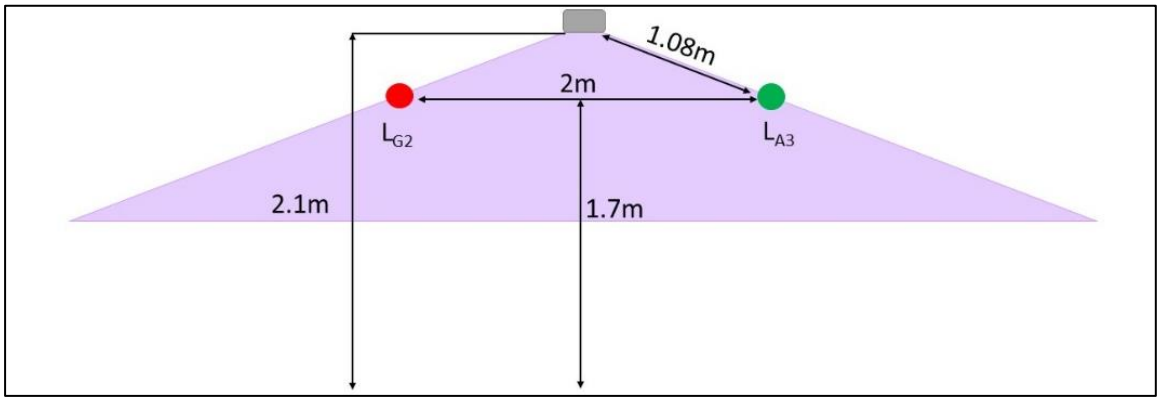
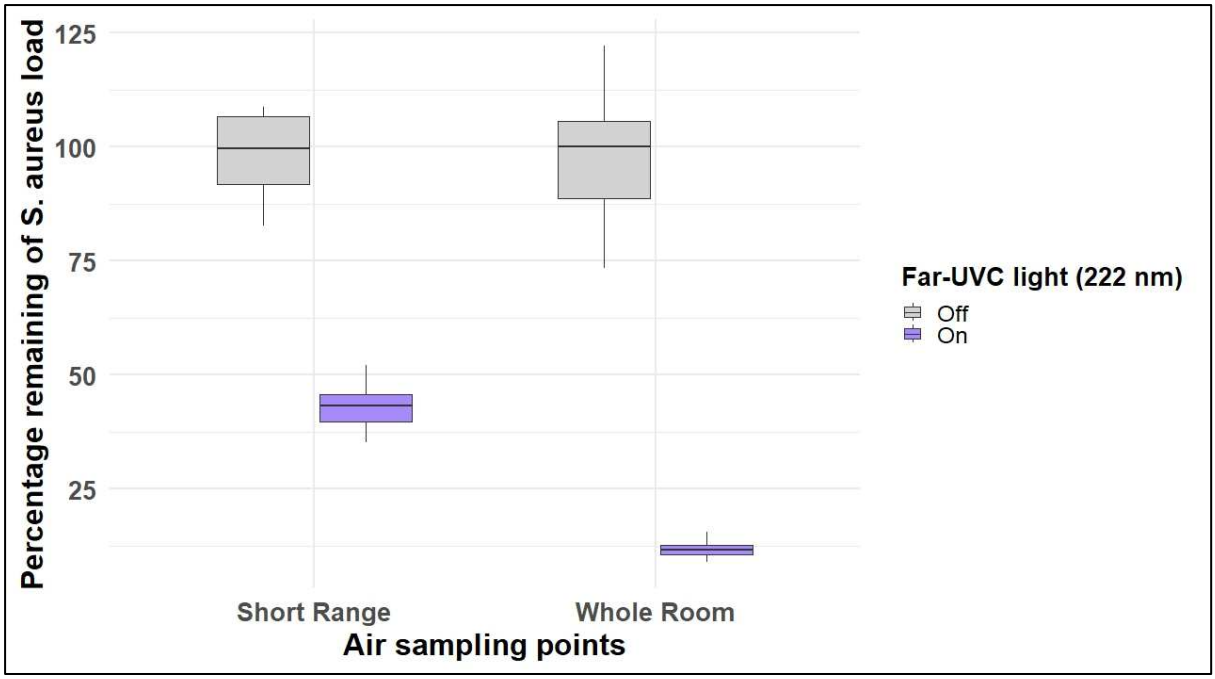


Figure 5: Graphical representation of the short-range distances experiment setup showing source (L_{G2}: near the middle Far-UVC lamp), Far-UVC lamp and sample locations (L_{A3}: near the middle Far-UVC lamp).



303

304 **Figure 6:** Far-UVC (222 nm) effectiveness in reducing airborne S. aureus at 3 ACH across

305 varying distances.

306 **Far-UVC and different pathogens**

307 Results from the final set of experiments with two different microbial species (*S. aureus* and
308 *P. aeruginosa*) at 3 ACH are shown in **Figure 7** and **Table S2**. While the inactivation of both
309 microbial species was significantly impacted by the Far-UVC light, the reductions were
310 substantial. The reduction achieved for *S. aureus* was 95.5% with one lamp and 94.9% for
311 *P. aeruginosa*. As the numbers of lamps used increased to five, reductions for *S. aureus*
312 were 97.8% and for *P. aeruginosa* were 100%. Care must be taken when comparing these
313 results because experimentally the release locations of the two pathogens differed. The
314 source of *P. aeruginosa* was released at the centre of the wall (L_{G3}) and *S. aureus* near the
315 high-level supply (L_{G1}), which could affect efficacy. This is because *P. aeruginosa* proved to
316 be sensitive to the tube initially used from outside the chamber used to release
317 microorganisms leading to very low concentrations prior to the device being switched on.

However, it is important to note that in both cases we report the relative concentration rather than absolute numbers, enabling better comparison within and between experiments.

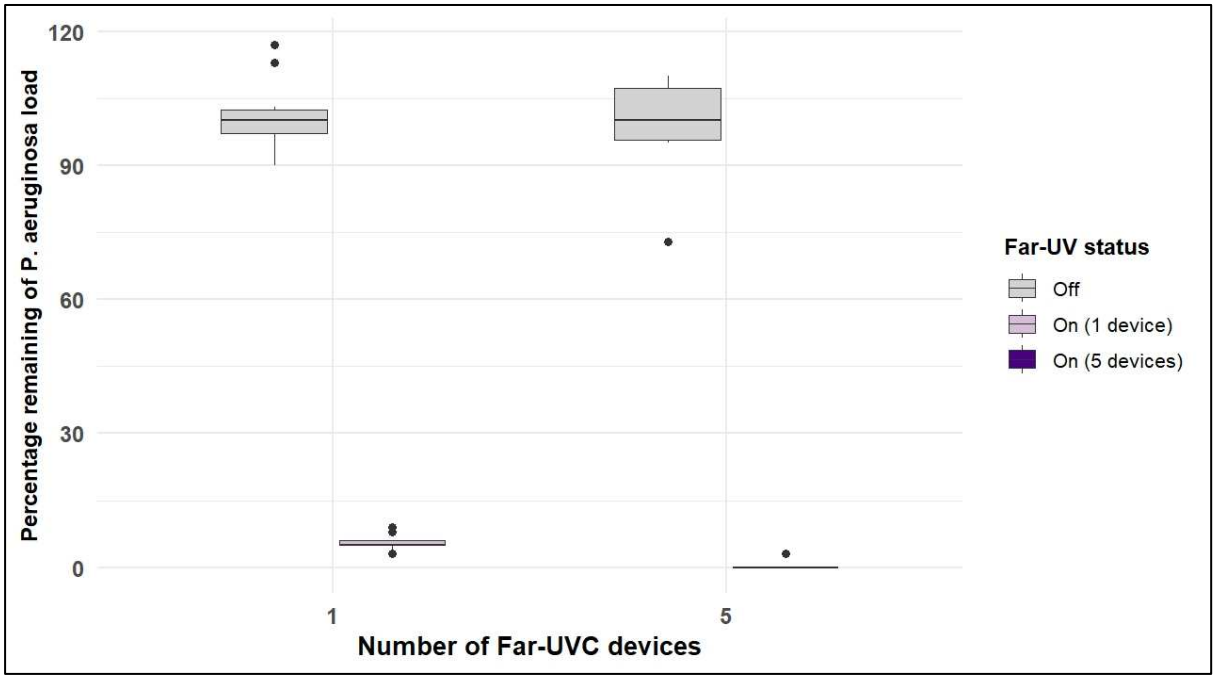


Figure 7: The performance of Far-UVC (222 nm) radiation in reducing the concentration of *P. aeruginosa* in the air at 3 ACH.

4. Discussion

The experimental study shows that Far-UVC effectively reduces the airborne pathogen load in a room under controlled conditions. We have tested devices against two microorganisms, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, with all experiments carried out to measure inactivation under continuous contamination conditions. The results demonstrate both are inactivated, with room scale reductions of over 90% in the majority of experiments, suggesting that Far-UVC is very likely to inactivate pathogens that are relevant to healthcare settings. Lab scale studies carried out by other groups internationally suggest that Far-UVC is also effective against a range of viruses and other pathogens [29] although we have not been able to test these at chamber scale.

The results from our preliminary work that demonstrated inactivation at one ventilation rate Eadie et al [18] have been shown in this study to be robust to changes in ventilation regime, ventilation rate and sample location. As expected Far-UVC is more effective when more lamps are used and hence there is a higher level of UVC in the room; experiments with 5 lamps lead to typical reductions of over 95% except at the highest air change rate, while those with one lamp are closer to 90%. We also see in experiments that having lamps

distributed across the room leads to results that have less variability than having a single UVC lamp in the room. Experimental results show that the difference with ventilation regime and sample location are small, and it is likely that the differences we see are driven by variations in experiments more than the influence of the set-up. As expected, the relative performance of the Far-UVC is better at a lower ventilation rate, where we also see less variation in the results.

Initial experiments to explore the ability of a Far-UVC device to inactivate microorganisms at closer proximity to a source show promise, with a reduced but still substantial reduction in concentration seen at the closer source-sampling distance set up. Similar findings were also seen in Xia et al [19] who measured *E.coli* concentrations of a small aerosol at much closer distances. Experiments to measure the influence of distance are challenging to set-up and a more detailed study is required to evaluate the impact of distance and orientation from a directional source to represent for example a cough. Results to show the impact of Far-UVC at reducing microbial deposition onto surfaces are also promising, however the very low values measured both with and without the devices in operation mean that confidence in these findings is lower. Further experiments carried out over longer time periods or using a different aerosol source that generates larger particles may enable a higher particle deposition rate and hence a more reliable determination of the effect of the Far-UVC devices.

A number of recent studies have shown that Far-UVC lamps can produce small amounts of ozone in indoor environments and that this may be a concern, particularly when ventilation rates are low [30]. As part of our studies, we attempted to measure ozone concentrations in the chamber under comparable conditions to the biological experiments. We detected small increases in ozone with generally higher concentrations with more lamps. However, our experimental results were inconsistent and further investigation suggested that the data was significantly influenced by the external air being brought into the chamber. We therefore calculated the theoretical ozone production rate, using the measured spectral irradiance of the lamp and the oxygen cross-section, as described by Peng et al [31]. The result was a ratio of ozone generation rate to fluence rate of 7.13 ppb $h^{-1}/(\mu Wcm^{-2})$. This is in excellent agreement with Peng et al., where a similar lamp (Ushio B1.5 (with diffuser)) had a ratio of 7.7 ppb $h^{-1}/(\mu Wcm^{-2})$. The resulting production rate is 4 ppb h^{-1} or 18 ppb h^{-1} for 1 (0.56 μWcm^{-2}) or 5 (2.54 μWcm^{-2}) lamps respectively [31], which produces a range of additional steady state ozone values of between 0.4 ppb (1 lamp, 0.56 μWcm^{-2} at 9 ACH) and 12 ppb (5 lamps, 2.54 μWcm^{-2} at 1.5 ACH) for our experimental conditions. It is likely to be feasible to implement Far-UVC to provide a good inactivation of microorganisms but without posing

an unacceptable risk from the generation of ozone or secondary chemistry. This trade-off may depend on the setting where the Far-UVC is deployed and may need to consider other air quality risks together with the vulnerability of occupants and the time of exposure to identify an appropriate balance. Further experiments are needed to more robustly quantify the generation of ozone in chamber scale studies; however, this is a longer-term goal for our studies as it will require significant modification to the chamber environment to provide a better control and measurement of the chemical composition of the outdoor air that enters the facility.

There were a number of limitations in our experiments. We have only considered two microorganisms, both bacteria in the timescale of this study. Although there is small scale laboratory data against a wider range of microorganisms including viruses [32, 33] it would be important to test against further microorganisms at full scale, as in Lu et al. [34].

Our experiments were all carried out at normal-to-warm room temperatures and normal indoor humidity (22°C ± 1°C and 48% ± 2%, respectively), so are representative of a large proportion of indoor spaces. Further research is needed at various temperatures and humidity levels, particularly as evidence from 254nm UVC work suggests that performance may be lower in higher humidity environments [35]. We have focused on the impact of Far-UVC on microorganisms in air, and alongside the air samples we have measured the impact on deposition onto surfaces which suggests a beneficial effect. However, we have not looked at the impact of Far-UVC on surface contamination over time and in environments where contamination can happen due to hand contacts as well as deposition. As Far-UVC is a technology which exposes the whole room to UVC light, this means that it has the potential to more widely contribute to surface hygiene. It is not considered as a decontamination technology in this study; however, it would be beneficial to understand the routine impacts on surface bioburden.

A further limitation is that our experiments are carried out using aerosolisation of the microorganisms in distilled water using a Collison nebuliser. This is a very common approach for aerosol chamber studies as it is a reliable method that generates a consistent aerosol with a narrow size range 0.3-3 µm with mean of 0.6 µm with geometric standard deviation of 1.6 [36]. However, this does not fully represent the aerosol size range or composition of human respiratory aerosols [37, 38] or other aerosol sources such as bed making or toilets that may be present in real world settings. Future experiments that consider a wider aerosol size at source and more realistic respiratory or environmental fluids are needed.

Our experiments were designed to consider the average whole-room reduction in pathogen load for a well-mixed room. This provides controlled experimental conditions which enabled us to investigate and compare the variables described here. Our experiments were not designed to consider real-life situations, where an infectious source and one or more recipients could be located anywhere within the room. Previous published studies have investigated such scenarios [39, 19, 40]. It will be important for future studies to investigate pathogens that are more resistant to UVC than those used in our study, as well as explore inactivation in realistic scenarios that consider different source locations and characteristics as well as the risk reduction for different occupants due to the Far UVC.

The study carried out here also focused primarily on the relationships with ventilation but did not consider detailed aspects around design, particular around how to optimise the application of Far UVC in terms of number and positioning of lamps or with other technologies such as filtration. Considering the results of our previous work [18] with our current study, we can see a 92% reduction in steady-state pathogen load when 5 lamps operate at around 14% (Medium) of their total output. In such a scenario the whole room average fluence rate is approximately 0.35 µWcm-2, producing 2.49 ppb h-1 ozone, which is less than a 1 ppb increase in the steady state ozone concentration. This provides excellent pathogen reduction without apparent significant photochemistry. Operating in a room at a lower ventilation rate would increase the photochemistry but allow for less Far-UVC to achieve the same inactivation (**Figure 3**). Further research is needed to investigate pathogens that are more resistant to UVC than those used in our study, as well as to look in detail at design parameters and interactions with other technologies. Approaches that use design optimisation, such as we have applied to 254nm upper room UVC [41, 42] could be a viable approach to exploring these trade-offs and interactions between technologies.

5. Conclusions

From the results in this study, we conclude that Far-UVC has substantial potential to reduce the concentration of microorganisms in the air. Experiments show that Far-UVC can effectively inactivate both *S. aureus* and *P. aeruginosa* bacteria in air at 3ACH, and for testing under different ventilation rates and ventilation regimes *S. aureus* was used in the experiments.

The Far-UVC light technology has shown significant microbial inactivation particularly at lower ventilation rates. This shows its robust applicability and efficacy in indoor settings. The effectiveness of inactivation increases when using a higher number of lamps which confirms the importance of the applied dose in the inactivation efficacy. A balance can be struck

between sufficient pathogen inactivation with minimal additional photochemistry. The differences in results with varying ventilation regime and sample location are marginal, and it may be due to variations in experiments more than the influence of changing the ventilation regimes. The microbial inactivation at short distances between the source and the collection point (such as a cough situation) showed a lower efficacy due to the short contact time with the Far-UVC light. Application of Far-UVC in real-life requires pairing it with ventilation and filtration systems to achieve maximum coverage through multiple low-powered lamps which maintain exposure safety parameters. User safety depends on correct positioning of lamps and proper ozone management during implementation. Further research is still necessary to understand interactions between Far UVC and environmental conditions and with other technologies such as filtration, and to confirm the findings in this study in real-world settings.

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Author contributions

KW, EE, DJB, CJN conceived the study and secured funding, KW, EE, CJN, LAF, CSA designed the experimental protocol, WH, ET carried out the experiments, WH, EE, MFK carried out the experimental analysis, KW, EE, CJN, WH led the evaluation of results, all authors contributed to writing and editing the manuscript.

Competing interest declaration

CJN was co-chair of the Environment and Modelling sub-group for the Scientific Advisory Group for Emergencies (SAGE) and has provided scientific advice on transmission of Covid-19 across UK government and to WHO. DJB and other coinventors have a granted US patent entitled ‘Apparatus, method and system for selectively affecting and/or killing a virus’ (US10780189B2). Columbia University (the parent institution of DJB) has licensed aspects of filtered UV light technology to USHIO Inc, and has received a research gift from Lumen Labs, a company producing Far-UVC sources. The remaining authors have no competing interests to declare.

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482 **Supplementary information**

483 **Table S1:** The performance of one Far-UVC light device to reduce the concentration of
484 airborne microorganisms at different distances between source and sample with mechanical
485 ventilation of 3 ACH.

Far-UVC light (222 nm)	Air sampling collection point	Bioaerosols load (cfu/m3), Mean ± SD (Min-Max)	% Reduction			Experiment Resolution
			Median	LOG	IQR	
Off	2m away from the source, 1.08m from the Far-UVC device (L_{A3}: Near the middle device [2.68 m, 2.1 m, 1.7 m])	2436 ± 227 (2054 - 2696)				
	2.87m away from the source, 2.46m away from the Far-UVC device (L_{A1}: Near the low air extract [2.85 m, 0.65 m, 0.5 m])	2348 ± 351 (1768 - 2946)				
On	2m away from the source, 1.08m from the Far-UVC device (L_{A3}: Near the middle device [2.68 m, 2.1 m, 1.7 m])	1045 ± 124 (857 - 1268)	57.4%	0.4	55.1% - 61.0%	0.7%
	2.87m away from the source, 2.46m away from the Far-UVC device (L_{A1}: Near the low air extract [2.85 m, 0.65 m, 0.5 m])	282 ± 44 (214 - 375)	88.5%	0.9	87.4%- 89.4.7%	0.7%

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Table S2: The performance of Far-UVC light to reduce the concentration of airborne microorganisms for different species with mechanical ventilation of 3 ACH.

No. of device	Far-UVC light (222 nm)	Generation source	Species	Bioaerosols load (cfu/m3), Mean ± SD (Min-Max)	% Reduction			Experiment Resolution
					Median	LOG	IQR	
1	Off	L_{G1}: Through a tube and near the supply fresh air (0.5 m, 3.55 m, 1.7 m).	*SA*	1711 ± 391 (1286 - 2393)				
		L_{G3}: Through a hole in the wall directly to the chamber (0 m, 2.1 m, 1.2 m).	*PA*	567 ± 48 (507 - 657)				
	On	L_{G1}: Through a tube and near the supply fresh air (0.5 m, 3.55 m, 1.7 m).	*SA*	75 ± 32 (36 - 143)	95.5%	1.3	93.3% - 97.8%	2.2%
		L_{G3}: Through a hole in the wall directly to the chamber (0 m, 2.1 m, 1.2 m).	*PA*	31 ± 11 (14 - 50)	94.9%	1.3	93.6% - 94.9%	1.4%
5	Off	L_{G1}: Through a tube and near the supply fresh air (0.5 m, 3.55 m, 1.7 m).	*SA*	3339 ± 424 (2714 - 4000)				

		L_{G3}: Through a hole in the wall directly to the chamber (0 m, 2.1 m, 1.2 m).	*PA*	471 ± 51 (345 - 524)				
	On	L_{G1}: Through a tube and near the supply fresh air (0.5 m, 3.55 m, 1.7 m).	*SA*	64 ± 38 (0 - 107)	97.8%	1.7	97.0% - 98.9%	1.1%
		L_{G3}: Through a hole in the wall directly to the chamber (0 m, 2.1 m, 1.2 m).	*PA*	2 ± 6 (0 - 12)	100.0%	-	-	2.5%

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