

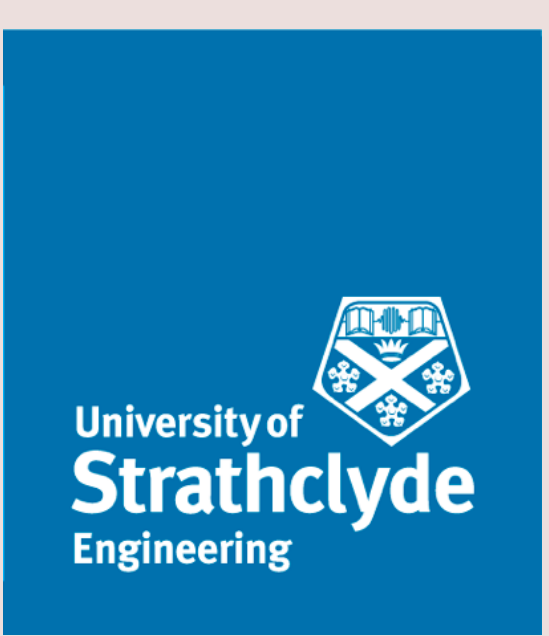
# Efficacy of a Low Irradiance Antimicrobial 405-nm Visible Light System for Inactivation of Bacteriophage Phi6 as a Surrogate for SARS-CoV-2

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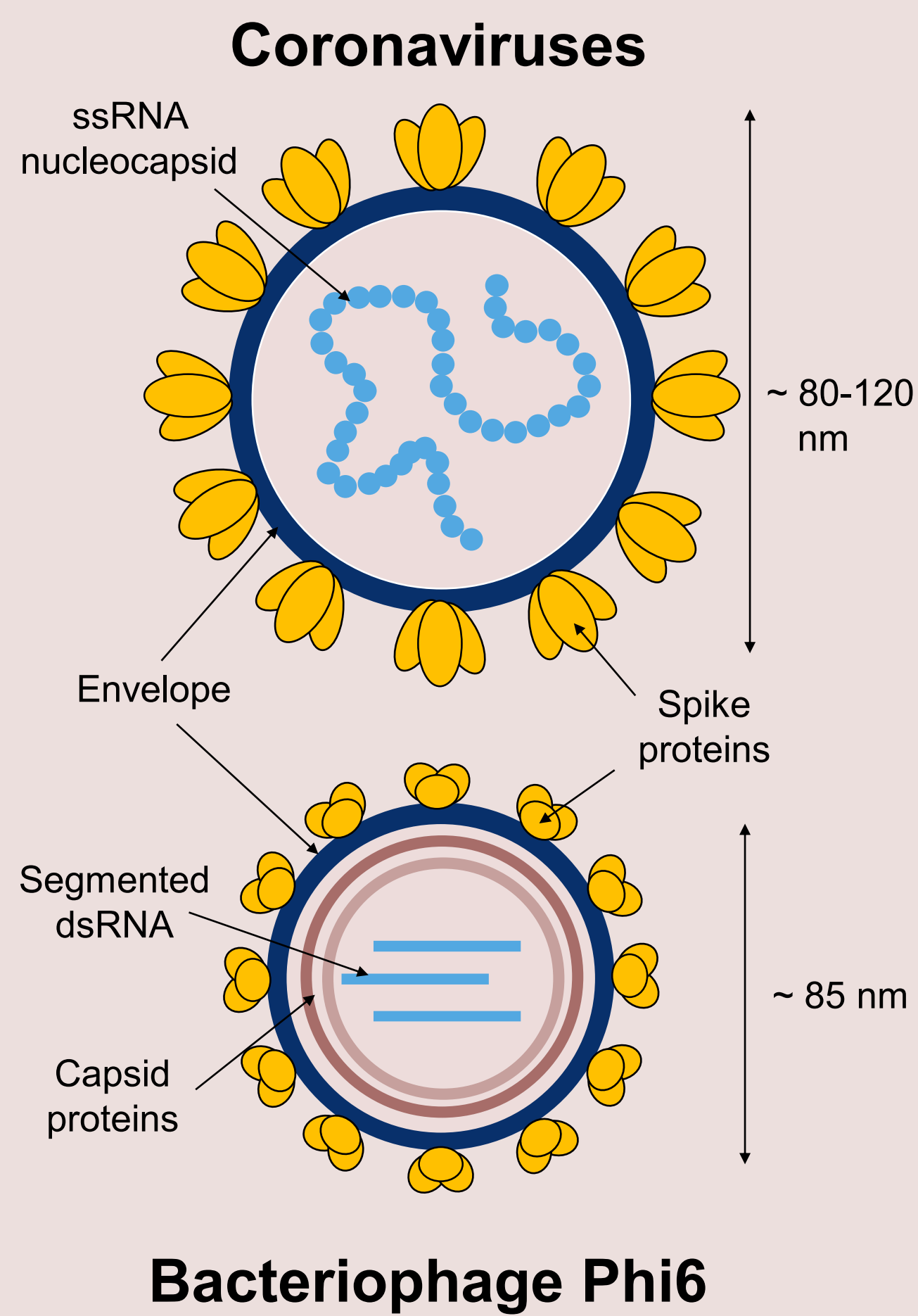
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## INTRODUCTION

- The COVID-19 pandemic has increased the necessity for novel strategies to safely decontaminate public areas.
- Low irradiance 405-nm light environmental decontamination systems (EDS) have recently been developed, with studies demonstrating their ability to safely reduce bacterial levels within occupied healthcare environments<sup>1-5</sup>.
- The 405-nm light emitted from these systems induces the excitation of photosensitive molecules, namely porphyrins, within microbial cells, initiating the production of reactive oxygen species and ultimately cell death<sup>6,7</sup>.
- Due to the absence of porphyrin molecules from viral structures, the viricidal properties of this technology are less understood.



## METHODS

- A ceiling-mounted 405-nm light EDS in 'blue-only' mode (Fig. 1A) was used to expose phage samples.
- Bacteriophage phi6 was suspended in SM buffer and artificial human saliva at low ( $\sim 10^3$  PFU mL<sup>-1</sup>) and high ( $\sim 10^7$  PFU mL<sup>-1</sup>) densities, and 3 mL volumes were light-exposed at  $\sim 1.5$  m below the light source (Fig. 1B).
- At this distance, the irradiance provided was  $\sim 0.5$  mW cm<sup>-2</sup>, which is representative of the irradiance levels which can illuminate high-touch surfaces within clinical areas (e.g., bed rails, tray table, bedside table, IV poles).
- Post-exposure, the surviving phage population was determined using a double agar overlay plaque assay, overnight incubation and subsequent enumeration.

This study investigates the efficacy of low irradiance 405-nm light systems for the inactivation of bacteriophage phi6 as a surrogate for SARS-CoV-2.

## RESULTS

Control (non-exposed) SM buffer (exposed) Artificial saliva (exposed)

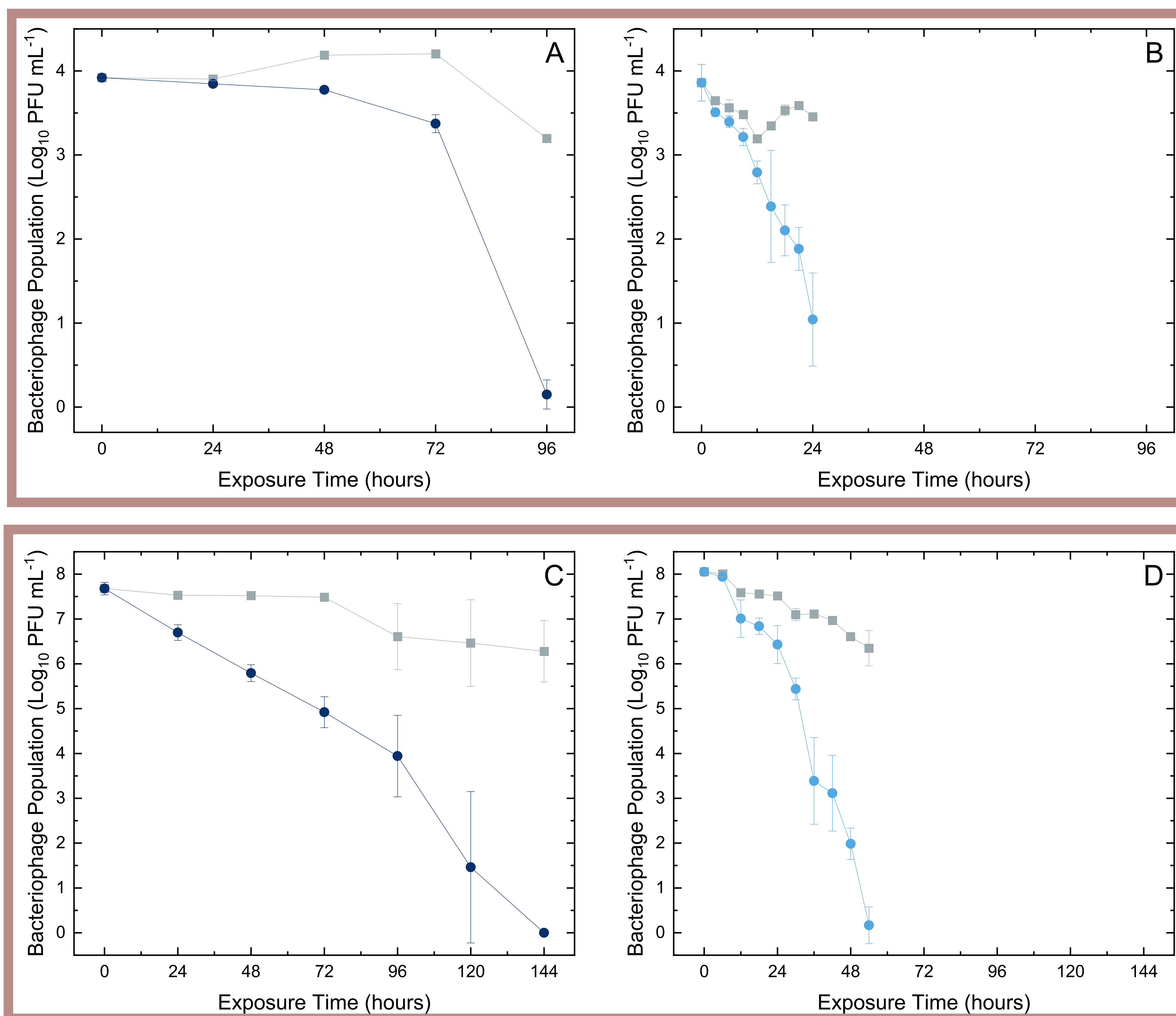


Fig. 2. Inactivation of bacteriophage phi6 suspended at  $10^{3-4}$  PFU mL<sup>-1</sup> in (A) SM buffer and (B) artificial saliva, and at  $10^{7-8}$  PFU mL<sup>-1</sup> in (C) SM buffer and (D) artificial saliva, upon exposure to increasing doses of  $\sim 0.5$  mWcm<sup>-2</sup> 405-nm light ( $n \geq 4 \pm SD$ ).

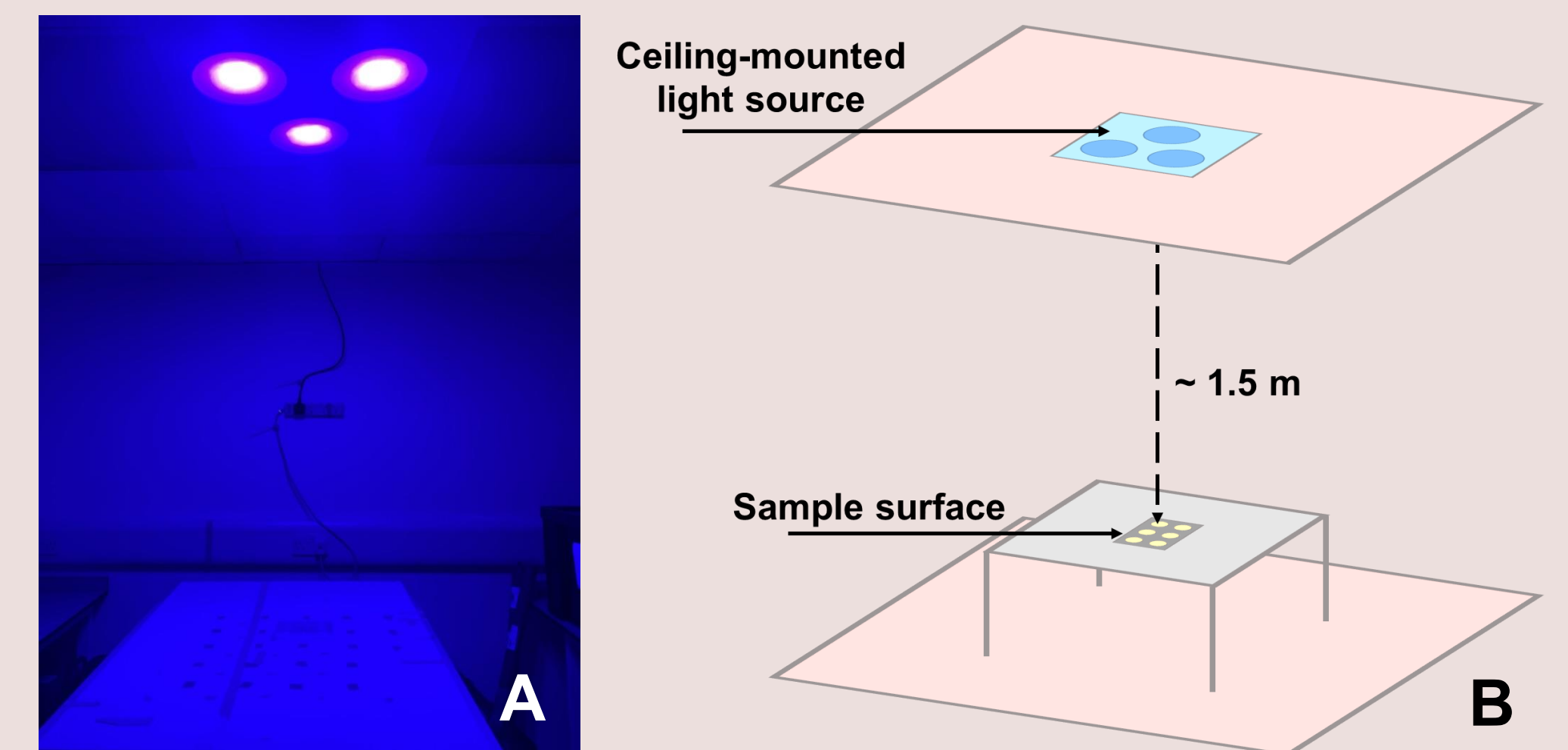


Fig. 1. Experimental set-up (A) photographed and (B) modelled.

Table 1. Comparison of mean percentage phage reductions when exposed at low and high seeding densities in SM buffer and artificial saliva. Asterisks (\*) represents a significant reduction in comparison to non-exposed controls ( $P \leq 0.05$ ).

Exposure Time (hours)	Mean Percentage Phage Reduction (%)			
	SM buffer $10^3$ PFU mL <sup>-1</sup>	SM buffer $10^7$ PFU mL <sup>-1</sup>	Artificial saliva $10^3$ PFU mL <sup>-1</sup>	Artificial saliva $10^7$ PFU mL <sup>-1</sup>
6			31.36*	12.12
12			58.63*	61.87*
18			95.60*	78.95*
24	12.19*	84.04*	99.41*	87.69*
36				99.94*
48	60.48*	97.97*		99.99*
72	83.06*	99.64*		
96	99.32*	98.98*		
120		99.89*		

- Low-irradiance 405-nm light significantly reduced ( $P \leq 0.05$ ) phi6 suspended in both artificial saliva and SM buffer at both low and high seeding densities.
- Inactivation was significantly enhanced ( $P \leq 0.05$ ) when exposed in artificial saliva: **83.3-87.5%** and **50% less dose** was required for a 1-log<sub>10</sub> reduction of phi6 at low and high seeding densities, respectively.
- Significantly greater exposure times were required for complete/near-complete inactivation at higher densities ( $P \leq 0.05$ ); however, the dose requirements for a 1-log<sub>10</sub> reduction were similar for both low and high seeding densities.

## CONCLUSIONS

- Low-irradiance 405-nm light systems can successfully reduce phi6 at irradiances typically implemented for whole-room decontamination.
- Inactivation was successfully achieved in SM buffer; suggesting 405-nm light viral inactivation is possible in the absence of external photosensitisers, possibly due to light interactions with the phage envelope<sup>8</sup>.
- An increased inactivation efficacy was observed when exposed in saliva, suggesting photosensitive components within this suspension can act as external photosensitisers and impart localised oxidative damage to the phage; highlighting its potential for decontamination of SARS-CoV-2 in environmental respiratory droplets.
- These findings establish a basis for further investigation into the viricidal properties of 405-nm light and demonstrate that, with further investigation, low irradiance 405-nm light systems hold potential as a novel approach to tackling COVID-19 transmission within occupied settings.

## References

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