

RESEARCH NOTE

Open Access



# Blue light inactivation of the enveloped RNA virus Phi6

Petra Vatter, Katharina Hoenes and Martin Hessling\*

## Abstract

**Objective:** Ultraviolet radiation is known for its antimicrobial properties but unfortunately, it could also harm humans. Currently, disinfection techniques against SARS-CoV-2 are being sought that can be applied on air and surfaces and which do not pose a relevant threat to humans. In this study, the bacteriophage phi6, which like SARS-CoV-2 is an enveloped RNA virus, is irradiated with visible blue light at a wavelength of 455 nm.

**Results:** For the first time worldwide, the antiviral properties of blue light around 455 nm can be demonstrated. With a dose of 7200 J/cm<sup>2</sup>, the concentration of this enveloped RNA virus can be successfully reduced by more than three orders of magnitude. The inactivation mechanism is still unknown, but the sensitivity ratio of phi6 towards blue and violet light hints towards an involvement of photosensitizers of the host cells. Own studies on coronaviruses cannot be executed, but the results support speculations about blue-susceptibility of coronaviruses, which might allow to employ blue light for infection prevention or even therapeutic applications.

**Keywords:** Phi6, Photoinactivation, Blue light, Coronavirus, SARS-CoV-2

## Introduction

Since December 2019, a new coronavirus capable of causing the severe pulmonary infection CoVid-19 has been spreading worldwide, and is therefore referred to as SARS-CoV-2 (severe acute respiratory syndrome coronavirus). As the number of infected and fatalities continues to rise, with more than 100 million infections and more than 2 million fatalities at the beginning of February 2021 [1], disinfection options are being sought to contain the further spread of the virus. Chemical disinfectants, heat, and ultraviolet radiation are successful against the virus [2–6], but can also be harmful to humans.

In recent years, visible blue and violet light has been employed to inactivate bacteria and fungi without particularly harming human cells [7–16]. The mechanism of action, which is similar for prokaryotic and eukaryotic cells, is based on endogenous photosensitizers naturally

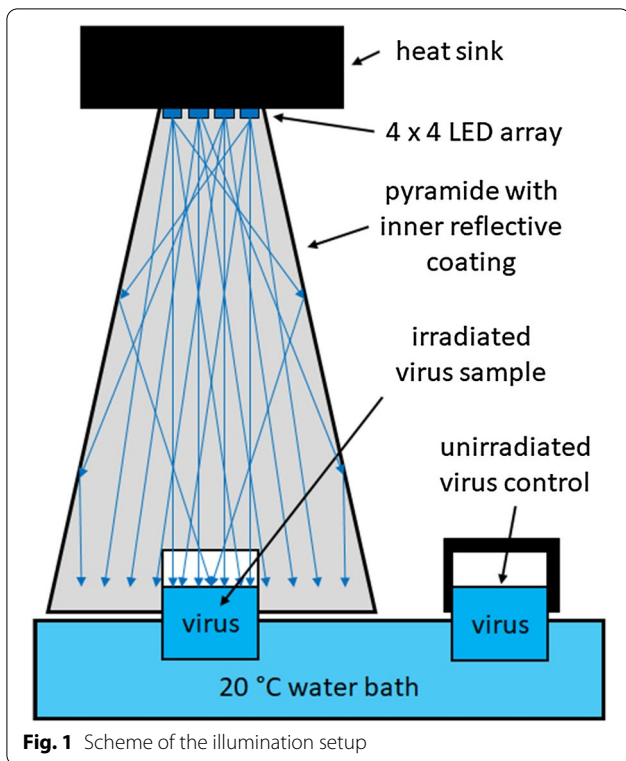
occurring in these microorganisms, such as porphyrins or flavins [17–23]. These photosensitizers absorb visible light of specific wavelengths and generate so-called reactive oxygen species (ROS) in the presence of oxygen, including <sup>1</sup>O<sub>2</sub>, O<sub>2</sub><sup>•-</sup>, H<sub>2</sub>O<sub>2</sub> and HO<sup>•</sup>, which attack and kill the cells from inside.

Initial studies reveal that violet light with a wavelength of 405 nm has an inactivating effect on viruses [24–26]. This is even true for the bacteriophage phi6, which, like the SARS-CoV-2 virus, is an enveloped RNA virus [27]. Therefore, it is hoped that SARS-CoV-2 is also sensitive to violet light.

Studies on the effect of blue, non-violet light on any viruses do not exist so far, although this wavelength range (450–470 nm) has advantages over violet light. It is even less harmful to human cells [9–11, 28] and exhibits a higher penetration depth into human tissue, which might lead to future local therapies that try to fight coronaviruses in the human body, if coronaviruses exhibit a sensitivity to visible light. At least, some local blue or violet illumination applications have been investigated for the

\*Correspondence: Martin.Hessling@thu.de  
Institute of Medical Engineering and Mechatronics, Ulm University of Applied Sciences, Ulm, Germany





**Fig. 1** Scheme of the illumination setup

treatment of bacterial or fungal infections, e. g. as therapy for acne [29, 30], *Helicobacter pylori* infections in the stomach [31], vaginal infections [32] and for the prevention of ventilator-associated pneumonia [33].

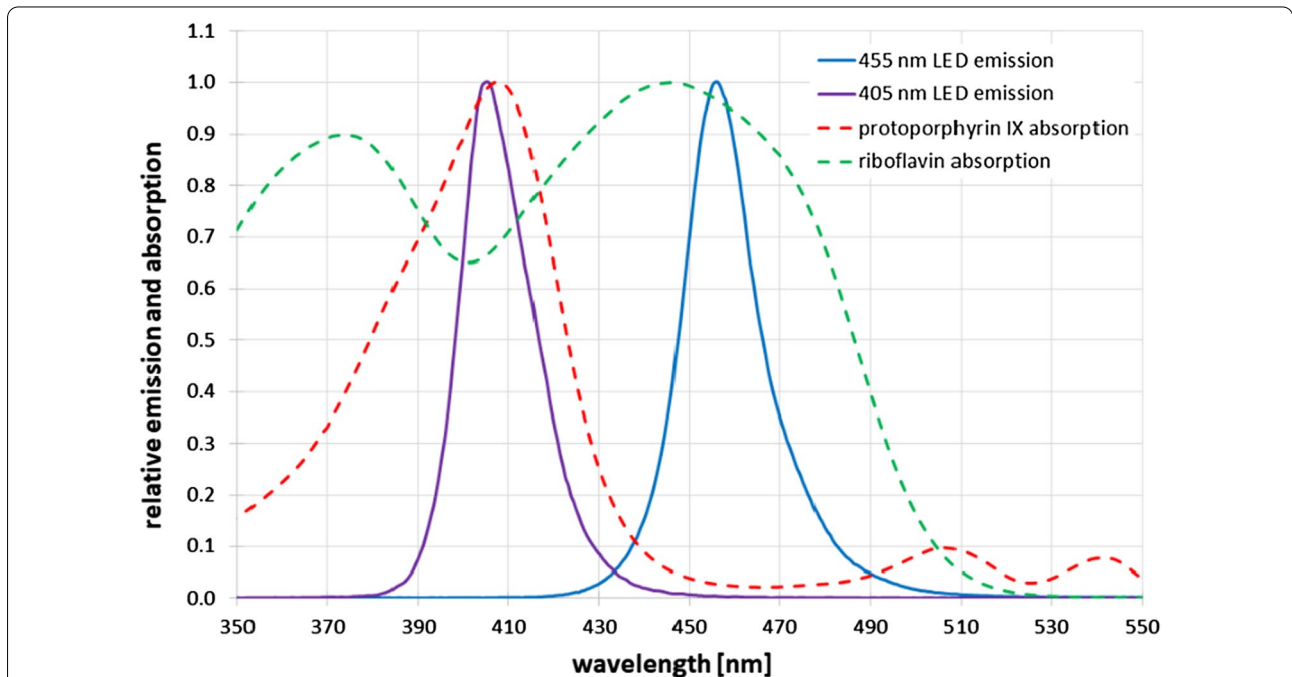
Unfortunately, we are not allowed to work with coronaviruses in our laboratory. Therefore, in the study presented here, experiments on the inactivation of phi6—as a non-pathogenic coronavirus surrogate—are performed with 455 nm blue light and compared to the results of a previous 405 nm investigation [27].

**Main text**

**Method**

**Irradiation setup**

The description of the irradiation setup is presented schematically in Fig. 1. Two glass beakers containing a virus-containing solution are kept at approximately 20 °C using a temperature-controlled water bath. One of the samples is irradiated from above by an array of 16 (4 × 4) 455 nm LEDs of RP-Technik GmbH (Rodgau, Germany). A hollow pyramid with reflective coating at the inside provides a homogeneous irradiance of up to 50 mW/cm<sup>2</sup> in the sample plane at a distance of 28 cm. The emission spectrum of the employed 455 nm LEDs is given in Fig. 2, together with the emission spectrum of the 405 nm LED employed by Vatter et al. in a former study [27] and the absorption of known bacterial photosensitizers. This illustrates that at least for bacteria 405 nm and blue 455 nm irradiation involve different photosensitizers. The second beaker glass is shielded from light and serves as a control.



**Fig. 2** 455 nm LED emission spectrum, with additional spectrum of the 405 nm LED of [27] and typical bacterial (!) photosensitizer absorption spectra [34] for comparison

**Microbiological experiments**

Test virus is the bacteriophage phi6 (DSM 21518), which is multiplied by its host bacterium *Pseudomonas syringae* (DSM 21482). For the experiments, approximately  $1.5 \times 10^7$  viruses or plaque forming units (PFU) per ml of a phosphate buffered saline (PBS) solution are prepared. Samples are drawn for 0 h, 8 h, 16 h, 24 h, 32 h and 40 h of irradiation. At the end of each irradiation experiment, a double agar overlay plaque assay is performed: small volumes of the irradiated and non-irradiated virus samples are first mixed with *Pseudomonas syringae* and then poured as a liquid agar layer onto solid agar plates. In the absence of replicable viruses, bacteria will multiply in the agar and provide detectable turbidity. However, existing phi6 can infect and lyse bacteria. This creates holes/plaques in the agar turbidity from which the concentration of replicable phi6 in the samples and thus the disinfection effect of the 455 nm radiation can be calculated [27, 35].

**Results**

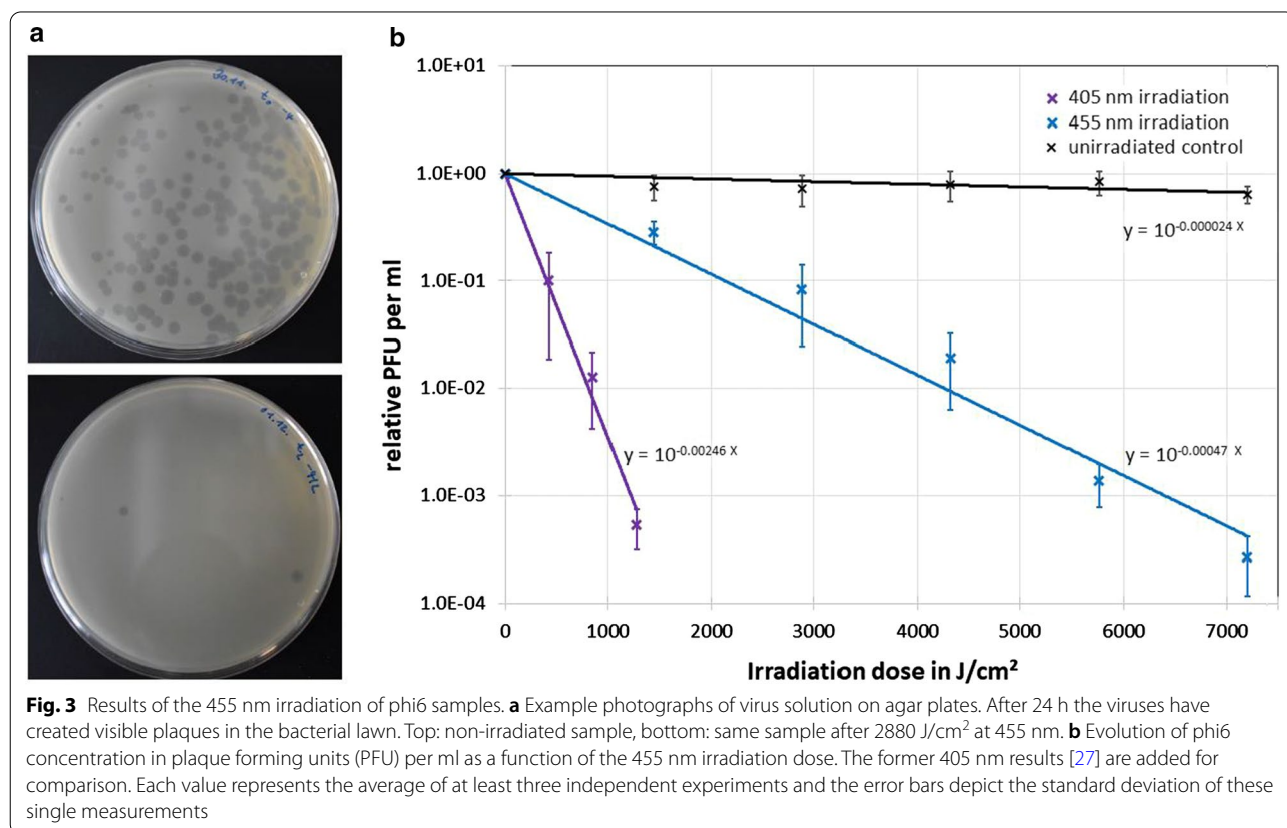
At least three technical replicates were performed of each individual irradiation dose up to  $7200 \text{ J/cm}^2$  over a period of up to 40 h and each series of measurements was

executed three times. Typical results for a non-irradiated and an irradiated virus sample with the double agar overlay plaque assay are illustrated in Fig. 3. The difference in the number of plaques—and therefore viruses—between non-irradiated and irradiated sample is evident. Figure 3b reveals the quantitative results. The phi6 concentrations in the non-irradiated samples hardly changed during the 40 h duration of the experiment, but in the irradiated samples the virus concentration was successfully reduced by more than three orders of magnitude after  $7200 \text{ J/cm}^2$  at 455 nm. The necessary log-reduction dose is about  $2130 \text{ J/cm}^2$  at 455 nm—compared to a log-reduction dose of approximately  $430 \text{ J/cm}^2$  at 405 nm according to Vatter et al. [27].

**Discussion**

For the first time, it could be demonstrated that the enveloped RNA virus phi6 is sensitive to visible blue light with a wavelength of 455 nm. The sensitivity is about 5 times lower than its 405 nm sensitivity, which was observed in a previous study [27]. This ratio is a typical sensitivity ratio known for pseudomonads and bacteria in general between these wavelengths [36, 37].

Nevertheless, the virus sensitivity to visible light is unexpected. In bacteria the presence of endogenous



photosensitizers like porphyrins and flavins is well known, because they are results of the bacterial metabolism. The virus however exhibits no metabolism and should not need or produce such photosensitizers. Even if it contains one photosensitizer this should be effective either at 405 nm or 455 nm—but not at both wavelengths. The fact that the virus concentration is reduced at both wavelengths and the sensitivity ratio between 455 and 405 nm, which is similar to the above mentioned typical bacterial ratios, gives room for the speculation that the virus unintentionally takes along the bacterial photosensitizers of its host (*Pseudomonas syringae*) when it assembles its envelope.

## Conclusion

Whether the more important SARS-CoV-2 also contains photosensitizers and exhibits photoinactivation sensitivity towards blue or visible light is unknown so far, but there are hints that this coronavirus might at least contain porphyrins [38], which would possibly result in a sensitivity towards 405 nm irradiation. The advantage of a 455 nm light sensitivity could be the higher penetration depth of blue light in human tissue compared to violet light in a—speculative—future antiviral therapy.

## Limitations

Coronaviruses and phi6 are both enveloped RNA viruses, and phi6 has often been applied as coronavirus surrogate in the past, but so far there is no prove for any sensitivity of coronaviruses towards blue or violet light. Unfortunately, our lab does not have the required security clearance for coronavirus experiments.

## Abbreviations

LED: Light emitting diode; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; CoVid 19: Covid coronavirus disease 2019; RNA: Ribonucleic acid; ROS: Reactive oxygen species; LED: Light emitting diode; DSM: Deutsche Sammlung von Mikroorganismen und Zellkulturen; PBS: Phosphate buffered saline.

## Acknowledgements

Not applicable.

## Authors' contributions

PV, KH and MH designed the experiments. PV carried out the experiments and analyzed the data. PV and MH drafted the manuscript. PV, KH and MH revised the final manuscript. All authors read and approved the final manuscript.

## Funding

Open Access funding enabled and organized by Projekt DEAL. The 455 nm irradiation experiments were financially supported by RP-Technik GmbH (Rodgau, Germany). The article processing charge was funded by the Baden-Württemberg Ministry of Science, Research and Culture and the Ulm University of Applied Sciences in the funding program Open Access Publishing.

## Availability of data and materials

All data generated or analyzed during this study are included in this published article.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that there is no conflict of interest.

Received: 10 March 2021 Accepted: 6 May 2021

Published online: 17 May 2021

## References

1. Coronavirus Resource Center. COVID-19 dashboard: (Global Map). 2021. <https://coronavirus.jhu.edu/map.html>. Accessed 6 Feb 2021.
2. Hessling M, Hönes K, Vatter P, Lingenfelder C. Ultraviolet irradiation doses for coronavirus inactivation—review and analysis of coronavirus photoinactivation studies. *GMS Hyg Infect Control*. 2020;15:Doc8. <https://doi.org/10.3205/dgkh000343>.
3. Kampf G, Todt D, Pfaender S, Steinmann E. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. *J Hosp Infect*. 2020;104:246–51. <https://doi.org/10.1016/j.jhin.2020.01.022>.
4. Kampf G, Voss A, Scheithauer S. Inactivation of coronaviruses by heat. *J Hosp Infect*. 2020. <https://doi.org/10.1016/j.jhin.2020.03.025>.
5. Kratzel A, Todt D, V'kovski P, Steiner S, Gultom M, Thao TTN, et al. Inactivation of severe acute respiratory syndrome coronavirus 2 by WHO-recommended hand rub formulations and alcohols. *Emerg Infect Dis*. 2020;26:1592–5. <https://doi.org/10.3201/eid2607.200915>.
6. Hessling M, Hoenes K, Lingenfelder C. Selection of parameters for thermal coronavirus inactivation—a data-based recommendation. *GMS Hyg Infect Control*. 2020. <https://doi.org/10.3205/dgkh000351>.
7. McDonald RS, Gupta S, Maclean M, Ramakrishnan P, Anderson JG, Macgregor SJ, et al. 405 nm Light exposure of osteoblasts and inactivation of bacterial isolates from arthroplasty patients: potential for new disinfection applications? *Eur Cell Mater*. 2013;25:204–14.
8. Wang T, Dong J, Yin H, Zhang G. Blue light therapy to treat candida vaginitis with comparisons of three wavelengths: an in vitro study. *Lasers Med Sci*. 2020. <https://doi.org/10.1007/s10103-019-02928-9>.
9. Liebmann J, Born M, Kolb-Bachofen V. Blue-light irradiation regulates proliferation and differentiation in human skin cells. *J Invest Dermatol*. 2010;130:259–69. <https://doi.org/10.1038/jid.2009.194>.
10. Bumah VV, Masson-Meyers DS, Awosika O, Zacharias S, Enwemeka CS. The viability of human cells irradiated with 470-nm light at various radiant energies in vitro. *Lasers Med Sci*. 2021. <https://doi.org/10.1007/s10103-021-03250-z>.
11. Makdoui K, Hedin M, Bäckman A. Different photodynamic effects of blue light with and without riboflavin on methicillin-resistant *Staphylococcus aureus* (MRSA) and human keratinocytes in vitro. *Lasers Med Sci*. 2019;34:1799–805. <https://doi.org/10.1007/s10103-019-02774-9>.
12. Ramakrishnan P, Maclean M, MacGregor SJ, Anderson JG, Grant MH. Differential sensitivity of osteoblasts and bacterial pathogens to 405-nm light highlighting potential for decontamination applications in orthopedic surgery. *J Biomed Opt*. 2014;19:105001. <https://doi.org/10.1117/1.JBO.19.10.105001>.
13. Dai T, Gupta A, Huang Y-Y, Yin R, Murray CK, Vrahas MS, et al. Blue light rescues mice from potentially fatal *Pseudomonas aeruginosa* burn infection: efficacy, safety, and mechanism of action. *Antimicrob Agents Chemother*. 2013;57:1238–45. <https://doi.org/10.1128/AAC.01652-12>.
14. Dai T, Gupta A, Huang Y-Y, Sherwood ME, Murray CK, Vrahas MS, et al. Blue light eliminates community-acquired methicillin-resistant *Staphylococcus aureus* in infected mouse skin abrasions. *Photomed Laser Surg*. 2013;31:531–8. <https://doi.org/10.1089/pho.2012.3365>.

15. Zhang Y, Zhu Y, Gupta A, Huang Y, Murray CK, Vrahas MS, et al. Antimicrobial blue light therapy for multidrug-resistant *Acinetobacter baumannii* infection in a mouse burn model: implications for prophylaxis and treatment of combat-related wound infections. *J Infect Dis*. 2014;209:1963–71. <https://doi.org/10.1093/infdis/jit842>.
16. Zhang Y, Zhu Y, Chen J, Wang Y, Sherwood ME, Murray CK, et al. Antimicrobial blue light inactivation of *Candida albicans*: in vitro and in vivo studies. *Virulence*. 2016;7:536–45. <https://doi.org/10.1080/21505594.2016.1155015>.
17. Ashkenazi H, Malik Z, Harth Y, Nitzan Y. Eradication of *Propionibacterium acnes* by its endogenous porphyrins after illumination with high intensity blue light. *FEMS Immunol Med Microbiol*. 2003;35:17–24. <https://doi.org/10.1111/j.1574-695X.2003.tb00644.x>.
18. Guffey JS, Wilborn J. In vitro bactericidal effects of 405-nm and 470-nm blue light. *Photomed Laser Surg*. 2006;24:684–8. <https://doi.org/10.1089/pho.2006.24.684>.
19. Maclean M, MacGregor SJ, Anderson JG, Woolsey G. High-intensity narrow-spectrum light inactivation and wavelength sensitivity of *Staphylococcus aureus*. *FEMS Microbiol Lett*. 2008;285:227–32. <https://doi.org/10.1111/j.1574-6968.2008.01233.x>.
20. Feuerstein O, Ginsburg I, Dayan E, Veler D, Weiss EI. Mechanism of visible light phototoxicity on *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. *Photochem Photobiol*. 2005;81:1186–9. <https://doi.org/10.1562/2005-04-06-RA-477>.
21. Amin RM, Bhayana B, Hamblin MR, Dai T. Antimicrobial blue light inactivation of *Pseudomonas aeruginosa* by photo-excitation of endogenous porphyrins: in vitro and in vivo studies. *Lasers Surg Med*. 2016;48:562–8. <https://doi.org/10.1002/lsm.22474>.
22. Plavskii VY, Mikulich AV, Tretyakova AI, Leusenka IA, Plavskaya LG, Kazyuchits OA, et al. Porphyrins and flavins as endogenous acceptors of optical radiation of blue spectral region determining photoinactivation of microbial cells. *J Photochem Photobiol B*. 2018;183:172–83. <https://doi.org/10.1016/j.jphotobiol.2018.04.021>.
23. Cieplik F, Spath A, Leibl C, Gollmer A, Regensburger J, Tabenski L, et al. Blue light kills *Aggregatibacter actinomycetemcomitans* due to its endogenous photosensitizers. *Clin Oral Investig*. 2014;18:1763–9. <https://doi.org/10.1007/s00784-013-1151-8>.
24. Richardson TB, Porter CD. Inactivation of murine leukaemia virus by exposure to visible light. *Virology*. 2005;341:321–9. <https://doi.org/10.1016/j.virol.2005.07.025>.
25. Tomb RM, Maclean M, Coia JE, Graham E, McDonald M, Atreya CD, et al. New proof-of-concept in viral inactivation: virucidal efficacy of 405 nm light against feline calicivirus as a model for norovirus decontamination. *Food Environ Virol*. 2016;9:159–67. <https://doi.org/10.1007/s12560-016-9275-z>.
26. Tomb RM, Maclean M, Herron PR, Hoskisson PA, MacGregor SJ, Anderson JG. Inactivation of Streptomyces phage C31 by 405 nm light: requirement for exogenous photosensitizers? *Bacteriophage*. 2014;4:e32129. <https://doi.org/10.4161/bact.32129>.
27. Vatter P, Hoenes K, Hessling M. Photoinactivation of the coronavirus surrogate phi6 by visible light. *Photochem Photobiol*. 2021;97:122–5. <https://doi.org/10.1111/php.13352>.
28. Opländer C, Hidding S, Werners FB, Born M, Pallua N, Suschek CV. Effects of blue light irradiation on human dermal fibroblasts. *J Photochem Photobiol B*. 2011;103:118–25. <https://doi.org/10.1016/j.jphotobiol.2011.02.018>.
29. Elman M, Slatkine M, Harth Y. The effective treatment of acne vulgaris by a high-intensity, narrow band 405–420 nm light source. *J Cosmet Laser Ther*. 2003;5:111–7.
30. Kawada A, Aragane Y, Kameyama H, Sangen Y, Tezuka T. Acne phototherapy with a high-intensity, enhanced, narrow-band, blue light source: an open study and in vitro investigation. *J Dermatol Sci*. 2002;30:129–35.
31. Ganz RA, Viveiros J, Ahmad A, Ahmadi A, Khalil A, Tolkooff MJ, et al. *Helicobacter pylori* in patients can be killed by visible light. *Lasers Surg Med*. 2005;36:260–5. <https://doi.org/10.1002/lsm.20161>.
32. Robatto M, Pavie MC, Garcia I, Menezes MP, Bastos M, Leite HJD, et al. Ultraviolet A/blue light-emitting diode therapy for vulvovaginal candidiasis: a case presentation. *Lasers Med Sci*. 2019;34:1819–27. <https://doi.org/10.1007/s10103-019-02782-9>.
33. Sicks B, Hoenes K, Spellerberg B, Hessling M. Blue LEDs in endotracheal tubes may prevent ventilator associated pneumonia. *Photobiomodul Photomed Laser Surg*. 2020. <https://doi.org/10.1089/pho.2020.4842>.
34. Hessling M, Wenzel U, Meurle T, Spellerberg B, Hönes K. Photoinactivation results of *Enterococcus moraviensis* with blue and violet light suggest the involvement of an unconsidered photosensitizer. *Biochem Biophys Res Commun*. 2020;533:813–7. <https://doi.org/10.1016/j.bbrc.2020.09.091>.
35. Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages by double agar overlay plaque assay. In: Walker JM, Cloukie MRJ, Kropinski AM, editors. *Bacteriophages*. Totowa: Humana Press; 2009. p. 69–76. [https://doi.org/10.1007/978-1-60327-164-6\\_7](https://doi.org/10.1007/978-1-60327-164-6_7).
36. Hessling M, Spellerberg B, Hoenes K. Photoinactivation of bacteria by endogenous photosensitizers and exposure to visible light of different wavelengths—a review on existing data. *FEMS Microbiol Lett*. 2016;364:fnw270. <https://doi.org/10.1093/femsle/fnw270>.
37. Hoenes K, Bauer R, Meurle T, Spellerberg B, Hessling M. Inactivation effect of violet and blue light on ESKAPE pathogens and closely related non-pathogenic bacterial species—a promising tool against antibiotic-sensitive and antibiotic-resistant microorganisms. *Front Microbiol*. 2020;11:612367. <https://doi.org/10.3389/fmicb.2020.612367>.
38. Wenzhong L, Hualan L. COVID-19: attacks the 1-beta chain of hemoglobin and captures the porphyrin to inhibit human heme metabolism. Preprint. ChemRxiv; 2020. <https://doi.org/10.26434/chemrxiv.11938173.v9>.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

