SASTA OLIPHANT SCIENCE AWARDS

Prize Winner

Scientific Inquiry

Year 11-12

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Prince Alfred College

OSA Scientific Inquiry

Subject: Biology

Topic: Effectiveness of visible light treatment on *Staphylococcus epidermidis* population (CFU/mL)

Research Question: To what extent does different coloured light (red, yellow, green, blue and violet) affect the population of *Staphylococcus epidermidis* after 30 minutes of light treatment, as measured by the absorbance at 600 nm and colony forming units per mL after 72 hours of incubation at 25°C?

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1. Chapter One: Introduction

1.1. Background Information

1.1.1. Epidemiology and Pathogenesis of Acne Vulgaris

Acne vulgaris is a skin condition that occurs when pilosebaceous units become blocked by sebum, dead skin cells and bacteria.[1] Sebum is an oily substance produced by sebaceous glands in the skin to provide a protective moisturising coating.[2] A pilosebaceous unit consists of a hair follicle and its associated sebaceous gland.[3] High sebum production causes pilosebaceous units to become blocked, thus forming acne lesions (pimples).[4]

Cutibacterium acnes is the main bacteria involved in pathogenesis of acne vulgaris.[5] It is anaerobic, living in the pilosebaceous units,[6] and thrives/proliferates in oily, nutritive environments created by high sebum production. This causes dysbiosis,[7] or an imbalance of bacteria, in the skin, stimulating inflammation of pimples.

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[1] Kahawita, T. (2021) *What bacteria causes acne? HealthMatch.* Available at: https://healthmatch.io/acne/what-bacteria-causes-acne#what-is-acne (Accessed: 19 December 2023).

[2] Cleveland Clinic Medical (2022) *Sebaceous glands: Function, location & secretion, Cleveland Clinic*. Available at: https://my.clevelandclinic.org/health/body/24538-sebaceous-glands (Accessed: 19 December 2023).

[3] Oakley, A. (2024) *Acne vulgaris: Features, types, and treatments - dermnet, DermNet®*. Available at: https://dermnetnz.org/topics/acne-vulgaris (Accessed: 19 December 2023).

[4] Elsaie, M. (2016) 'Hormonal treatment of acne vulgaris: An update', *Clinical, Cosmetic and Investigational Dermatology*, Volume 9, pp. 241–248. doi:10.2147/ccid.s114830.

[5] Platsidaki, E. and Dessinioti, C. (2018) 'Recent advances in understanding Propionibacterium acnes (cutibacterium acnes) in acne', *F1000Research*, 7, p. 1953. doi:10.12688/f1000research.15659.1.

[6] Ahle, C.M., Feidenhansl, C. and Brüggemann, H. (2023) 'Cutibacterium acnes', *Trends in Microbiology*, 31(4), pp. 419–420. doi:10.1016/j.tim.2022.10.006.

[7] Wang, Y. *et al.* (2016) 'A precision microbiome approach using sucrose for selective augmentation of Staphylococcus epidermidis fermentation against Propionibacterium acnes', *International Journal of Molecular Sciences*, 17(11), p. 1870. doi:10.3390/ijms17111870.

1.1.2. Role of *Staphylococcus epidermidis*

Recent studies have demonstrated *Staphylococcus epidermidis* has inhibitory effects on *Cutibacterium acnes*.[8] *Staphylococcus epidermidis* is the most common member of the Coagulase-Negative Staphylococci family found on human skin, [9] with a rate of doubling of 55 minutes.[10] It has a temperature range for growth between 15°-45°C, growing optimally between 30°-37°C,[11] and is a facultative anaerobe, meaning it respires aerobically in presence of oxygen, but is also capable of anaerobic respiration. According to Wang et al., the anaerobic environment within acne lesions that facilitates *Cutibacterium acnes* growth triggers *Staphylococcus epidermidis*[12] to undergo fermentation of glycerol produced naturally in the skin.[13] This produces several short-chain fatty acid by-products – most notably, succinic acid, which inhibits *Cutibacterium acnes* growth.[14] Thus, increasing research suggests

[8] Wang, Y. *et al.* (2013) 'Staphylococcus epidermidis in the human skin microbiome mediates fermentation to inhibit the growth of Propionibacterium acnes: Implications of probiotics in acne vulgaris', *Applied Microbiology and Biotechnology*, 98(1), pp. 411–424. doi:10.1007/s00253-013-5394-8.

[9] Kahawita, T. (2021) *What bacteria causes acne- and is it contagious?*, *HealthMatch*. Available at: https://healthmatch.io/acne/what-bacteria-causes-acne#what-is-acne (Accessed: 19 December 2023).

[10] Oliveira, F., França, Â. and Cerca, N. (2017) 'Staphylococcus epidermidis is largely dependent on iron availability to form biofilms', *International Journal of Medical Microbiology*, 307(8), pp. 552–563. doi:10.1016/j.ijmm.2017.08.009.

[11] Kundrat, L. (2021) *Environmental isolate case files: Staphylococcus epidermidis*, *Microbiologics Blog*. Available at: https://blog.microbiologics.com/environmental-isolate-case-files-staphylococcus-epidermidis/#:~:text=Conditions%20for%20Growth%3A,°C%20in%20aerobic%20conditions (Accessed: 18 December 2023).

[12] Nishijima, S. *et al.* (2000) 'The bacteriology of acne vulgaris and antimicrobial susceptibility of *propionibacterium acnes*and *staphylococcus epidermidis* isolated from acne lesions', *The Journal of Dermatology*, 27(5), pp. 318–323. doi:10.1111/j.1346-8138.2000.tb02174.x.

[13] Blank-Porat, D. *et al.* (2007) 'The anticancer prodrugs of butyric acid an-7 and an-9, possess antiangiogenic properties', *Cancer Letters*, 256(1), pp. 39–48. doi:10.1016/j.canlet.2007.05.011.

[14] Wang, Y. *et al.* (2013) 'Staphylococcus epidermidis in the human skin microbiome mediates fermentation to inhibit the growth of Propionibacterium acnes: Implications of probiotics in acne vulgaris', *Applied Microbiology and Biotechnology*, 98(1), pp. 411–424. doi:10.1007/s00253-013-5394-8.

Staphylococcus epidermidis regulates proliferation of *Cutibacterium acnes*, and, by extension, acne vulgaris.[15]

1.2. Relevance of Investigation into Visible Light Treatment

Visible light treatment is emerging as a viable, non-invasive acne vulgaris treatment. It involves shining direct light on skin affected by acne, generally the face. This is absorbed by photosensitisers produced naturally by *Cutibacterium acnes* in the pilosebaceous unit, such as cytochromes, porphyrins, and NADH[16], which, when photoexcited, catalyse production of toxic agents such as reactive oxygen species (ROS). ROS are highly unstable molecules containing unpaired valence electrons, e.g., oxygen free radicals and peroxides. ROS undergo various chemical processes which exert bactericidal effects on *Cutibacterium acnes*,[17] reducing inflammation of acne.[18]

Current studies suggest absorption of light by these photosensitisers span the entire visible range, peaking in the blue region, with a minimum in the red.[19] This suggests blue light likely induces release of most ROS, leading to greatest bactericidal effects on *Cutibacterium acnes*.[20]

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[15] Marito, S. *et al.* (2021) *Electricity-producing Staphylococcus epidermidis counteracts cutibacterium acnes* [Preprint]. doi:10.21203/rs.3.rs-393212/v1.

[16] Lubart, R. *et al.* (2011) 'A possible mechanism for the bactericidal effect of Visible light', *LASER THERAPY*, 20(1), pp. 17–22. doi:10.5978/islsm.20.17.

[17] Slauch, J.M. (2011) 'How does the oxidative burst of macrophages kill bacteria? still an open question', *Molecular Microbiology*, 80(3), pp. 580–583. doi:10.1111/j.1365-2958.2011.07612.x.

[18] Tsoukas, M.M. *et al.* (2015) 'Light-based therapies in acne treatment', *Indian Dermatology Online Journal*, 6(3), p. 145. doi:10.4103/2229-5178.156379.

[19] Tsoukas, M.M. *et al.* (2015) 'Light-based therapies in acne treatment', *Indian Dermatology Online Journal*, 6(3), p. 145. doi:10.4103/2229-5178.156379.

[20] Eichler, M. *et al.* (2005) 'Flavins are source of visible-light-induced free radical formation in cells', *Lasers in Surgery and Medicine*, 37(4), pp. 314–319. doi:10.1002/lsm.20239.

1.3. Significance of Investigation

Currently, visible light treatment for acne vulgaris only targets *Cutibacterium acnes*.[21] Effects of different coloured light on *Staphylococcus epidermidis* and their beneficial role in limiting *Cutibacterium acnes* proliferation are relatively unknown. By observing impacts of different coloured light on *Staphylococcus epidermidis* population, overall effectiveness of light treatment on acne may be evaluated and improved to allow more effective, safe treatments.

1.4. Mechanism for Photoinactivation of *Staphylococcus epidermidis*

There are currently no established studies on the exact mechanism for photoinactivation, or killing by light, of *Staphylococcus epidermidis* specifically. However, studies on morphologically similar bacteria, e.g., *Staphylococcus aureus*,[22] have similarities to *Cutibacterium acnes*, involving generation of ROS[23] by photosensitisers, which exert bactericidal effects.[24]

1.5. Research Question

"To what extent does different coloured light (red, yellow, green, blue and violet) affect *Staphylococcus epidermidis* population after 30 minutes of light treatment, as measured by absorbance at 600 nm and colony forming units per mL after 72 hours of incubation at 25°C?"

[21] Xu, H. and Li, H. (2019) 'Acne, the skin microbiome, and antibiotic treatment', *American Journal of Clinical Dermatology*, 20(3), pp. 335–344. doi:10.1007/s40257-018-00417-3.

[22] Slauch, J.M. (2011a) 'How does the oxidative burst of macrophages kill bacteria? still an open question', *Molecular Microbiology*, 80(3), pp. 580–583. doi:10.1111/j.1365-2958.2011.07612.x.

[23] Josefsen, L.B. and Boyle, R.W. (2008) 'Photodynamic therapy and the development of metal-based Photosensitisers', *Metal-Based Drugs*, 2008, pp. 1–23. doi:10.1155/2008/276109.

[24] Lubart, R. *et al.* (2011) 'A possible mechanism for the bactericidal effect of Visible light', *LASER THERAPY*, 20(1), pp. 17–22. doi:10.5978/islsm.20.17.

1.6. Rationale for Chosen Methodology

See Appendix G. Absorbance and plate count were used to measure population based on prior research. 600 nm is the most suitable wavelength for measuring *Staphylococcus epidermidis* absorbance without harming the culture.[25] Using two different measurements of population will increase reliability of data and corroborate any trends.

1.7. Expected Morphology of *Staphylococcus epidermidis*

Staphylococcus epidermidis colonies are expected to be white, spherical, 1-2mm in diameter with complete edges[26] after 72 hours of incubation at 25°C and organized into clusters (Figure 1).[27] Only bacteria matching known morphological structures of *Staphylococcus epidermidis* will be included in plate count.

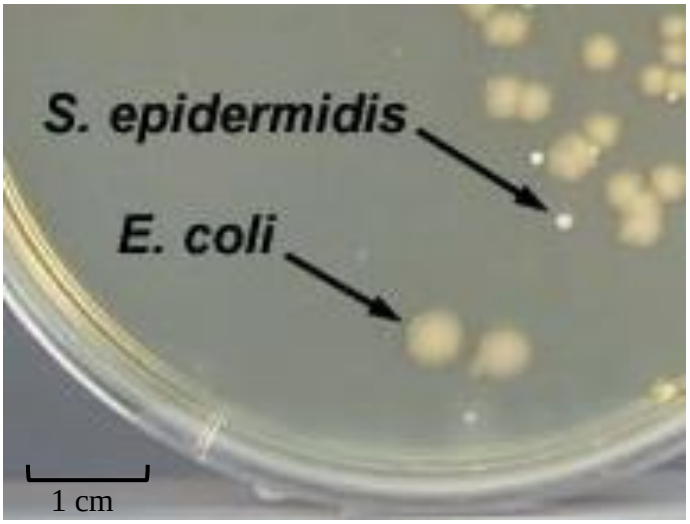

Figure 1: Expected morphology of *Staphylococcus epidermidis:* white, spherical raised colonies organised into clusters.[28]

[25] Uribe-Alvarez, C. *et al.* (2015) 'Staphylococcus epidermidis: Metabolic adaptation and biofilm formation in response to different oxygen concentrations', *Pathogens and Disease*, 74(1). doi:10.1093/femspd/ftv111.

[26] Akbar, M.U. *et al.* (2022) 'Biofilm formation by *staphylococcus epidermidis* and its inhibition using carvacrol, 2-aminobenzemidazole, and 3-indole acetonitrile', *ACS Omega*, 8(1), pp. 682–687. doi:10.1021/acsomega.2c05893.

[27] Zhou, X. and Li, Y. (2022) *Atlas of Oral Microbiology: From healthy microflora to disease*. S.l.: SPRINGER VERLAG, SINGAPORE.

[28] microbiology pictures (2015) *Colony morphology of S. epidermidis and S. aureus, Staphylococcus epidermidis on agar plate with tryptic soy agar (trypticase soy agar, TSA). growth of s.epidermidis in*

2. Chapter Two: Methodology

2.1. Aim and Objectives

To investigate effects of different coloured light (red, yellow, green, blue and violet) on *Staphylococcus epidermidis* population. This may influence development of more effective visible light treatments for acne.

2.2. Hypothesis

2.2.1. Colony forming units per mL according to absorbance at 600 nm.

Table 1: Table of hypotheses for absorbance at 600 nm.

Null Hypothesis (H_0):	There is no statistically significant difference in effects of different coloured light treatments on *Staphylococcus epidermidis* population (CFU/mL).
Alternative Hypothesis (H_1):	Blue light treatment will cause a significantly reduced population (CFU/mL) of *Staphylococcus epidermidis* compared to other colours.

2.2.2. Colony forming units per mL according to plate count after 72 hours of incubation at 25°C.

Table 2: Table of hypotheses for plate count after 72 hours of incubation at 25°C.

Null Hypothesis (H_0):	There is no statistically significant difference in effects of different coloured light treatments on *Staphylococcus epidermidis* population (CFU/mL).
Alternative Hypothesis (H_1):	Blue light treatment will cause a significantly reduced population (CFU/mL) of *Staphylococcus epidermidis* compared to other colours.

2.3. Variables

2.3.1. Independent Variable

Table 3: Independent variable of the experiment

What	How
Colour of light treatment	5 different coloured cellophane light filters were used; red, yellow, green, blue, violet. Unfiltered light acted as a control. Filters were attached to a 240V MTA LED light box and covered by a lid (Figure 2 and Figure 3).

Petri dish on nutrient agar medium. appearance and morphology of Staphylococcus epidermidis and s.aureus colonies. Available at: https://www.microbiologyinpictures.com/bacteria-photos/staphylococcus-epidermidis-photos/staph-epidermidis-tsa.html (Accessed: 27 March 2024).

2.3.2. Dependent Variable

Table 4: Dependent variable of the experiment

What	How
Staphylococcus epidermidis population following treatment under each colour	Measured by CFU/mL according to both absorbance at 600 nm and plate count after 72 hours of incubation at 25°C.

2.3.1. Controlled Variables

Table 5: Controlled variables for the experiment

What	How
Bacterial strain:	Different strains may differ in response to light treatment. This would impact the bactericidal effects of treatment on population. More responsive bacteria would have lower population than less responsive bacteria, as reflected by decreased CFU/mL according. Thus, all samples were extracted from the same culture to ensure the same strain.
Treatment and incubation temperature:	Bacteria was treated at 21°C. Incubation was maintained at 25°C according to IB guidelines. If bacteria are grown at higher temperatures, e.g., 30°C, bacteria would grow faster, likely causing significant overlap between colony forming units according to plate count.
Treatment and incubation length:	Treatment lasted 30-minutes. Plates were incubated for 72 hours. If treatment length is increased, population will likely decrease, as bactericidal effects of treatment will be more pronounced. Thus, CFU/mL according to both absorbance and plate count will be higher than expected. If incubated for longer, e.g. 120 hours, bacteria would likely grow larger, causing significant overlap between colony forming units.
Same light source:	All samples were treated simultaneously (Figure 3). Additionally, the light panel was homogenous (Appendix A).
Type of agar:	Nutrient agar was used throughout. This is the best medium for *Staphylococcus epidermidis* growth.[29]

2.3.2. Uncontrolled Variable

Table 6: Uncontrolled variable in the experiment

What	Why
Lux of each colour:	Different coloured filters impacted brightness of light according to Appendix A. Thus, effects of treatment on population likely would have differed from expected. For example, yellow light had significantly higher Lux than green, thus impacts of yellow may have been disproportionately more pronounced than green.

[29] Jenkins, C.L. and Bean, H.D. (2019) 'Influence of media on the differentiation of Staphylococcus spp. by volatile compounds', *Journal of Breath Research*, 14(1), p. 016007. doi:10.1088/1752-7163/ab3e9d.

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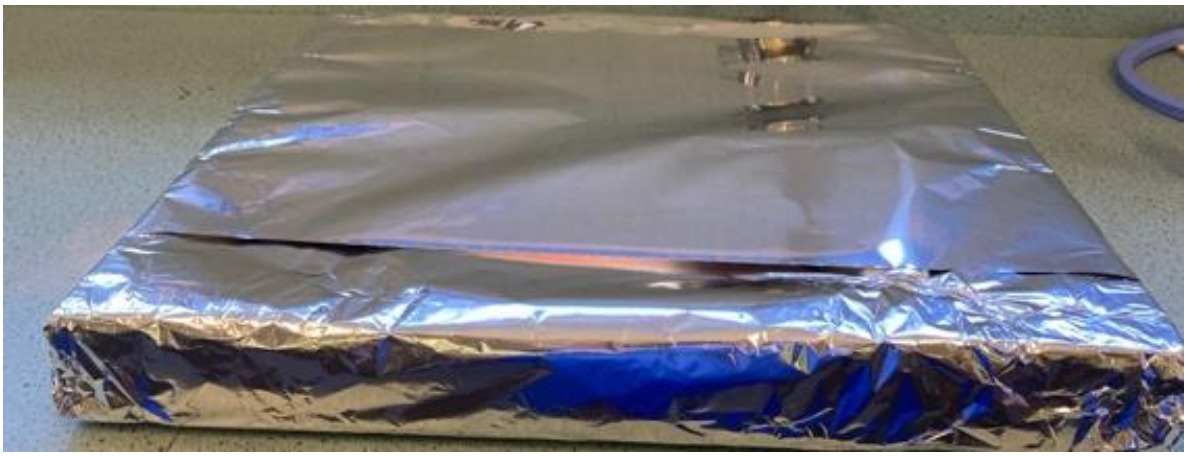


Figure 2: Aluminium foil was used to wrap the lightbox lid to minimise contamination from surroundings and interference from external light whilst bacteria was undergoing treatment.

Figure 3: Experimental setup of light treatment. *Staphylococcus epidermidis* samples were treated using a 240V backlit MTA light panel (KD1022). Colour was changed using 5 different coloured filters: red, yellow, green, blue and violet. A control sample was treated with unfiltered white light. Samples were placed on a watch glass during treatment.

2.4. Apparatus and Materials

x42 90.00mm polystyrene nutrient agar petri dishes

x1 Bunsen burner

x1 A3 240V MTA backlit lightbox (KD1022)

x5 10x10cm cellophane light filters (red, yellow, green, blue, violet)

3000.00mm^3 *Staphylococcus epidermidis* culture (Southern Biological)

x1 1000.00mm^3 micropipette ($\pm 0.16\%$)

x20 1000.00mm^3 micropipette tips

x12 10cm^3 test tubes

x1 spectrophotometer ($\pm 0.003AU, \pm 0.5nm$)

x18 polystyrene cuvettes

x1 incubator

x6 watch glasses

x12 sterile cell spreaders

x1 roll aluminium foil

x1 roll masking tape

2.5. Procedure

2.5.1. Conducting light treatment

2.5.1.1. Preparation of light source

1. 10x10cm red cellophane was taped over an MTA lightbox, ensuring to minimise creasing.
2. Step 1 was repeated for yellow, green, blue and violet cellophane.
3. 6 watch glasses were placed on each cellophane sheet.

2.5.1.2. Treating of bacteria

1. A Bunsen burner was lit.
2. 500.00mm^3 bacterial broth from the original culture was added to each sterilised watch glass from 2.5.1.1 using a 1000.00mm^3 micropipette (Figure 3).
3. The lightbox was turned on. A 30-minute timer was started, before covering with the lid.

2.5.2. Creating 1:10 and 1:100 serial dilutions of light treated bacterial culture.

1. 350.00mm^3 bacterial culture was extracted from the watch glass containing *Staphylococcus epidermidis* treated with red light using a 1000.00mm^3 micropipette, and added to a 10cm^3 test tube (Figure 4)
2. 3150.00mm^3 distilled water was added to that test tube using a 5000.00mm^3 micropipette, making a 1:10 dilution.
3. 350.00mm^3 was extracted from that 1:10 dilution into a separate test tube using a separate 5000.00mm^3 micropipette tip.
4. Step 2 was repeated to make a 1:100 dilution.
5. Steps 1-4 were repeated for other colours.

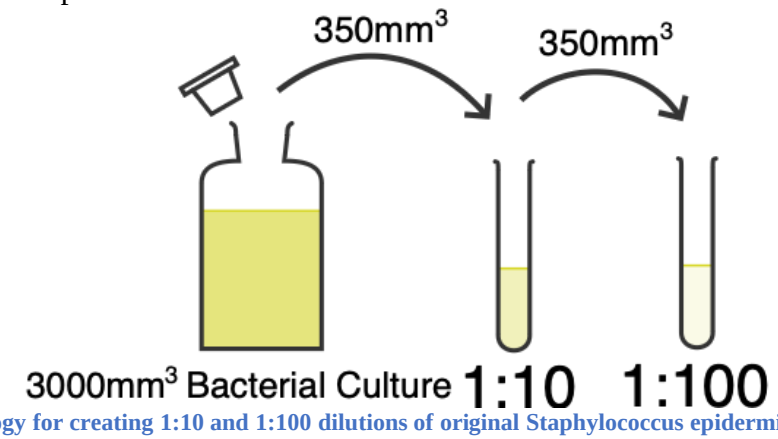


Figure 4: Methodology for creating 1:10 and 1:100 dilutions of original Staphylococcus epidermidis culture. Diagram created using chemix website[30]

2.5.3. Conducting absorbance measurements of CFU/mL at 600 nm

2.5.3.1. Measuring spectrophotometric absorbance of 1:10 dilutions of *Staphylococcus epidermidis* after 30 minutes of light treatment

1. 1000.00mm^3 of the 1:10 dilution of bacteria treated with red light was added to a cuvette using a 1000.00mm^3 micropipette.
2. Step 1 was repeated twice to fill three total cuvettes.
3. These cuvettes were placed in the spectrophotometer, along with a blank cuvette containing distilled water.
4. The spectrophotometer was blanked before readings were completed at 600 nm.
5. Steps 2-5 were repeated for other colours.

[30] *Draw lab diagrams. simply.* (no date) *Chemix*. Available at: https://chemix.org/ (Accessed: 23 June 2024).

2.5.3.2. Conversion of absorbance at 600 nm to CFU/mL

1. Absorbance was converted to CFU/mL according to the standard calibration curve of absorbance versus CFU/mL by Pan et al.[31], which stated the relationship: $CFU/mL = (2.35 \times 10^9)a - (4.60 \times 10^7)$. a represents absorbance at 600 nm.

2.5.4. Conducting plate count of CFU/mL

2.5.4.1. Spreading of bacteria on petri dishes:

1. Using a sterile cell spreader, 3 petri dishes were inoculated with 10.00mm^3 of the 1:10 dilution of red light treated broth (Figure 5).
2. Step 2 was repeated for the 1:100 dilution using a new sterile cell spreader.
3. Steps 1-2 were repeated for samples treated with other colours.
4. 6 dishes were left uninoculated as a control.
5. The 42 total plates were incubated at 25°C for 72 hours.

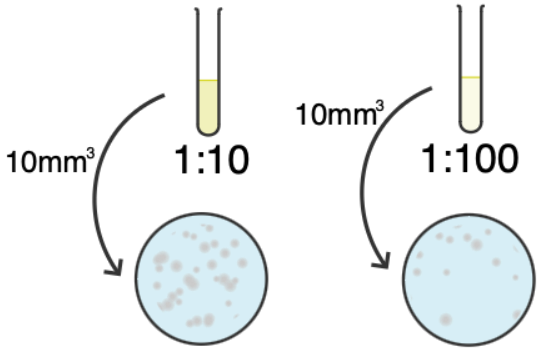


Figure 5: Methodology for spreading of bacteria on petri dishes. Diagram created using chemix website.[32]

2.5.4.2. Counting population of each petri dish

1. All non-anomalous plates (Appendix F) with 30-300 colonies were counted.
2. Results were inputted into an Excel spreadsheet and converted to CFU/mL[33]:

$$CFU/mL = \frac{number\ of\ colonies\ \times\ total\ dilution\ factor}{volume\ of\ culture\ plated\ (mL)}$$

[31] Pan, H. *et al.* (2014) 'A comparison of conventional methods for the quantification of bacterial cells after exposure to metal oxide nanoparticles', *BMC Microbiology*, 14(1). doi:10.1186/s12866-014-0222-6.

[32] *Draw lab diagrams. simply.* (no date) *Chemix*. Available at: https://chemix.org/ (Accessed: 23 June 2024).

[33] Libretexts (2023) *1.15: Determination of bacterial numbers*, *Biology LibreTexts*. Available at: https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_Laboratory_Manual_(Hartline)/01%3A_Labs/1.15%3A_Determination_of_Bacterial_Numbers (Accessed: 23 June 2024).

2.5.5. Conducting statistical analysis of CFU/mL data from absorbance at 600 nm and plate count after 72 hours of incubation at 25°C

1. Statistical analysis was conducted using the ANOVA: Single Factor Analysis tool[34] in Microsoft Excel, followed by a Tukey-Kramer post-hoc[35].
2. The alpha value used was 0.05.

2.6. Safety Concerns

Table 7: Table of safety concerns

What	Why	How
Use of Bunsen burner.	Potential fire risk and burn hazard.	Left on safety flame throughout.
Presence of *Staphylococcus epidermidis.*	Risk of contamination and/or transmission of bacteria.	Bunsen burner used to sterilise environment. Ventilation turned off when handling bacteria. Gloves, face mask, lab coat and safety glasses worn.
Use of ethanol and isopropyl (for sterilisation).	Highly flammable, can cause discomfort if ingested.	Potential fire hazards considered. Bunsen burner only lit after sterilisation/sanitisation. Appropriate safety equipment used.

2.7. Ethical, Environmental and Social Concerns

Table 8: Table of ethical, environmental and social concerns

Concern	How it was mitigated
Ethical	Risk of releasing bacteria into surroundings/environment. Mitigated by thorough sanitisation of laboratory before and after handling bacteria, use of appropriate safety equipment, and regular hand sanitisation.
Environmental	Major environmental concerns surrounded releasing bacteria into the environment. This was mitigated by autoclaving all equipment after usage before disposal.
Social	No social concerns.

[34] Bobbitt, Z. (2021a) *How to perform a one-way ANOVA in Excel*, *Statology*. Available at: https://www.statology.org/one-way-anova-excel/ (Accessed: 24 May 2024).

[35] Bobbitt, Z. (2021b) *How to perform a Tukey-Kramer Post Hoc Test in Excel*, *Statology*. Available at: https://www.statology.org/tukey-kramer-post-hoc-test-excel/ (Accessed: 24 May 2024).

3. Chapter Three: Data Collection and Processing

3.1. Qualitative Observations from Conducting Light Treatment

Table 9: Qualitative observations during light treatment

Observation	Evidence
Following light treatment, significant broth was lost from each watch glass	500.00mm^3 was originally added to each, however just over 350.00mm^3 remained for extraction after treatment. Furthermore, remaining volumes of broth differed between watch glasses

3.2. Impacts of Different Coloured Light on *Staphylococcus epidermidis*, as measured by absorbance (AU) at 600 nm.

3.2.1. Raw Data

See Appendix B for raw data for absorbance following treatment.

3.2.2. Sample Calculations

1. Mean absorbance (AU) at 600 nm for samples treated with red light:

$$\overline{x} = \frac{\sum fx}{n}$$

$$\therefore mean = \frac{0.038 + 0.038 + 0.038 + 0.035 + 0.035 + 0.035 + 0.035 + 0.035 + 0.035}{9}$$

$$= 0.036$$

2. Standard deviation (AU) of absorbance at 600 nm for samples treated with red light:

$$\sigma = \frac{\sqrt{\sum(x - \overline{x})^2}}{n}$$

$$= \frac{\sqrt{(0.036 - 0.038)^2 + (0.036 - 0.038)^2 + (0.036 - 0.038)^2 + (0.036 - 0.035)^2 + (0.036 - 0.035)^2 + (0.036 - 0.035)^2 + (0.036 - 0.035)^2 + (0.036 - 0.035)^2 + (0.036 - 0.035)^2}}{5}$$

$$= 0.002$$

3. Conversion of absorbance (AU) at 600 nm for samples treated with red light to CFU/mL[36]:

$$CFU/mL = (2.35 \times 10^9)a - (4.60 \times 10^7)$$

$$= (2.35 \times 10^9)(0.036) - (4.60 \times 10^7)$$

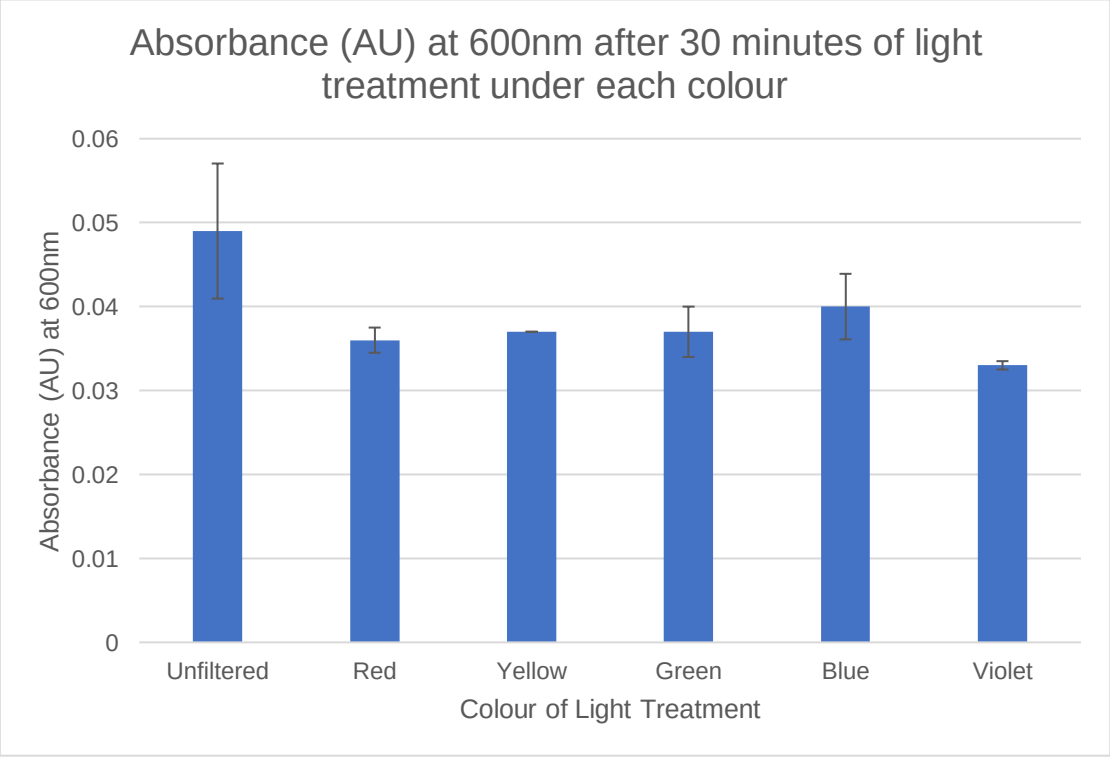
$$= 3.86 \times 10^7$$

[36] Pan, H. *et al.* (2014) 'A comparison of conventional methods for the quantification of bacterial cells after exposure to metal oxide nanoparticles', *BMC Microbiology*, 14(1). doi:10.1186/s12866-014-0222-6.

3.2.3. Processed Data

Table 10: Impacts of each coloured light treatment on absorbance (AU) at 600 nm, and the standard deviation of each coloured treatment.

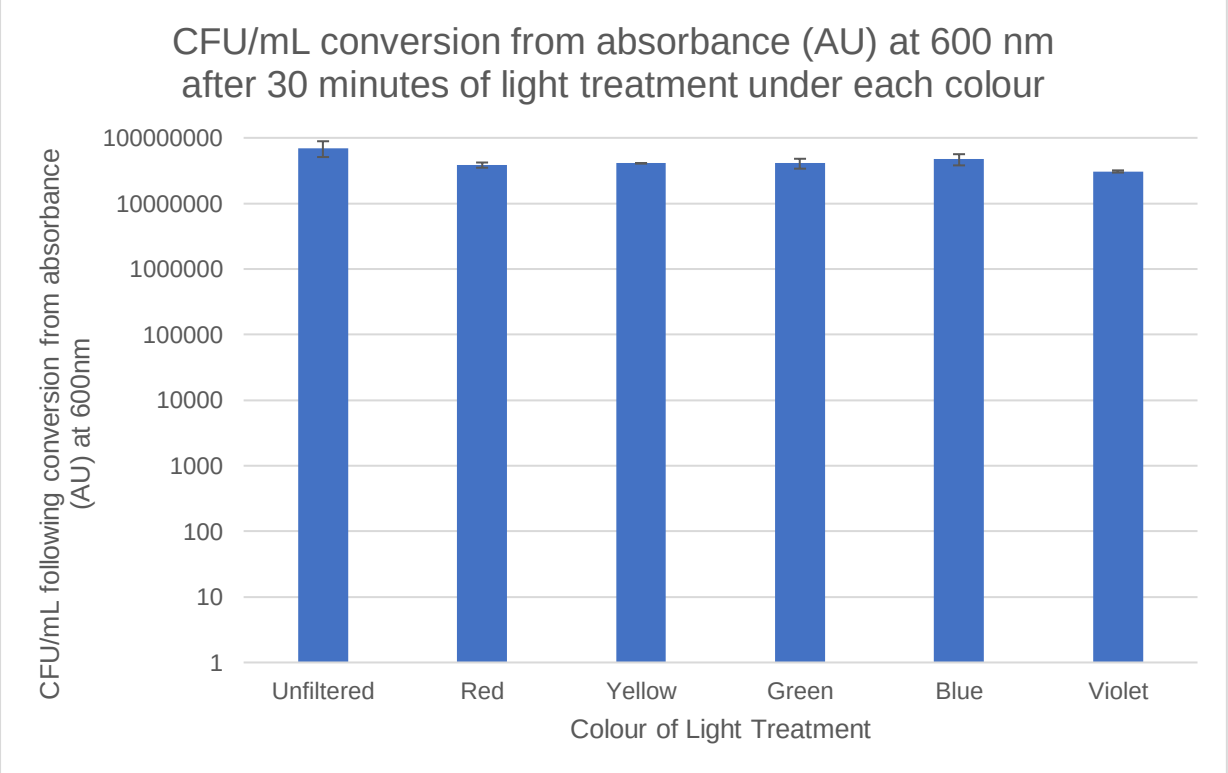
		Mean Absorbance (AU) at 600 nm	Standard Deviation (AU)
Colour of Light Treatment	Unfiltered	0.049	0.008
	Red	0.036	0.002
	Yellow	0.037	0.000
	Green	0.037	0.003
	Blue	0.040	0.004
	Violet	0.033	0.001



Graph 1: Conventional presentation of absorbance using data from Table 10. Error bars represent standard deviation across 3 trials of absorbance (AU) of each sample at 600 nm following 30 minutes of light treatment with unfiltered, red, yellow, green, blue and violet light.

Table 11: Processed absorbance (AU) data including mean absorbance and standard deviation.

		Conversion of Absorbance (AU) to CFU/mL[37]	Standard Deviation (CFU/mL)
Colour of Light Treatment	Unfiltered	6.99×10^7	1.89×10^7
	Red	3.86×10^7	3.53×10^6
	Yellow	4.10×10^7	0.00
	Green	4.10×10^7	7.05×10^6
	Blue	4.72×10^7	9.18×10^6
	Violet	3.08×10^7	1.18×10^6



Graph 2: Effects of light treatment on CFU/mL of *Staphylococcus epidermidis* according to conversion from absorbance to CFU/mL, depicting Table 11 data on a logarithmic y-axis. This allows direct comparison of population according to absorbance to population according to plate count. Error bars represent standard deviation following conversion.

[37] Pan, H. *et al.* (2014) 'A comparison of conventional methods for the quantification of bacterial cells after exposure to metal oxide nanoparticles', *BMC Microbiology*, 14(1). doi:10.1186/s12866-014-0222-6.

3.3. Impacts of Different Coloured Light on *Staphylococcus epidermidis*, as measured by standard plate count after 72 hours of incubation at 25°C

3.3.1. Qualitative Data

Some plates had indiscrete colonies which could not be easily counted (Figure 6) and/or grew in a vertical line (Figure 7).

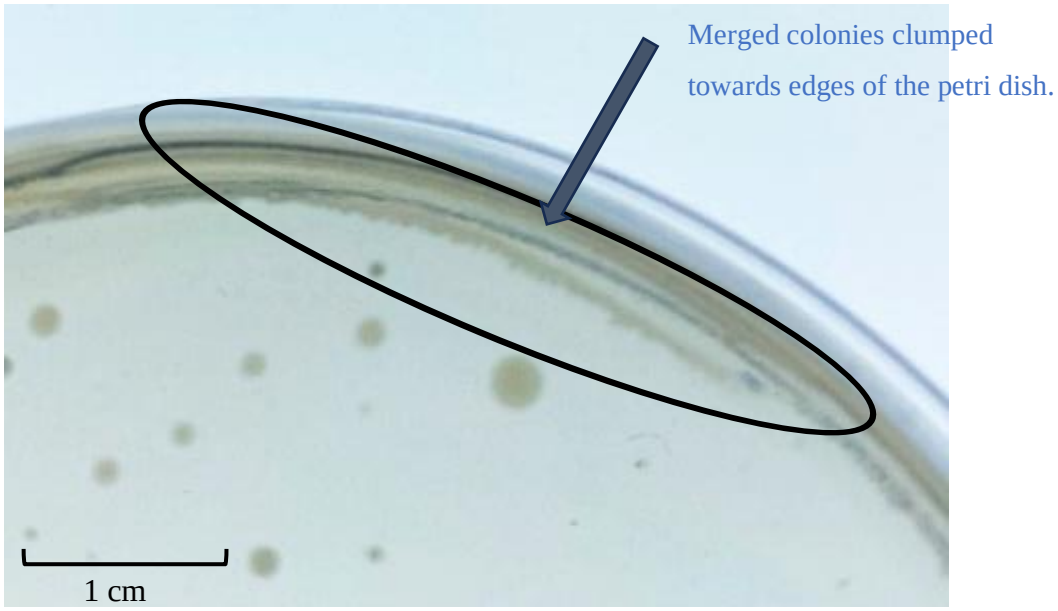


Figure 6: 1:100 dilution of *Staphylococcus epidermidis* sample treated under violet light following 72 hours of incubation at 25°C. Displays evidence of excessive growth of colonies around edges of the plate which seemingly 'merged' together.

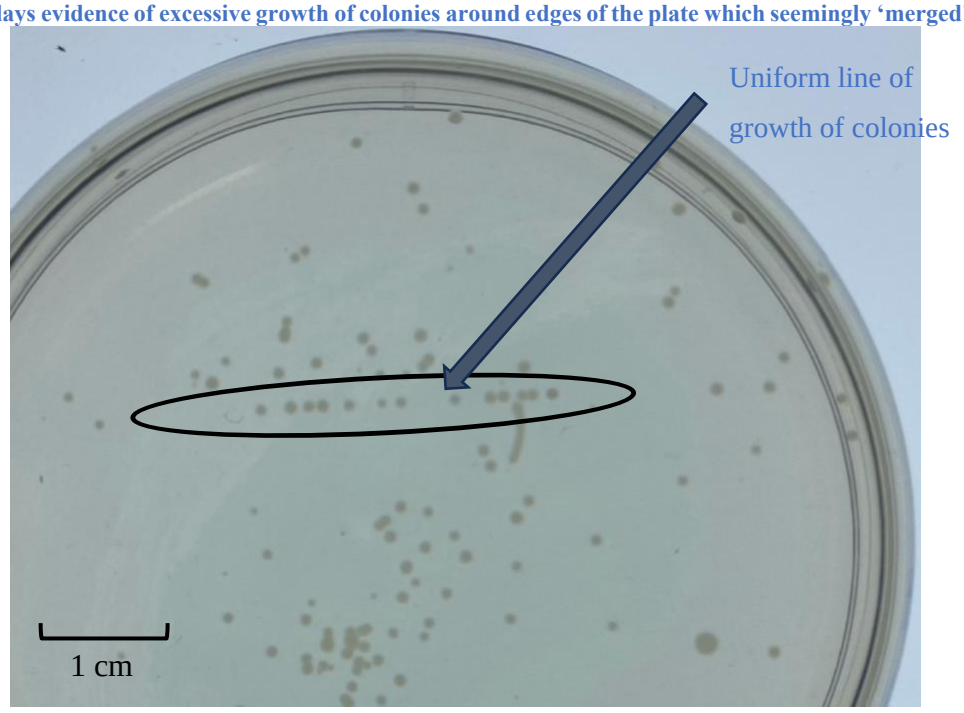


Figure 7: 1:100 dilution of *Staphylococcus epidermidis* sample treated under blue light. Displays evidence of bacterial colonies growing in a uniform straight line

At least five different microorganisms were present within plates (Figure 8): the white, yellow, orange, black and fluffy colonies.

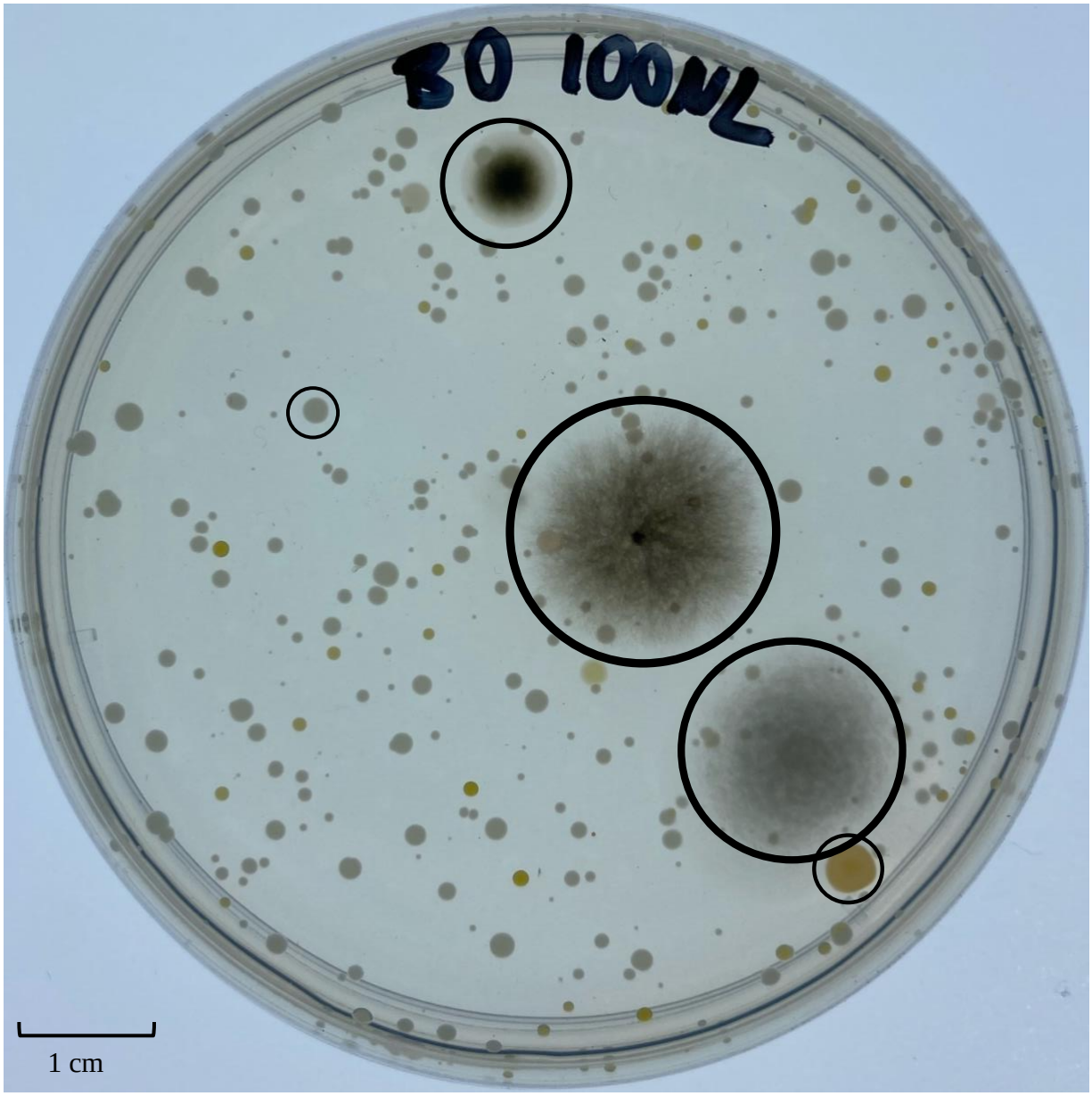


Figure 8: 1:10 dilution of *Staphylococcus epidermidis* sample treated under blue light following 72 hours of incubation at 25°C. At least 4 different microorganisms are evident in this plate, indicating significant contamination.

All 6 control plates experienced no bacterial growth.

3.3.2. Raw Data

See Appendix C for raw plate count data of 1:10 and 1:100 dilutions.

3.3.3. Sample Calculations

1. CFU/mL calculation for plate count of trial 1 of the 1:100 serial dilution of bacterial broth treated with red light after 72 hours of incubation at 25°C (Appendix C):

$$CFU/mL = \frac{number\ of\ colonies\ \times\ total\ dilution\ factor}{volume\ of\ culture\ plated\ (mL)}$$

$$= \frac{94 \times 100}{0.1}$$

$$= 9.4 \times 10^4$$

2. Range (CFU/mL) of plate count data after 72 hours of incubation at 25°C for 1:100 serial dilutions of samples treated under red light:

$$Range = maximum - minimum$$

$$= 1.59 \times 10^5 - 9.4 \times 10^4$$

$$= 6.50 \times 10^4$$

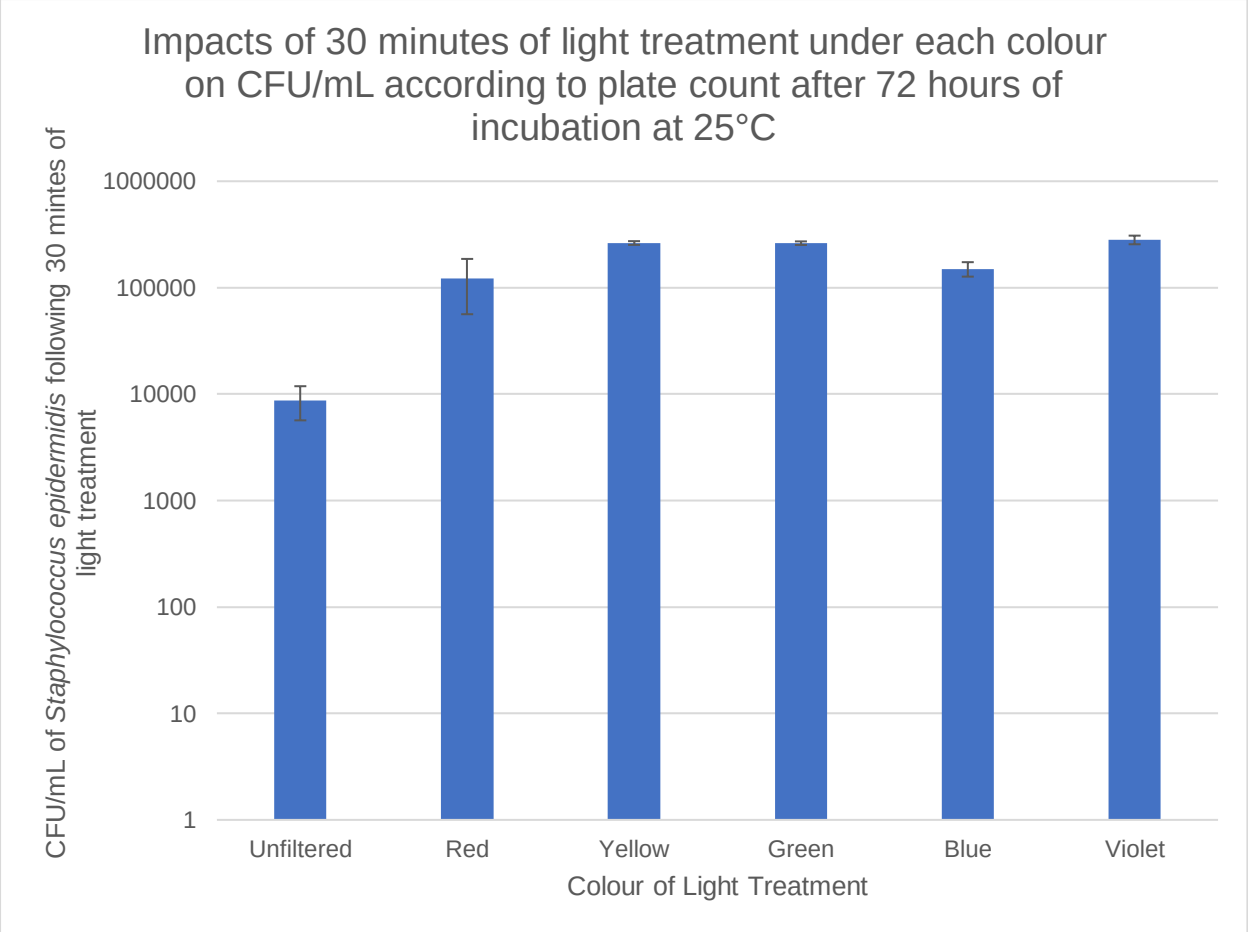
3.3.4. Processed Data

The most suitable dilution selected was whichever contained more countable trials.

Table 12: Processed data from standard plate count, including average CFU/mL, percentage change in CFU/mL compared to unfiltered white light and range

	Average CFU/mL (3 s.f.)	Range (CFU/mL)
Unfiltered	8.77×10^3	3.10×10^3
Red	1.21×10^5	6.50×10^4
Yellow	2.63×10^5	1.06×10^4
Green	2.62×10^5	1.02×10^4
Blue	1.50×10^5	2.33×10^4
Violet	4.08×10^5	2.63×10^4

Spread was measured by range instead of standard deviation as insufficient datapoints were available to produce meaningful standard deviations (due to limited bacterial broth available).



Graph 3: Effects of 30 minutes of unfiltered, red, yellow, green, blue and violet light treatment on CFU/mL of *Staphylococcus epidermidis* on a logarithmic y-axis scale, as derived from a standard plate count (Table 12). Error bars represent range of CFU/mL

3.4. Statistically Tested Data:

3.4.1. Sample Calculations

1. Sample Calculation for Q Critical Value:

$$Q\ Critical\ Value = Q\sqrt{\frac{S^2_{pooled}}{n}}$$

$$= 4.197\sqrt{\frac{8.42 \times 10^{13}}{9}}$$

$$= 1.28 \times 10^7$$

2. Absolute mean difference for the comparison between CFU/mL of samples treated under unfiltered light and red light according to absorbance at 600 nm.

$$|Mean\ Difference| = Average\ Unfiltered\ Absorbance - Average\ Red\ Absorbance$$

$$= 6.99 \times 10^7 - 3.86 \times 10^7$$

$$= 3.13 \times 10^7$$

3.4.2. One-way analysis of variance (ANOVA test) for impacts of different coloured light on *Staphylococcus epidermidis* population (CFU/mL), as measured by absorbance (AU) at 600 nm.

An ANOVA test was conducted[38] on absorbance data (Table 13).

Table 13: Single Factor ANOVA for the Absorbance (AU) at 600 nm of *Staphylococcus epidermidis* bacteria following 30 minutes of light treatment under different colours.

Source of Variation	SS	df	MS	F	p-value	F-crit
Between Groups	8.12×10^{15}	5	1.62×10^{15}	19.290	1.763×10^{-10}	2.409
Within Groups	4.04×10^{15}	48	8.42×10^{13}			
Total	1.22×10^{16}	53				

As the p-value (1.763×10^{-10})<alpha level (0.05), there is sufficient evidence to reject the null hypothesis. A further Tukey-Kramer post-hoc was conducted[39] to determine which specific treatments had statistically significant effects on population (Table 14). If Absolute Mean Difference > Q Critical Value, differences in Table 11 and Graph 1 are statistically significant, and not due to variance/uncertainties in data.

[38] Bobbitt, Z. (2021a) *How to perform a one-way ANOVA in Excel*, *Statology*. Available at: https://www.statology.org/one-way-anova-excel/ (Accessed: 24 May 2024).

[39] Bobbitt, Z. (2021b) *How to perform a Tukey-Kramer Post Hoc Test in Excel*, *Statology*. Available at: https://www.statology.org/tukey-kramer-post-hoc-test-excel/ (Accessed: 24 May 2024).

Table 14: Tukey-Kramer post-hoc test to determine which results were statistically significant.

Comparison	Absolute Mean Difference	Q Critical Value	Significant?
Unfiltered vs Red	3.13×10^7	1.28×10^7	Yes
Unfiltered vs Yellow	2.90×10^7	1.28×10^7	Yes
Unfiltered vs Green	2.90×10^7	1.28×10^7	Yes
Unfiltered vs Blue	2.27×10^7	1.28×10^7	Yes
Unfiltered vs Violet	3.92×10^7	1.28×10^7	Yes
Blue vs Violet	1.65×10^7	1.28×10^7	Yes

See Appendix D for full Tukey-Kramer post-hoc.

3.4.3. One-way analysis of variance (ANOVA test) for impacts of different coloured light on *Staphylococcus epidermidis* population, as measured by standard plate count after 72 hours of incubation at 25°C

A similar statistical test was completed on plate count data (Table 15).

Table 15: Single Factor ANOVA for plate count after 72 hours of incubation at 25°C of *Staphylococcus epidermidis* following 30 minutes of light treatment under different coloured light.

Source of Variation	SS	df	MS	F	p-value	F-crit
Between Groups	1.73×10^{11}	5	3.46×10^{10}	5.371	8.03×10^{-3}	3.11
Within Groups	7.74×10^{10}	12	6.45×10^9			
Total	2.50×10^{11}	17				

The p-value (8.03×10^{-3})<alpha level, thus the null hypothesis is rejected. A further Tukey-Kramer post-hoc was conducted.

Table 16: Tukey-Kramer post-hoc test to determine which results were statistically significant.

Comparison	Absolute Mean Difference	Q Critical Value	Significant?
Unfiltered vs Red	3.38×10^5	2.21×10^5	Yes
Unfiltered vs Yellow	7.63×10^5	2.21×10^5	Yes
Unfiltered vs Green	7.61×10^5	2.21×10^5	Yes
Unfiltered vs Blue	4.25×10^5	2.21×10^5	Yes
Unfiltered vs Violet	8.20×10^5	2.21×10^5	Yes
Red vs Yellow	4.25×10^5	2.21×10^5	Yes
Red vs Green	4.23×10^5	2.21×10^5	Yes
Red vs Violet	4.82×10^5	2.21×10^5	Yes
Yellow vs Blue	3.38×10^5	2.21×10^5	Yes
Green vs Blue	3.36×10^5	2.21×10^5	Yes
Blue vs Violet	3.95×10^5	2.21×10^5	Yes

See Appendix E for full Tukey-Kramer post-hoc.

4. Chapter Four: Analysis

4.1. Impacts of Different Coloured Light on *Staphylococcus epidermidis* population, as measured by absorbance (AU) at 600 nm.

Absorbance at 600 nm is directly correlated with *Staphylococcus epidermidis* population.[40] Lower absorbance means less light is absorbed, indicating fewer bacteria present within each sample.

According to Graph 1, all colours tested caused samples to have lower absorbance, thus CFU/mL following conversion (Graph 2), than unfiltered light, although to varying extents. This suggested all colours were bactericidal, as population decreased compared to the unfiltered control. Violet caused the lowest average CFU/mL (3.08×10^7), while blue caused the highest (4.72×10^7). Green and yellow caused similar CFU/mL, both slightly more than red.

Differing effects of each colour on population were evaluated in an ANOVA test, which suggested significant differences existed. A Tukey-Kramer post-hoc revealed coloured light significantly decreased CFU/mL compared to unfiltered light. Moreover, violet significantly decreased population compared to blue. All other colours had no statistically significant differences. As blue caused no significant reduction in population compared to red, yellow and green, the alternative hypothesis was only partially supported.

[40] Swolana, D. *et al.* (2020) 'The antibacterial effect of silver nanoparticles on Staphylococcus epidermidis strains with different biofilm-forming ability', *Nanomaterials*, 10(5), p. 1010. doi:10.3390/nano10051010.

Currently, no established studies exist on the mechanism for photoinactivation of *Staphylococcus epidermidis* specifically, or why different coloured lights have different effects on *Staphylococcus epidermidis*. However, studies on similar bacteria, e.g., *Staphylococcus aureus*, suggest it is likely due to light being absorbed by photosensitisers,[41] which when photoexcited, catalyse production of ROS.[42] These ROS are thought to exert bactericidal effects on *Staphylococcus aureus*. Blue light is believed to be more effectively absorbed by photosensitisers in *Staphylococcus aureus* than other colours,[43] thus causes greater ROS production and bactericidal effects.[44]

Although, *Cutibacterium acnes*, which causes proliferation of acne vulgaris, has the same mechanism. Further research is needed to determine whether there is a differential impact of light treatment on *Staphylococcus epidermidis* and *Cutibacterium acnes*.

This experiment largely corroborated a similar study by Angarano,[45] which found violet caused the most bactericidal effects on *Staphylococcus epidermidis* biofilms. Angarano also found blue, green, yellow and red caused no bactericidal effects on biofilms.

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[41] McClary, J.S., Sassoubre, L.M. and Boehm, A.B. (2017) 'Staphylococcus aureus strain Newman photoinactivation and cellular response to sunlight exposure', *Applied and Environmental Microbiology*, 83(17). doi:10.1128/aem.01052-17.

[42] Josefsen, L.B. and Boyle, R.W. (2008) 'Photodynamic therapy and the development of metal-based Photosensitisers', *Metal-Based Drugs*, 2008, pp. 1–23. doi:10.1155/2008/276109.

[43] Ramakrishnan, P. *et al.* (2016) 'Cytotoxic responses to 405nm light exposure in mammalian and bacterial cells: Involvement of reactive oxygen species', *Toxicology in Vitro*, 33, pp. 54–62. doi:10.1016/j.tiv.2016.02.011.

[44] Dai, T. *et al.* (2012) 'Blue Light for Infectious Diseases: Propionibacterium acnes, helicobacter pylori, and beyond?', *Drug Resistance Updates*, 15(4), pp. 223–236. doi:10.1016/j.drup.2012.07.001.

[45] Angarano, V. *et al.* (2020) 'Visible light as an antimicrobial strategy for inactivation of pseudomonas fluorescens and Staphylococcus epidermidis biofilms', *Antibiotics*, 9(4), p. 171. doi:10.3390/antibiotics9040171.

4.2. Impacts of Different Coloured Light on *Staphylococcus epidermidis* population, as measured by standard plate count after 72 hours of incubation at 25°C.

Qualitative data corroborated expected morphological features of *Staphylococcus epidermidis*, despite presence of other microbes in some plates. Only colonies which matched known morphological structures were counted to ensure *Staphylococcus epidermidis* specifically was measured. Additionally, only plates containing between 30-300 colonies were counted. This ensured a sufficient sample size, providing accurate representations of the original culture without being too time-consuming to count and difficult to differentiate between individual colonies. Abnormal plates (Appendix F) were not counted to minimise random errors.

Graph 3 shows all samples treated with coloured light caused more CFU/mL than unfiltered light. Violet caused the largest population of 2.82×10^5 CFU/mL, whereas red caused the smallest of 1.21×10^5 CFU/mL, closely followed by blue. Green and yellow had relatively similar impacts on CFU/mL of 2.62×10^5 and 2.63×10^5 respectively.

These differences were evaluated in an ANOVA, which suggested significant differences existed. A Tukey-Kramer post-hoc revealed all colours significantly increased CFU/mL, thus promoted population growth, compared to unfiltered light. Furthermore, red and blue significantly reduced CFU/mL compared to all other colours. However, blue caused no significant reduction in population compared to red and unfiltered light, thus the alternative hypothesis was only partially supported.

Plate count results largely contradicted Angarano's study, as stated above, which found light treatment was bactericidal rather than promoting population growth as observed.[46] These differences are likely due to random and systematic errors, which will be discussed in Chapter Five: Evaluation.

4.3. Comparison between absorbance at 600 nm and plate count after 72 hours of incubation at 25°C

Absorbance and plate count data largely contradicted. The unfiltered control caused the most CFU/mL according to absorbance, implying coloured treatment was bactericidal, and fewest according to plate count, implying treatment promoted population growth.

According to both measures, yellow and green had nearly identical relative impacts on *Staphylococcus epidermidis* population. Red also had similar impacts, however violet and blue had opposing effects. Violet was most bactericidal and blue least according to absorbance, and vice versa according to plate count. Potential reasons will be discussed in Chapter Five: Evaluation.

[46] Angarano, V. *et al.* (2020) 'Visible light as an antimicrobial strategy for inactivation of pseudomonas fluorescens and Staphylococcus epidermidis biofilms', *Antibiotics*, 9(4), p. 171. doi:10.3390/antibiotics9040171.

5. Chapter Five: Evaluation

5.1. Strengths of Methodology:

Table 17: Table of strengths

What	Why
All 6 control plates experienced no bacterial growth	Indicates no contamination occurred when preparing plates.
Not all plates were contaminated	Means contamination likely only occurred during plating, thus absorbance was likely unaffected by contamination.
Two methods of data collection (absorbance and plate count) used.	Provided a more comprehensive insight into effects of each coloured light on population than each one alone.

5.2. Weaknesses of Methodology:

5.2.1. Precision of Data

Precision was measured using standard deviation and range. Absorbance had relatively low standard deviation (Graph 2), indicating high precision. In comparison, plate count had high range, as seen in Appendix C and reflected in Graph 3 error bars. Low precision of plate count may have also impacted reliability of conclusions drawn from its' ANOVA. Thus, absorbance was likely more precise. Precision of both measures were impacted by random errors.

Table 18: Weaknesses of methodology which impacted precision of results

Error	Evidence	Effect on Results	Improvements
Uneven relative distribution of bacteria throughout broth.	Due to bacteria having a relatively higher density, thus being more concentrated near the bottom of the broth.	Absorbance likely varied between samples, with samples containing more concentrated bacteria having higher absorbance. Decreased precision of absorbance.	Minimised by shaking the broth before extraction, however, could not be eliminated, thus was intrinsic to the experiment, decreasing reliability of conclusions drawn from statistical tests.
Uneven distribution of bacteria during inoculation (2.5.4.1).	Indiscrete colonies in Figure 6 and Figure 7.	Some 'merged' colonies may have been considered a single colony, decreasing precision of the plate count.	An improvement could be reducing incubation to 48 hours, as bacteria would likely not grow as large, reducing merging.

5.2.2. Accuracy of Data

A direct comparison of results to literature could not be made, as no theoretical values were obtained. However, comparison of results to similar studies suggests absorbance more closely follows expected bactericidal trends than plate count.[47] Therefore, absorbance was likely more accurate. Accuracy of both measures was impacted by systematic errors.

Table 19: Weaknesses of methodology which impacted accuracy of results

Error	Evidence	Effect on Results	Improvements
Contamination of bacteria	Figure 8, and reflected in Appendix C	Some anti-toxin[48] properties of foreign microbes may have interfered with *Staphylococcus epidermidis* growth, likely causing fewer CFU/mL than expected.	Taking greater caution when handling bacteria and preparing plates, e.g., using a laminar airflow hood.
Heat produced by the light source whilst samples underwent treatment caused evaporation of the broth.	3.1. Each watch glass containing different volumes of remaining broth indicates each coloured filter caused different evaporation rates, thus absorbed differing amounts of heat.	Likely increased bacterial concentration, meaning each sample contained more CFU/mL than expected, but by different amounts for each colour. Thus, effects of light treatment were not accurately represented, impacting validity of conclusions.	Adding water after treatment to maintain initial volume of broth, however, this could also introduce more random errors, i.e., contamination. Thus, this error was intrinsic to the experiment and could not be readily improved.
Intensity of light bacteria was exposed to (Appendix A)..	Each coloured cellophane filter caused differing light intensity (Lux), by absorbing different amounts of light	Likely reduced impacts of treatment, making CFU/mL higher according to absorbance and lower according to plate count	Using better equipment, e.g., a light source that selectively emits different wavelengths without a filter.

[47] Angarano, V. *et al.* (2020) 'Visible light as an antimicrobial strategy for inactivation of pseudomonas fluorescens and Staphylococcus epidermidis biofilms', *Antibiotics*, 9(4), p. 171. doi:10.3390/antibiotics9040171.

[48] Sułkowska-Ziaja, K. *et al.* (2023) 'Natural compounds of fungal origin with antimicrobial activity—potential cosmetics applications', *Pharmaceuticals*, 16(9), p. 1200. doi:10.3390/ph16091200.

5.3. Limitations:

5.3.1. Limitations of Absorbance at 600 nm

Table 20: Limitations of absorbance at 600 nm as a measure of *Staphylococcus epidermidis* population

What	Why
Indirectly measured absorbance of all particles suspended in solution, did not directly measure live *Staphylococcus epidermidis* population[49].	This meant absorbance likely included dead and/or foreign contaminants present in the broth. Thus, all readings were likely higher than expected, limiting validity of conclusions.
Conversion from absorbance to CFU/mL[50] did not necessarily reflect the specific strain of *Staphylococcus epidermidis* used.	This was evidenced by several sources citing different absorbance to CFU/mL conversions, however, has little impact on overall conclusions. The specific conversion selected was the only one which did not produce negative CFU/mL values when applied to raw data.

These limitations were intrinsic to the experiment. Improvements include fluorescent labelling to exclude absorbance of foreign particles and creating a standard curve for the specific strain used. However, this is costly and unrealistic for a school laboratory.

5.3.2. Limitations of Standard Plate Count after 72 Hours of Incubation at 25°C

Table 21: Limitations of plate count after 72 hours of incubation at 25°C as a measure of population

What	Why
Incubation temperature was restricted to 25°C, rather than optimal conditions for *Staphylococcus epidermidis* growth (30-37°C) or normal human skin temperature (37°C).	Plate count provided a limited representation of impacts of light treatment on *Staphylococcus epidermidis* that is likely not applicable to real world impacts of light treatment on the beneficial role of *Staphylococcus epidermidis* in regulating acne vulgaris proliferation.
Although counted colonies matched expected morphology of *Staphylococcus epidermidis*, it was not completely certain they were	Without further testing, although it is highly likely counted colonies were Staphylococcus epidermidis, it cannot be said for sure, thus applications of the findings are limited.

These limitations were intrinsic to the experiment and available resources, limiting validity of conclusions.

[49] Li, R. *et al.* (2018) 'In situ detection of live-to-dead bacteria ratio after inactivation by means of synchronous fluorescence and PCA', *Proceedings of the National Academy of Sciences*, 115(4), pp. 668–673. doi:10.1073/pnas.1716514115.

[50] Libretexts (2023a) *1.15: Determination of bacterial numbers*, *Biology LibreTexts*. Available at: https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_Laboratory_Manual_(Hartline)/01%3A_Labs/1.15%3A_Determination_of_Bacterial_Numbers (Accessed: 23 June 2024).

5.4. Conclusion

Colours causing highest CFU/mL are most effective for acne vulgaris treatment, as increased *Staphylococcus epidermidis* leads to greatest bactericidal effects on *Cutibacterium acnes*, reducing proliferation of acne. Results from this experiment are inconclusive, as the two measures of CFU/mL, absorbance and plate count, contradicted each other.

Absorbance was likely more precise, as more repeats were conducted, and results varied less compared to plate count. It also corroborated more closely with expected trends,[51] therefore was also more accurate. Thus, conclusions from absorbance were likely more valid.

Both measures produced results demonstrating statistically significant differences in effects of different colours on *Staphylococcus epidermidis* population, leading to rejection of the null hypotheses. However, blue light did not significantly reduce *Staphylococcus epidermidis* population compared to all other colours, so alternative hypotheses were only partially supported. Therefore, more research should be done on the photoinactivation mechanism of *Staphylococcus epidermidis*, and whether there are differential impacts of light treatment on *Staphylococcus epidermidis* and *Cutibacterium acnes*. If so, it could be determined whether inactivating both bacteria is more beneficial in treating acne vulgaris than not employing any light treatment at all, and therefore whether light treatment is a viable acne treatment option.

[51] Angarano, V. *et al.* (2020) 'Visible light as an antimicrobial strategy for inactivation of pseudomonas fluorescens and Staphylococcus epidermidis biofilms', *Antibiotics*, 9(4), p. 171. doi:10.3390/antibiotics9040171.

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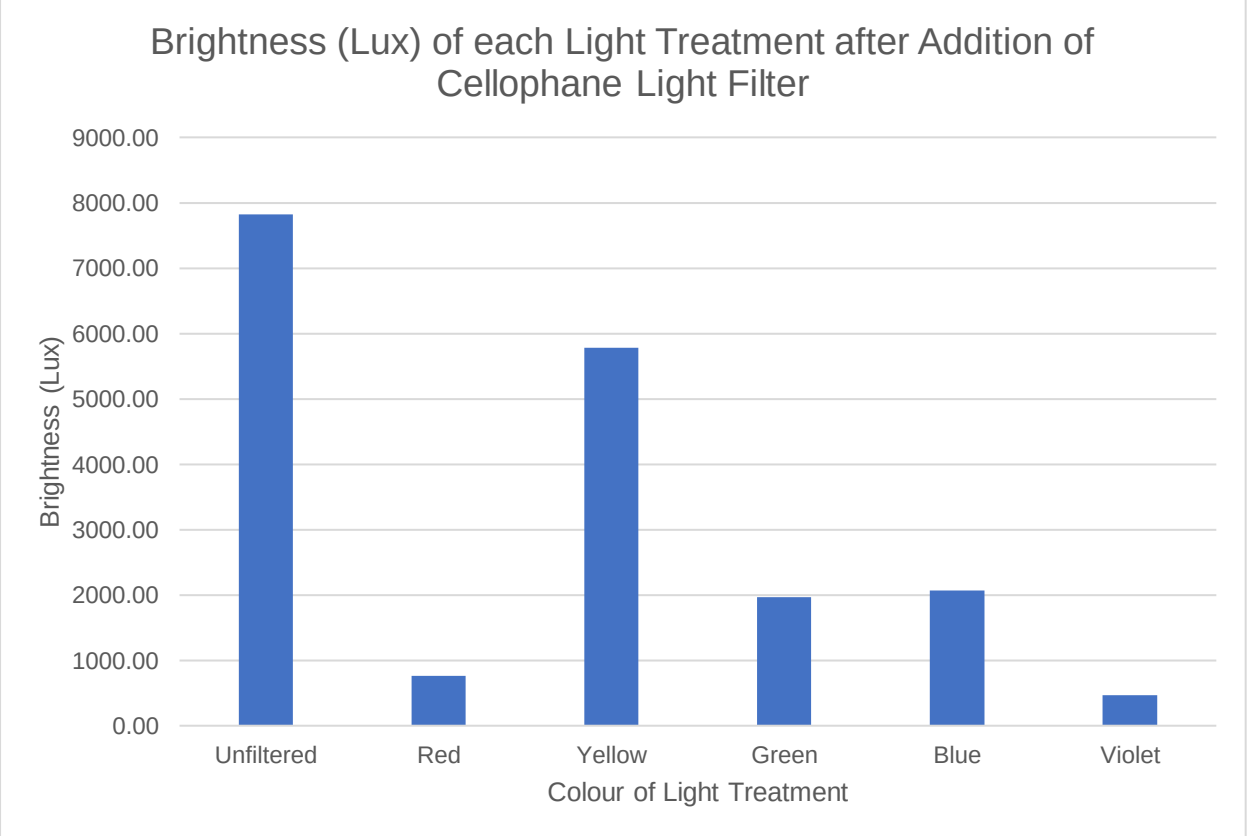
7. Appendices

7.1. Appendix A

Table 22: Lux measurements using a light sensor to determine both the homogeneity of the light panel and the effects of different coloured cellophane filters on the intensity of light bacterial samples are exposed to

		Colour of Light Treatment					
		Unfiltered	Red	Yellow	Green	Blue	Violet
Brightness (Lux)	Trial 1	7604.95	768.22	5768.28	1984.57	2039.76	456.96
	Trial 2	7673.39	768.22	5779.32	1982.37	2061.84	463.58
	Trial 3	7688.84	761.60	5781.53	1958.08	2114.82	472.41
	Trial 4	7991.27	764.42	5772.70	1961.19	2041.97	461.37
	Trial 5	7882.69	766.36	5780.91	1960.23	2064.04	463.58
	Average	7768.23	765.76	5776.55	1969.29	2064.49	463.58



Graph 4: Graph of brightness (Lux) of different coloured light treatments when using cellophane filters to change colour.

7.2. Appendix B

Table 23: Raw data for absorbance of 1:10 dilutions of *Staphylococcus epidermidis* following 30 minutes of light treatment under each colour.

			Absorbance (AU) ($\pm 0.003 AU$) at 600 nm ($\pm 0.5 nm$)		
			Trial 1	Trial 2	Trial 3
Colour of Light Treatment	Unfiltered Light	Scan 1	0.045	0.043	0.060
		Scan 2	0.045	0.043	0.060
		Scan 3	0.045	0.043	0.060
		Average	0.045	0.043	0.060
	Red Light	Scan 1	0.038	0.035	0.035
		Scan 2	0.038	0.035	0.035
		Scan 3	0.038	0.035	0.035
		Average	0.038	0.035	0.035
	Yellow Light	Scan 1	0.037	0.037	0.037
		Scan 2	0.037	0.037	0.037
		Scan 3	0.037	0.037	0.037
		Average	0.037	0.037	0.037
	Green Light	Scan 1	0.039	0.033	0.039
		Scan 2	0.039	0.033	0.039
		Scan 3	0.039	0.033	0.039
		Average	0.039	0.033	0.039
	Blue Light	Scan 1	0.035	0.040	0.044
		Scan 2	0.035	0.040	0.044
		Scan 3	0.035	0.040	0.044
		Average	0.035	0.040	0.044
	Violet Light	Scan 1	0.033	0.032	0.033
		Scan 2	0.033	0.032	0.033
		Scan 3	0.033	0.032	0.033
		Average	0.033	0.032	0.033

7.3. Appendix C

Table 24: Raw data for standard plate count of 1:10 and 1:100 dilutions of *Staphylococcus epidermidis* following 30 minutes of light treatment under each colour.

			Colony count of *Staphylococcus epidermidis* in each petri dish after 30 minutes of light treatment under each colour			
			1:10	Average 1:10	1:100	Average 1:100
Colour of Light Treatment	Unfiltered	Sample 1	100	87.67	TNTC	205.50
		Sample 2	69		115	
		Sample 3	94		296	
	Red	Sample 1	TNTC	146.50	94	121.33
		Sample 2	140		111	
		Sample 3	153		159	
	Yellow	Sample 1	124	124.00	314	263.00
		Sample 2	TNTC		267	
		Sample 3	TNTC		208	
	Green	Sample 1	TNTC	TNTC	199	262.33
		Sample 2	TNTC		287	
		Sample 3	TNTC		301	
	Blue	Sample 1	TNTC	TNTC	43	150.33
		Sample 2	TNTC		276	
		Sample 3	TNTC		132	
	Violet	Sample 1	TNTC	TNTC	309	282.00
		Sample 2	TNTC		137	
		Sample 3	TNTC		400	

Highlighted data points are plates which experienced fungal growth or contamination from other foreign particles.

7.4. Appendix D

Table 25: Tukey-Kramer post-hoc test for CFU/mL conversion from absorbance at 600 nm to determine which results were statistically significant.

Comparison	Absolute Mean Difference	Q Critical Value	Significant?
Unfiltered vs Red	3.13×10^7	1.28×10^7	Yes
Unfiltered vs Yellow	2.90×10^7	1.28×10^7	Yes
Unfiltered vs Green	2.90×10^7	1.28×10^7	Yes
Unfiltered vs Blue	2.27×10^7	1.28×10^7	Yes
Unfiltered vs Violet	3.92×10^7	1.28×10^7	Yes
Red vs Yellow	2.35×10^6	1.28×10^7	No
Red vs Green	2.35×10^6	1.28×10^7	No
Red vs Blue	8.62×10^6	1.28×10^7	No
Red vs Violet	7.83×10^6	1.28×10^7	No
Yellow vs Green	0.00	1.28×10^7	No
Yellow vs Blue	6.27×10^6	1.28×10^7	No
Yellow vs Violet	1.02×10^7	1.28×10^7	No
Green vs Blue	6.27×10^6	1.28×10^7	No
Green vs Violet	1.02×10^7	1.28×10^7	No
Blue vs Violet	1.65×10^7	1.28×10^7	Yes

7.5. Appendix E

Table 26: Tukey-Kramer post-hoc test on CFU/mL data from plate count after 72 hours of incubation at 25°C to determine which results were statistically significant.

Comparison	Absolute Mean Difference	Q Critical Value	Significant?
Unfiltered vs Red	3.38×10^5	2.21×10^5	Yes
Unfiltered vs Yellow	7.63×10^5	2.21×10^5	Yes
Unfiltered vs Green	7.61×10^5	2.21×10^5	Yes
Unfiltered vs Blue	4.25×10^5	2.21×10^5	Yes
Unfiltered vs Violet	8.20×10^5	2.21×10^5	Yes
Red vs Yellow	4.25×10^5	2.21×10^5	Yes
Red vs Green	4.23×10^5	2.21×10^5	Yes
Red vs Blue	8.70×10^4	2.21×10^5	No
Red vs Violet	4.82×10^5	2.21×10^5	Yes
Yellow vs Green	2.00×10^3	2.21×10^5	No
Yellow vs Blue	3.38×10^5	2.21×10^5	Yes
Yellow vs Violet	5.70×10^4	2.21×10^5	No
Green vs Blue	3.36×10^5	2.21×10^5	Yes
Green vs Violet	5.90×10^4	2.21×10^5	No
Blue vs Violet	3.95×10^5	2.21×10^5	Yes

7.6. Appendix F

Some plates had unusual, potentially anomalous bacterial growth (Figure 9 and Figure 10).

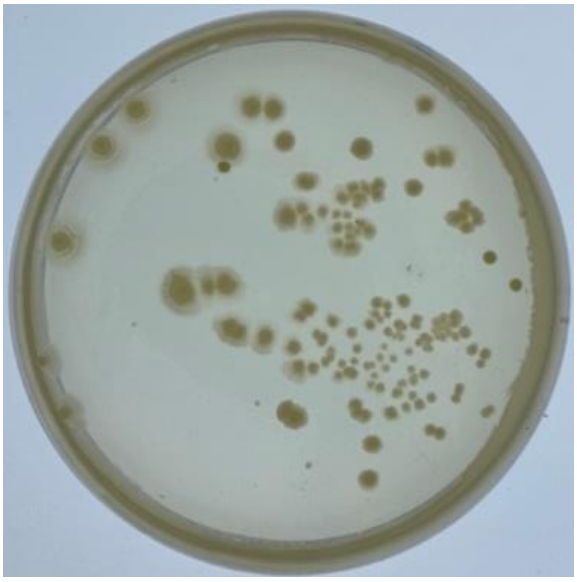


Figure 9: 1:100 dilution of petri dish containing *Staphylococcus epidermidis* sample treated under unfiltered white light after 30 minutes of incubation at 25°C. Displays evidence of anomalous colony morphology, thus was excluded from counting.

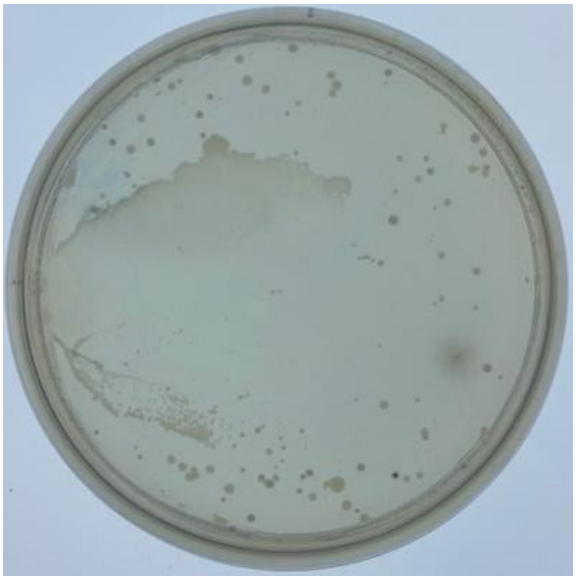


Figure 10: 1:100 dilution of petri dish containing *Staphylococcus epidermidis* sample treated under green light after 30 minutes of incubation at 25°C. Appears anomalous/unusual compared to other plates, thus was excluded from counting.

7.7. Appendix G

Preliminary trials involved determining suitable bacterial dilutions to obtain measurable colony counts and absorbance. 30-minute treatment length was selected due to being the typical length of current clinical light treatment[52]. 1:10, 1:100, 1:10,000 and 1:100,000 dilutions were trialled. Employing a method identical to 2.4, except only repeating each dilution once, 1:10 dilutions were found most suitable for absorbance, and 1:10 and 1:100 for plate count after 72 hours of incubation at 25°C. All other dilutions had negligible absorbance, and plate counts either too few to count (TFTC) or too numerous to count (TNTC), increasing random error.

[52] Handler, M. (2022) *Lasers and lights: How well do they treat acne?*, *American Academy of Dermatology*. Available at: https://www.aad.org/public/diseases/acne/derm-treat/lasers-lights (Accessed: 26 June 2024).

3/2/24

My younger brother was recently prescribed antibiotics for his eczema infection by the bacteria *Staphylococcus aureus*. Since undergoing work experience at a microbiology lab, I developed an interest in bacterial growth/antibiotics and thought it would be interesting to investigate the effectiveness of different eczema treatments on bacteria extracted from my brother's infection or *Staphylococcus aureus* grown in culture. However, discussions with my supervisor revealed IB guidelines prohibit use of pathogenic bacteria or bacteria cultured from unknown sources. Through further research I discovered *Staphylococcus epidermidis*, a non-pathogenic bacterium which inhibits acne growth - something I personally am affected by. Whilst talking to my friend I learnt about a handheld LED device he uses to treat his acne, which intrigued me due to its convenience and relative un-invasiveness. After reading secondary sources I discovered various wavelengths of light are used commercially in visible light therapy (420-700nm) and wanted to investigate why these different wavelengths are used and their effectiveness.

7/4/24

Due to limitations in equipment, my research question was modified slightly from investigating effects of specific wavelengths of light to different coloured light on colony size. This revised methodology was limited as different coloured lights differ in brightness/irradiance due to using different cellophane filters, potentially hindering the bactericidal effects of lower irradiance light.

When conducting light treatment, one unanticipated difficulty was that heat from the light source would cause some bacteria to evaporate. This decreased volume of bacterial broth available following treatment, thus limiting number of trials undertaken.

Results from the two different measurements of colony size (absorbance and standard plate count) initially appeared contradicting, however consultations with my supervisor revealed this confusion was from a simple calculation error, thus the results mostly corroborated.

I found absorption easier to measure while producing more consistent data, however standard plate counts allowed identification of growth/presence of other bacteria/fungi within the colony which indirect absorption measurements alone did not consider. Thus, I decided both measurements were important to include. However, due to limited bacterial broth, incorporating both measurements were at the expense of number of repetitions of each measurement conducted.

10/4/24

Since processing my raw data for both measures I have discovered that absorbance and plate count data seem to contradict each other. Regardless, I will continue with the write up of this practical, and evaluate some of the potential causes for these contrasting results.

12/5/24

My write up has been completed, not I just have to cut down my words. This will be extremely difficult. I am currently at 8000 words. However, reading through my whole practical I have noticed a lot of description that I can probably cut out for conciseness. A lot of analysis also needs to be rearranged.

23/5/24

I have finally managed to cut down my words. I have added a contents page and page numbers to my essay, and it felt so satisfying to scroll through my document and look at the quality of my work. I have spent way too much time on this assignment that I definitely could have used better in some of my other subjects at school, however this whole process of writing a research paper has really engaged me so much and is definitely something I could see myself potentially doing in future.

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☑ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

STUDENT(S) NAME: Caleb Tang ID: 0526-008

SCHOOL: Prince Alfred College

Activity: Give a brief outline of what you are planning to do.

Investigate the effects of different coloured light on the population of Staphylcococcus epidermidis bacteria

Population of growth will be measured in two different ways: absorbance at 600 nm and plate count after 72

hours of incubation at 25°C.

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead?
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

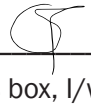
Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Risks	How I will control/manage the risk
Presence of Staphylococcus epidermidis bacteria. There is a risk of contamination and/or transmission of bacteria.	Bunsen burner used to sterilise environment. Ventilation turned off when handling bacteria. Gloves, face mask, lab coat and safety glasses worn, and thorough sanitisation of laboratory before and after handling bacteria. All equipment autoclaved after usage before disposal to minimise release of bacteria into the environment
Use of bunsen burner	Was left on safety flame throughout.

(Attach another sheet if needed.)

Risk Assessment indicates that this activity can be safely carried out

RISK ASSESSMENT COMPLETED BY (student name(s)): Caleb Tang

SIGNATURE(S): 

☑ By ticking this box, I/we state that my/our project adheres to the listed criteria for this Category.

TEACHER'S NAME: Fidias Andari

SIGNATURE:  DATE: 30/6/24