

# How Ultraviolet Rays Can Participate in Sterilisation Practices

Jojo Li & Anna Xu

## ABSTRACT

Past studies have shown that ultraviolet rays have the capabilities to provide disinfection properties towards microorganisms like bacteria. However, there are estimated 1513 bacterial pathogens known to infect humans described pre-2021 (Bartlett *et al.* 2022) and studies have also shown clear differences in time lengths required for specific species of bacteria to inactivate during the process of ultraviolet irradiation (Sousa *et al.* 2023). This work aims to determine the period of time it takes for bacterial microorganisms to disinfect under the exposure of UV-C light. It was



predicted that during this investigation, the longest time tested of 30 minutes would provide the lowest number of bacterial growths after exposure to UV-C light. This experiment was done by exposing agar plates contaminated with bacteria *Escherichia coli* and *Staphylococcus epidermidis* to UV-C light, using a sterilisation lamp and recording the amount of bacterial growth after varying time lengths of exposure. The results of this work show that the length of UV-C light exposure effectively influences bacterial growth. It was found that bacteria *E. coli* had a percentage coverage of 98% at time of exposure of 0 minutes, and after 30 minutes of UV-C light exposure, the average microbial growth percentage decreased to 35%. As for bacteria *S. epidermidis*, overall microbial coverage at 0 minutes of UV exposure was at an average of 93%, then descended to a mean percentage of 25% after 30 minutes of UV-C exposure. It can be obtained from this investigation that UV-C light rays have a sterilization effect on bacteria species *E. coli* and *S. epidermidis*, but longer periods of exposure time should be applied to ensure higher percentages of bacterial inactivation.

## INTRODUCTION

Cures like pharmaceutical drugs, surgeries, procedures, and vaccines are constantly being renewed by society, aiming to provide a stable treatment for specific harmful microorganisms. Over the last three years, the medical industry has been challenged by a global virus outbreak, COVID-19. This infamous upsurge led to several shortages in medical supplies, specifically regarding personal protective equipment (PPE) health professionals had on hand. Although many are still not familiar with the action of ultraviolet germicidal irradiation (UVGI). The deficit of medical equipment prompted the industry to regain interest in this method due to its disinfection properties and the potential use, sterilising not only PPE but also other surfaces and pathogens (Rowan & Laffey 2021).

The microbial inactivation method, UVGI, is mainly achieved through photochemical reactions between the UV light and the genetic material (DNA or RNA) within the microorganism (Raeiszadeh & Adeli 2020). The UV-C light breaks the adenine-thymine bond in the DNA leading to the formation of pyrimidine dimers, which ultimately affects the microorganisms' ability to transcribe and replicate RNA and DNA (G. Reed 2010). UV-C light is a specific subtype of UV light, with a wavelength range of 100-280 nm. Due to its short wavelength

compared to UV-A and UV-B, UV-C is the most damaging type of UV radiation (World Health Organization 2016). Due to this characteristic, it is commonly used in UVGI.

Multiple studies have shown that the longer the exposure of bacteria to short wavelength UV light the lower the survival rates. An experiment using *Escherichia coli* and UV lamp of 254nm demonstrated that an exposure time of 48 hours resulted in a lower number of colony-forming units (CFUs) compared to 24 hours (Kodoth & Jones 2014). A study using *Staphylococcus aureus* bacterium established that 10 minutes of UVGI eliminated 0.5% more bacterial growth compared to the period of 5 minutes, and all bacteria were inhibited after 3-4 hours of UV light exposure (Hardjawinata *et al.* 2012). Similarly, another study obtained the results of a different decrease in CFU/cm<sup>2</sup> of each bacteria *E. coli* and *S. epidermidis*, after the application of UVC light with the percentage of reduction greater for 5 minutes than for 1 minute of exposure (Mariana Sousa *et al.* 2023).

However, studies have also revealed that there are clear effectiveness differences that UVGI has for individual species of bacteria (Mariana Sousa *et al.* 2023). There is an estimated amount of 1513 pathogenic bacterial species which infect humans (Bartlett *et al.* 2022), and common bacterial species like *E. coli* and *S. epidermidis* are the main cause of diseases like infective endocarditis (Lee & Anjum 2023) and food poisoning (Health Direct 2023). Hence, it is vital to validate a universal length of time that can be set for disinfection of general bacterial species using UVGI and establish clear timescales of disinfection for distinct bacteria.

This paper aims to substantiate and evaluate the percentage of bacterial inactivation across varying time intervals under UV-C exposure, using bacteria *E. coli* and *S. epidermidis*. This experiment measured the amount of bacterial growth of *E. coli* and *S. epidermidis* after varying periods of UV-C exposure. It is predicted that out of the ten varying intervals of UV light exposure, 30 minutes, the longest time tested, will result in the lowest bacterial growth percentage. This is because the longer the duration of UVC exposure, the more photochemical reactions will take place and pose a potential fault in the microorganism's biological processes, causing them to inactivate.

## METHOD

### Materials

- |   |   |
|---|---|
| <ul style="list-style-type: none"><li>- 42 Agar plates</li><li>- Escherichia Coli bacteria broth</li><li>- Staphylococcus Epidermidis bacteria broth</li><li>- Pipette</li><li>- Plastic Spreader</li><li>- UV Light Source (Xiaoda Sterilisation Lamp)</li><li>- Alcohol spray</li><li>- Stopwatch</li><li>- Incubator</li><li>- Thermometer</li></ul> | <ul style="list-style-type: none"><li>- Tape</li><li>- Sharpie</li><li>- Camera source</li><li>- Open cardboard box</li><li>- A2 Sheets of Black paper</li><li>- 100ml Beakers</li><li>- Masks</li><li>- Sunglasses</li><li>- Gloves</li><li>- Safety goggles</li></ul> |
|---|---|



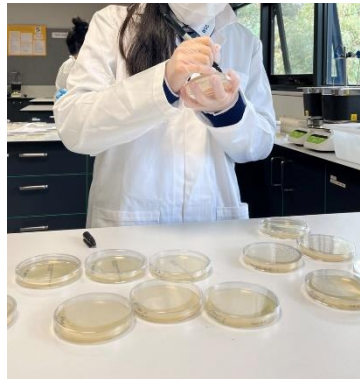
### Procedure

1. Safety precautions were taken regarding protective equipment.

2. Incubator was set at a temperature of 28°C, measured with a thermometer.



3. 42 agar plates were collected and distributed evenly across two lab tables, designating one station for each bacterium.
4. A sharpie was used to label the underside of each agar plate, commenting the duration of UV light exposure, type of bacteria and plate number.
  - a. Control Plate labelling example: '0 E. coli 1' (0 minutes, E. coli bacteria, plate 1)



5. A sterile pipette was used to place 3 controlled drops of *Escherichia Coli* broth onto the 21 assigned plates.



6. Bacterial broth was then spread evenly across the surface of nutritional plates, using a sterile plastic spreader.
  - a. Plastic spreader and pipette were placed in 100ml beakers when not in use to prevent cross contamination.



7. Once all 21 *Escherichia Coli* agar plates were evenly swabbed, tape was used to seal the plates.
8. Steps 5-7 were repeated for bacterium *Staphylococcus Epidermidis*.
9. The UV light was set up underneath a cardboard box.
  - a. Control plates were set aside ready for incubation during the UV light exposure process as it does not require any exposure to UV-C light
10. 3 plates of each bacterium of the same time intervals were placed around the UV light, with all the sides touching, creating a flower-like shape.



11. Sunglasses were worn during the activation of UV-C light source, participants were cautious to not look directly at the light source.
12. Exposure site was immediately covered with a cardboard box after UV-C light source was turned on.

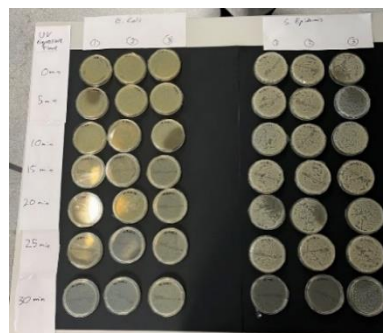
- a. A stopwatch was used to time the duration of UV exposure following the set 5-minute intervals.



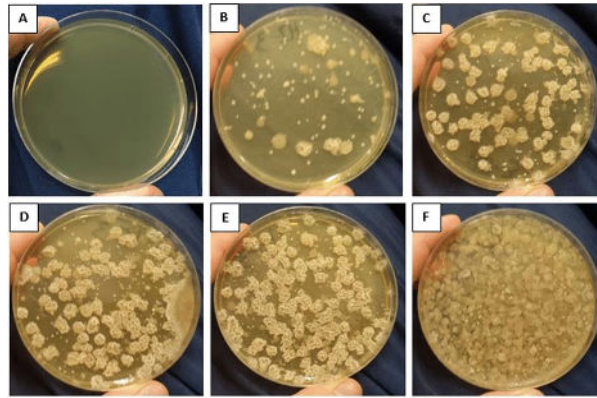
13. After timed exposure, UV light was turned off at once and agar plates were set aside until all remaining 30 agar plates were exposed.
14. When exposure process has been completed, all 42 agar plates were placed in the incubator at the same time for 72 hours. This was done to ensure that all the agar plates received the same amount of incubation time length.
15. Contaminated materials were disposed of via biohazard waste bags and surfaces were disinfected with alcohol liquid.



16. Gloves were taken off cautiously and required hand hygiene was taken.
17. After 72 hours of incubation, the 42 agar plates were collected and placed onto a black coloured surface to better observe results.



18. Amount of bacterial growth was recorded via reference photos.
- Percentage calculations were estimated by comparing bacterial growth on tested agar plates to the reference.
  - The average of the 3 agar plates of the same bacteria on which received the same exposure length to UV-C was calculated.
  - Reference photo used in this experiment is shown below:



Reference Photo

A) 0% plate coverage, B) 20% plate coverage, C) 40% plate coverage, D) 60% plate coverage, E) 80% plate coverage, and F) 100% plate coverage.

Graham, L. (2018). *Reference pictures used to determine the percentage of plate coverage*. ResearchGate. [https://www.researchgate.net/figure/Reference-pictures-used-to-determine-the-percentage-of-plate-coverage-on-TSA-plates\\_fig1\\_323817661](https://www.researchgate.net/figure/Reference-pictures-used-to-determine-the-percentage-of-plate-coverage-on-TSA-plates_fig1_323817661)

### Diagram

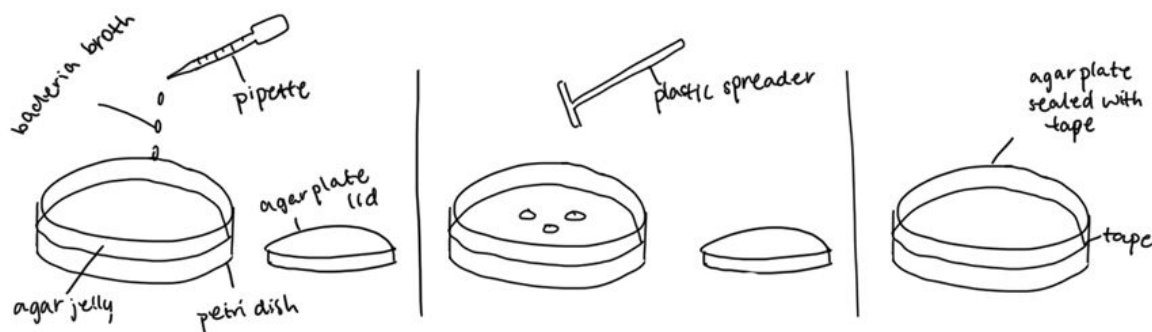


Figure 1. Swabbing Agar Plates with Bacteria Broth

### Safety

In this experiment, there are two main safety risks associated. The UV-C light used in this experiment can pose health risks to participants' eyes and skin. As well as the pathogenic microorganisms tested with can cause serious bacterial infections. To prevent this, protective equipment was worn accordingly throughout the whole of the experiment, including safety goggles, gloves, face masks, lab coats and sunglasses. In addition to this, experimenters' direct exposure to UV-C light did not exceed one second on occasions where the UV-C lamp



Figure 2. Contaminated agar plates after incubation period

Table 1. *E. coli* → 0 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
95	100	100	98

Table 2. *E. coli* → 5 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
100	100	100	100

Table 3. *E. coli* → 10 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
100	100	100	100

Table 4. *E. coli* → 15 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
70	70	75	72

Table 5. *E. coli* → 20 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
80	100	80	87

Table 6. *E. coli* → 25 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
70	60	80	70

Table 7. *E. coli* → 30 Minutes

Plate 1 (%)	Plate 2(%)	Plate 3(%)	Avg. (%)
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20	25	60	35
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Table 8. *S. epidermidis* → 0 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
100	100	80	93

Table 9. *S. epidermidis* → 5 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
100	100	70	90

Table 10. *S. epidermidis* → 10 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
90	100	95	95

Table 11. *S. epidermidis* → 15 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
95	95	95	95

Table 12. *S. epidermidis* → 20 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
75	90	90	85

Table 13. *S. epidermidis* → 25 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
95	100	100	97

Table 14. *S. epidermidis* → 30 Minutes

Plate 1(%)	Plate 2(%)	Plate 3(%)	Avg. (%)
15	20	40	25

Table 15. *E. coli* Plate Average

Time of exposure (minutes)	0	5	10	15	20	25	30
Average amount of bacterial growth coverage (%)	98	100	100	72	87	70	35

Table 16. *S. epidermidis* Plate Average

Time of exposure (minutes)	0	5	10	15	20	25	30
Average amount of bacterial growth coverage (%)	93	90	95	95	85	97	25

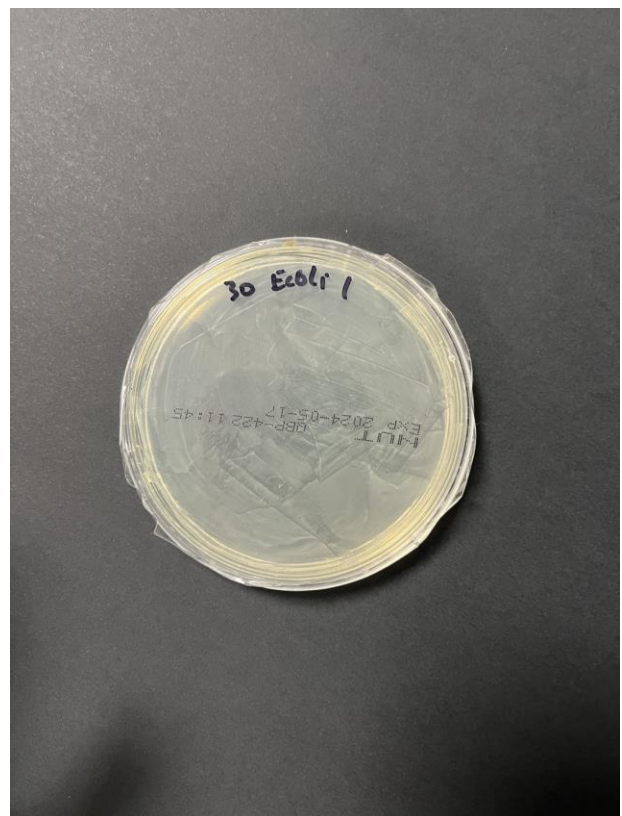


Figure 3. *E. coli* 0-30 minutes Change

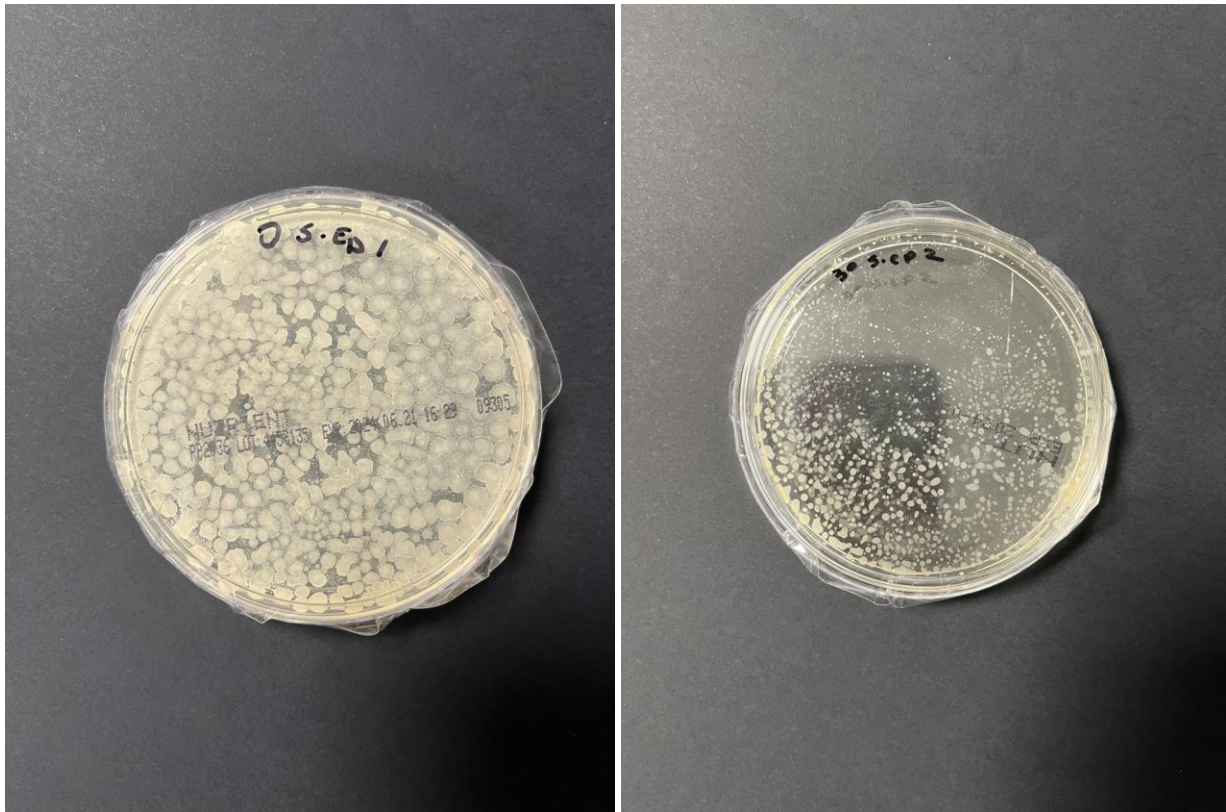


Figure 4. *S. epidermidis* 0-30 minutes Change

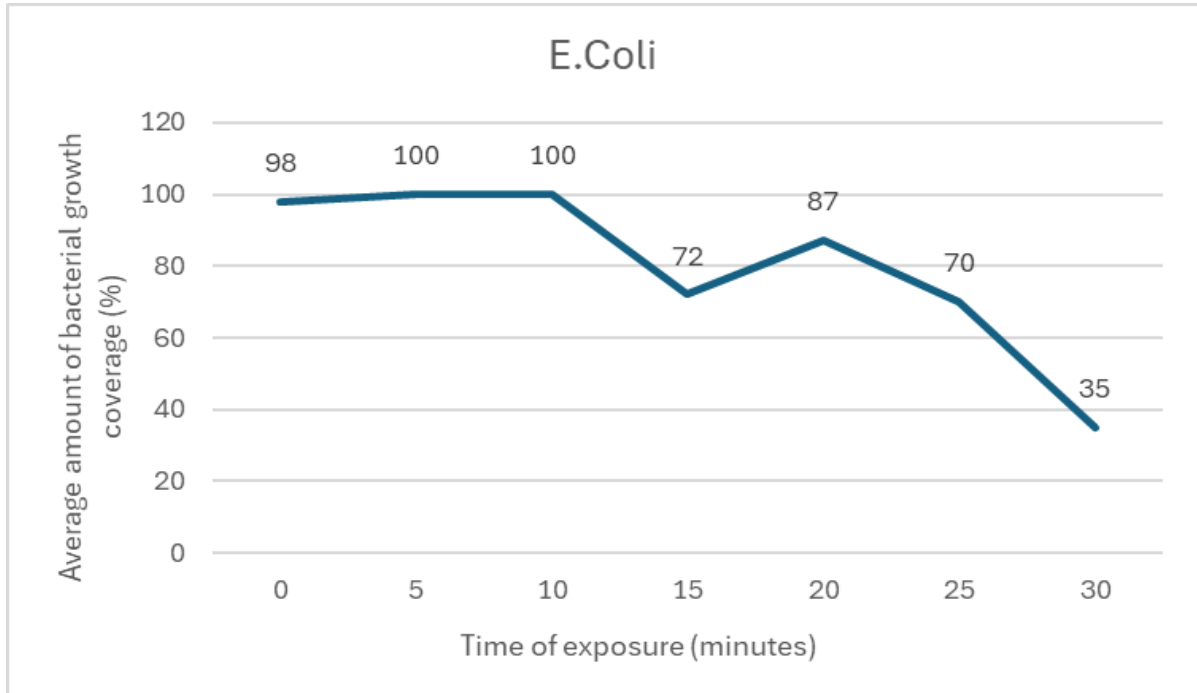


Figure 5. UV-C Exposure Against *E. coli*

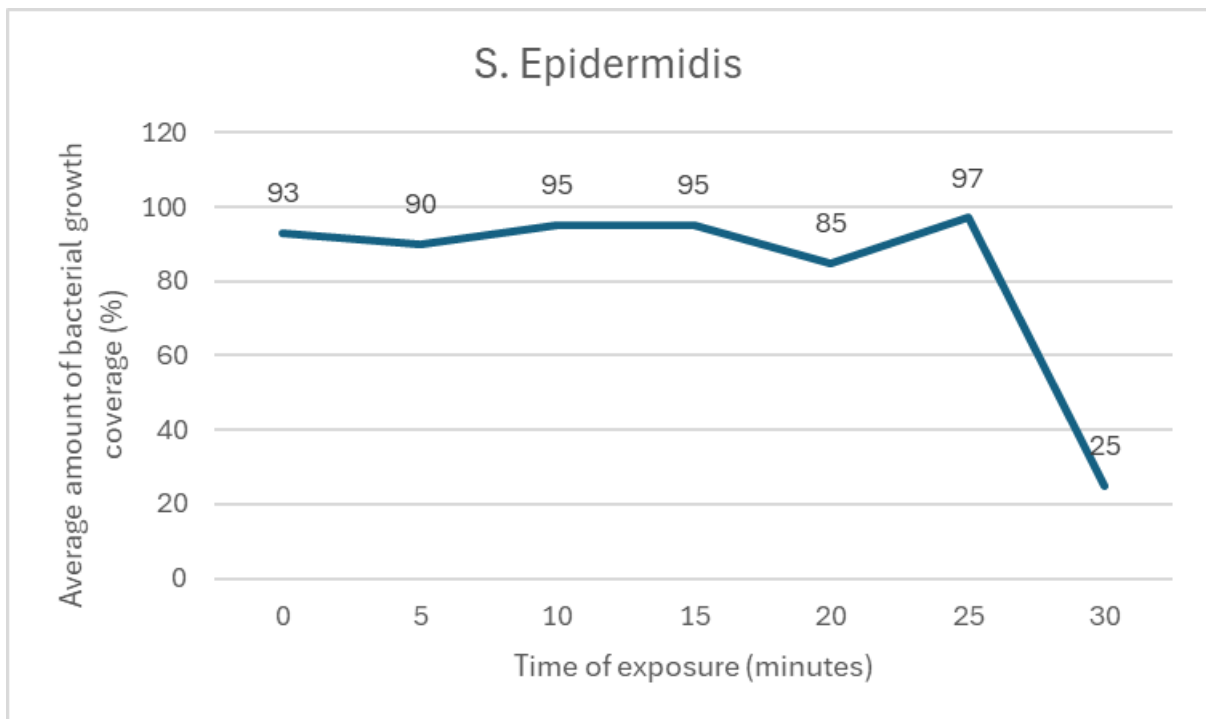


Figure 6. UV-C Exposure Against *S. epidermidis*

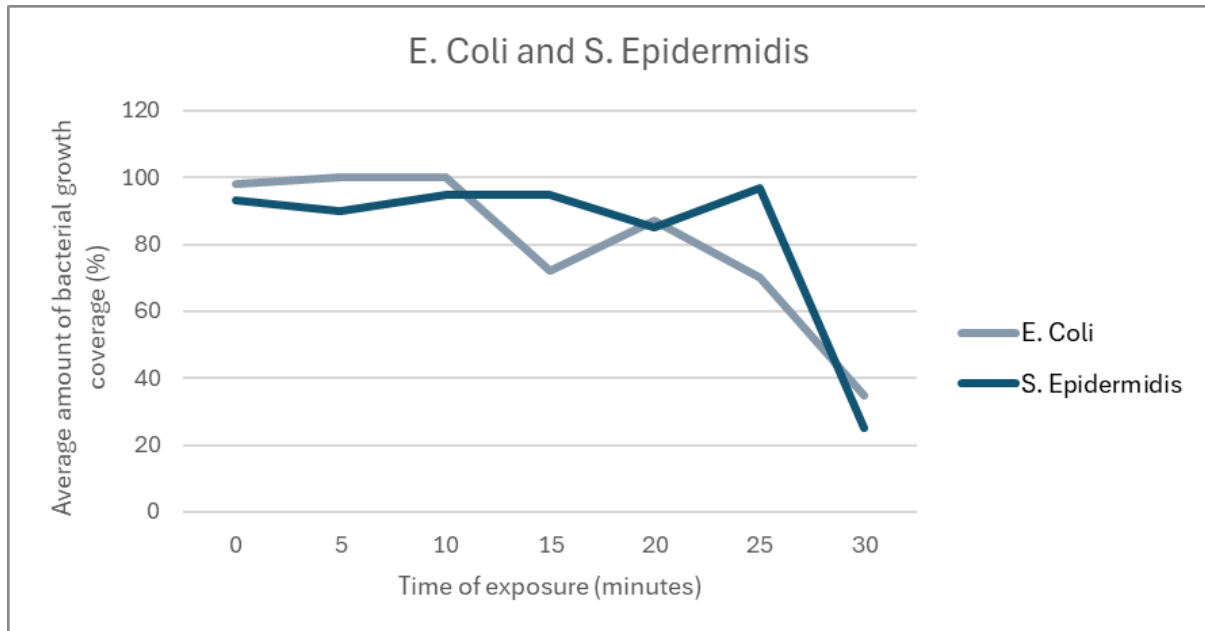


Figure 7. UV-C Exposure Against *E. coli* and *S. epidermidis*

## DISCUSSION

In this experiment, two types of bacteria *Escherichia coli* and *Staphylococcus epidermidis* were grown in an incubator after being exposed to UV-C light of varying time intervals. This was done to evaluate the disinfection time required for the different pathogenic microorganisms to inactivate. When evaluating the results, the hypothesis that the longest interval of UV light exposure of 30 minutes would be most effective in inhibiting the growth of bacteria was supported.

### Discussion for *Escherichia coli*

With the bacterium *E. coli*, the inactivation of this bacteria began shortly after 10 minutes, followed with a bacterial coverage decrease of 28% between the time periods 10 and 15 minutes. After exposing the contaminated agar plate for 30 minutes, the average bacterial coverage was set at a mean of 35%, decreasing greatly when compared to the control agar plate, which held 98% of microbial coverage, supporting the hypothesis of this experiment.

The results of this work indicated that, at approximately 10 to 15 minutes, the photochemical reactions caused from the ultraviolet radiation (Raeiszadeh & Adeli 2020) has begun to take effect on *E. coli*. This process then breaks down certain DNA bonds and hindering the microorganisms' capabilities of reproduction (G. Reed 2010) and results in bacterial inactivation.

### Discussion for *Staphylococcus epidermidis*

In comparison, the bacteria *S. epidermidis* began its inactivation process later, at a period after the 25-minute interval. During this period, the average bacterial growth percentage decreased by over 60%, as opposed to all intervals before this point, where CFU percentages ranged from 85% to 97%. These results gathered show that effective UVGI on bacterium *S. epidermidis* requests for a disinfection time longer than of bacterium *E. coli*. Despite this, the process of UV disinfection on *S. epidermidis* was shown to work rapidly

when effective bacterial inhibiting begins, at time intervals of 25-30 minutes, deflating by an average of 72% within the 5 minutes.

The reason of difference between the time taken for *E. coli* to be affected compared to *S. epidermidis* could be due to the structure of *S. epidermidis* having a mesh-like layer that acts as protection called the peptidoglycan layer (Schumann 2011). This layer is the major component of a bacteria's cell wall and is widely investigated by those experimenting the action of antibiotics and mechanisms of resistance. Its thickness is a determining factor in how susceptible bacteria is to UV radiation.

### **Errors and Improvements**

Two main random errors that affected the precision of this experiment was incubator inconsistencies and data collection method. Some agar plates were found to have inconsistent areas that lack bacteria growth. This cannot be explained by UV light exposure as the plates that were exposed to the same amount of time do not show similar defections. However, this could be due to incubator malfunctions, where some plates were exposed to higher temperatures that potentially killed the microorganisms. A potential improvement that can be made is to use a different incubator to run the experiment again.

Although precautions are taken to make data as accurate as possible when using the reference photos, the human eye can be unreliable. Therefore, the precision of the data can be increased by potentially pairing the human judgement with advanced technology such as AI algorithms. This experiment has multiple random errors that lower the precision but by avoiding systematic errors the investigation remains accurate.

### **Validity and Reliability**

Validity refers to the extent of a method accurately measuring what it is intended to measure. Experiments with high validity, produce results that are easily relatable to the characteristics and variations of the physical world. Reliability can be defined by how easily an experiment can be repeated and how consistently it measures something. A variable of the experiment that was controlled was the intensity of the UV light dosage, ensuring that all agar plates received equal strength exposure to UV-C light. The temperature of incubation was set to 28C for 72 hours to mimic real life circumstances where bacteria thrive at room temperature. To ensure that the bacteria in the broth was spread evenly across all plates, the broth was stirred beforehand. Other controlled variables include: the distance between UV light and plate, type of bacteria and number droplets of bacterial broth. Reliability is apparent in this experiment as the data shows plates with the same exposure interval provided similar results. Ultimately this experiment is valid and reliable due to its numerous control variables and carefully thought-out method.

### **Application**

Through demonstrating the efficiency of using UV light to sterilise surfaces, the wider population can greatly benefit from the usage of UVGI. These benefits can come in the forms of decreased rates of sickness, which is caused by cleaner environments, specifically regarding the recent Covid-19 outbreak. Following the pandemic, it is extremely vital to have multiple ways to combat the spread of pathogens so that the severity of the situation can remain low and avoid being worsened by other infectious microorganisms. Although the time

interval, 30 minutes, is adequate to inhibit bacterial growth in *E. coli* and *S. epidermis*, future investigations could determine the most effective disinfection times in other pathogens.

With the expectation to make the use of UV light as a disinfection practice more common and accessible, future experiments could also evaluate why bacteria have different levels of susceptibility to support the find of varying tolerances and time of UV light exposure needed, so that a universal length of disinfection time can be determined.

## **CONCLUSION**

The aim of this experiment was to determine the total percentage of bacterial inactivation across varying time intervals under the exposure of short wavelength UV, using bacteria *E. coli* and *S. epidermidis*. It was predicted that the contaminated agar plate receiving the longest duration of UV exposure of 30 minutes would eradicate the most colonies of bacteria and develop the lowest percentage of CFUs present on the nutrient plates. This was hypothesised because the increasing the length of UV-C exposure allows more time for chemical reactions to occur and hinder the microorganism's biological activities. According to the results, bacteria *E. coli* was found to have a percentage coverage of 98% at time of exposure of 0 minutes. Consequently, after 30 minutes of UV-C light exposure, the average growth percentage decreased to 35%. As for bacteria *S. epidermidis*, overall bacterial coverage at 0 minutes of UV exposure was calculated to be at an average of 93%, then descended to a mean percentage of 25% after 30 minutes of UV-C exposure. Therefore, the hypothesis is accepted.

## REFERENCE

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### **Image References**

*UVC lights* [image]. [https://avidbots.com/assets/Blog/74\\_avidbots-blog-what-you-need-to-know-about-uv-light-and-autonomous-floor-scrubbing-robots.png](https://avidbots.com/assets/Blog/74_avidbots-blog-what-you-need-to-know-about-uv-light-and-autonomous-floor-scrubbing-robots.png)

### **ACKNOWLEDGEMENTS**

We would like to acknowledge Mr Evan Leed (Waverley Christian College), Mr Mark Chalmers (Waverley Christian College) and Mrs Nataile Yan (Waverley Christian College) for their feedback and assistance throughout this experiment and report write up.

# APPENDIX



## Risk Assessment Form:

Name of Entry How Ultraviolet Rays Can Participate in Sterilisation Practices

Student Name: Jojo Li Signature: *Jojo* Date: 12/07/2024

Student Name: Anna Xu Signature: *A* Date: 12/07/2024

Your assessment should include sample handling, storage, disposal, spill procedures and use of machinery...

Use as many pages as necessary, a blank table provided on the next page.

Type of Risk	Hazard	Level of Risk	Precaution taken to control risk	Adjusted level of Risk
<input type="checkbox"/> Chemical or microorganism <input type="checkbox"/> Procedure or equipment X	UV light causes damage to the eye and skin.	Consequence: Major Likelihood: Likely Risk Level: Extreme Risk	Participants are to wear lab coats, gloves and sunglasses.  Participants are briefed on the dangers of UV light exposure.	Consequence: Minor Likelihood: Unlikely Risk Level: Low Risk
<input type="checkbox"/> Chemical or microorganism <input type="checkbox"/> Procedure or equipment X	Bacterial infections can occur.	Consequence: Major Likelihood: Likely Risk Level: Extreme Risk	Participants are to wear lab coats, gloves, masks and safety goggles.  Participants are briefed on the dangers of bacteria being experimented with.	Consequence: Minor Likelihood: Unlikely Risk Level: Low Risk
<input type="checkbox"/> Chemical or microorganism <input type="checkbox"/> Procedure or equipment				
<input type="checkbox"/> Chemical or microorganism <input type="checkbox"/> Procedure or equipment				

Possible sources of information to complete your risk assessment

- [www.riskassess.com.au](http://www.riskassess.com.au)
- Search: safety data sheet