WaterScope Sterilization

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WaterScope has tasked this team to develop a sterilisation method to sterilise the sample containers and the metal filter 'cartridge' used in its water testing equipment. The method has to be quick, require minimal training, and be cheaper than the current alternatives. Considering initial research of sterilisation methods and recommendation from WaterScope, UV Irradiation and the application of Dry Heat were chosen to be investigated further. Experiments showed the best UV LED given by WaterScope could only give a 3-orders-of-magnitude reduction in the number of bacteria after 15 minutes of exposure at 2.5cm, which was far from the target of 6-orders-of-magnitude reduction. Dry heat at as low as 130 ° C for 6 minutes however was found to give at least a 4-orders-of-magnitude reduction of bacteria.

Fig. 1. WaterScope sample pot and 'cartridge'

I. Introduction

OVER 10% of the world's population do not have access to an improved drinking water source within a 30 minute round walk [1]. Contaminated water transmits diseases and is estimated to cause over half a million deaths each year. Chemical contamination levels remain relatively stable over time, but bacterial contamination can vary locally and fluctuate over short times, hence the importance of frequent local water sampling.

The current leading DelAgua kits cost thousands of pounds, are contained within large trunks and can take 16-18 hours to yield results [2]. Petri dishes and media are sterilised in an autoclave or pressure cooker, while the kit's vacuum cup is sterilised by burning methanol without oxygen inside, which produces formaldehyde. Wet heat requires bulky equipment, mains electricity or a large heat source, and both methods take at least 20 minutes. The right amount of methanol and correct chemical must be used, introducing the need for some user skill and knowledge and possibility of human error.

The size, cost, difficulty, and time taken by this kit, which is considered the current 'gold standard', provide some restrictions to its use. Ideally, according to WHO recommendations [3], bacterial tests should be frequent and the community should be benefited by training on how to use the equipment.

Waterscope's product uses a vacuum pump to draw water samples through a filter held inside a cartridge, where bacteria are concentrated and can then be studied in a purpose-designed microscope. This process aims to be portable, affordable, quick, simple to use and automated wherever possible [4] - this increases the potential frequency of tests and reduces risk of human error impacting results. The plastic or metal sample containers and the stainless steel 'cartridge' must be sterilised between tests.

Having researched potential methods against project specification and taken insight from existing WaterScope research, this project will test feasibility of two methods: 1) UV Irradiation and 2) Dry Heat. The first stage of the project was to characterise the effectiveness of these two principles and the test results are presented in section V of the report. The second stage would involve building prototypes based on the results of the first stage testing.

II. UV Irradiation

A. The Theory

UV light is used to kill or deactivate microorganisms by disrupting their DNA, leaving them unable to carry out their vital functions [5].

Different UV wavelengths have a different germicidal effectiveness (as shown in Appendix 4), with peak effectiveness at around 265nm. Longer wavelengths can be used - even as high as 365nm – though they will be less effective [6]. This is important since LEDs and bulbs with a shorter wavelength (~265nm) are typically more expensive and more difficult to source, hence the initial testing was only carried out on the longer wavelength LEDs that were available to us.

Organisms require a certain dose of UV in order to be destroyed. Typical doses at a 254nm wavelength are

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shown in Appendix 4. It is measured in energy per unit area (typically μJcm^{-2} or μWscm^{-2}) as shown by *(1)*.

$$Dose\ (\mu J cm^{-2}) = \frac{UV\ Power\ (\mu W) \times Time\ (s)}{Area\ (cm^2)} \quad (1)$$

The dose the received therefore depends on the:

- Exposure time (<20min per WaterScope specification)
- Radiant power of the UV (dependent on source efficiency and distance between source and sample as light intensity follows inverse square law. Electrical power limited by battery packs available to WaterScope).
- Area exposed (dependent on distance to source).

Other independent variables are:

- The wavelength of the UV (as shown in Appendix 4)
- The physical factors affecting exposure of surfaces to UV, including part geometry, shade and dirtiness of the surface.

The physical factors will be significant, as the complex shape of filter cartridge causes a lot of surface area to be shaded from the UV source.

Some initial feasibility research was carried out to determine if components we had already or could purchase could provide the required UV dose in a short enough time. The calculations can be found in Appendix 4.

Table I shows that current UV bulbs are faster than LEDs but are more expensive and require AC voltage and power supply that is less feasible within WaterScope's equipment and specification. The expensive short wavelength LEDs (LUEVA) have too little power so sterilisation time increases. The longer wavelength LED (VISHAY) however may have enough power for rapid sterilisation, and while the longer wavelength will be less effective their low price makes it possible to include multiple LEDs if necessary, to increase the total power. Large mains-powered UV bulb sterilisers are already available, so it is more useful to test if a smaller, specialised device can be produced to the specification using LEDs. Therefore, some cheaper, longer wavelength LEDs will be tested.

TABLE I
ESTIMATED TIME TO PROVIDE LOG-3 REDUCTION IN TOBACCO MOSAIC VIRUS FOR DIFFERENT UV COMPONENTS

Component	Wavelength (nm)	Cost (£)	Rated Power (mW)	Radiant Power (mW)	Estimated time to provide log-3 reduction
Bulb Lawtronics	253.7	24.35	4000	-	2mins
LED LUEVA	278	46.51	130	2	17h20mins
LED VISHAY	370	1.71	70	20	1h33mins

B. Design

A rig had to be built before performing any UV sterilisation tests, with a primary focus on safety – a fully contained enclosure to mitigate risk of using unknown UV sources (See Appendix 5).

The enclosure itself is constructed from laser cut Medium Density Fibreboard (MDF) for simplicity and speed, and is designed around safety features such as having at least three overlapping pieces at any edge, with black tape covering the edges to ensure no gaps for UV to escape. The UV sources are mounted on their own interchangeable moveable shelves which allows their height to be changed (thus intensity and coverage) and UV sources to be swapped without having to make a new seal around the bulb each time. There is a permanent roof with a flexible front piece to press against the door and seal any gaps when it closes.

As an electrical safety feature the enclosure has its own circuit – banana plugs fit into a variable supply for input power, and a microswitch is used as an interlock on the door. Each bulb is fitted with a molex plug that fits into a socket on the enclosure. This way they are interchangeable and can only be powered through the safety circuit on the device.

UV has been tested on stainless steel sheet cut to the dimensions of the cartridge's largest side to imitate the cartridge. Thickness is not important, but surface reflectivity must be replicated exactly as it effects the UV dose absorbed. Further testing will be carried out on polypropylene and HDPE sheet to represent the plastic sample cup.

III. Dry Heat Sterilisation

A. The Theory

Dry heat sterilisation is one of the earliest sterilisation methods used [7] It is effective in killing all microorganisms, viruses and bacterial spores by oxidising the molecules, denaturing the proteins and destroying their cell constituents [8] This method of sterilisation depends on the transfer of heat from a heat source to the article to be sterilised. It is, therefore, a considerably simpler sterilisation method than UV irradiation, since the only goal is to heat up the whole specimen to reach a desired uniform temperature.

The disadvantage of this method is that it can take a

long time to bring specimens to the desired temperature. However, given the small volume of the WaterScope cartridge, it was calculated that, in an ideal case, it would only take a time (t) of 6 minutes, according to:

$$t = Q/P \quad (2)$$
where $Q = mc\Delta T$ and $P = IV$

t = time (s)
Q = energy required to heat specimen to 200°C (J)
P = heater power (W)

The mass of the cartridge (m) is 101g, its specific heat capacity (c) is 502.4 $Jkg^{-1}K^{-1}$ [9] and the difference in temperature (ΔT) is 177K (200-23K, where 200°C is the desired temperature and 23°C is the room temperature). WaterScope have specified a 12V supply providing 1-2A of current (2A used in calculation).

Initially, a dry heat oven at the Cambridge Vet lab was used to test the feasibility of this method over appropriate temperatures and timescales by sterilising contaminated metal blocks. At a later stage, our prototype will be tested for sterilisation. For testing samples so far, the stainless-steel blocks were cut to roughly the dimensions of the cartridge, as the thickness of the sample is an important parameter in the process of conduction.

B. Design

Sterilisation by dry heat depends on the penetration of enough heat to the whole article to be sterilised. Since the cartridge to be sterilised is made of stainless steel, this helps accelerate the process as this material is a good conductor of heat (thermal conductivity = 16.3 - 16.7 $Wm^{-1}K^{-1}$) [10], [11] However, given the specifications set by Waterscope, a dry heat oven in remote areas would be unavailable, heavy and expensive. Therefore, a design was created that would use conduction to heat up the cartridge quickly and cheaply and would be simple for any user to operate. Specifically, the cartridge to be sterilised is denoted by 3 in Fig. 2. A 40x40x40 steel block (EN1A type steel) was machined to create a socket 4 where the cartridge can be placed onto. In addition, a hole was drilled on one side of the block in order to place the heating element 5 in. In future prototypes the heating element will be permanently secured in the block but at this stage that was not deemed wise. Then, the steel 'box' 2 is placed on top of the block-cartridge assembly in order to reduce heat loss by convection somewhat. Finally, the whole assembly is placed onto the concrete block 1, given that it can withstand high enough temperatures, so that to avoid any burning of the surface below the assembly. This method seems to be the most efficient way of heating up the cartridge quickly, given the requirements by Waterscope.

Fig. 2. Waterscope sample pot and 'cartridge'

Other methods considered were discarded, such as placing and heating up a metal plate in between the cartridge gap, either because a lot of heat would escape to the surroundings, or because of their complexity. At this current stage, a variable power source is used for the heating element. We are, however, still considering other potential prototypes, for example possibly using 2 heating elements to give a more even temperature distribution. Our next aim, given the promising results from the lab, is to focus on the aspects of safety and ease of usage. Specifically, an enclosure needs to be designed such that to mitigate the risk of any burn injuries, while sterilising the cartridge. For the purpose of testing, this enclosure could be the oven used for the earlier testing turned off, but part of more advanced prototyping will be developing a smaller enclosure that could be used in the field.

This enclosure would have to be light, which precludes concrete, and would have to be made of an insulating material, leaving a limited set of options within our reach of access. We will also have to develop a method of varying the power supply from a 12V battery supply. At this stage the suggested solution is to use a potential divider with a variable resistor controlled by a feedback system. The feedback system will use signals from a temperature sensor (or a number of sensors) placed within the enclosure to indicate the temperature of the assembly. The intention is to use an NTC thermistor for the temperature sensor.

IV. Other Methods Considered

This section outlines a number of other methods we considered for further research.

A. Ethanol/IPA/Methanol

Alcohols such as ethanol or isopropyl alcohol (IPA) kill bacteria by denaturing their proteins. Both are relatively easy to procure from local suppliers in most countries [12] They also work and evaporate quickly, reducing the time required for sterilisation and meaning bacteria can be cultured on the surface afterwards, if required.

However, it was concluded fairly rapidly that alcohols are unsuitable for sterilisation as they are generally "not recommended for medical use as [they] lack sporicidal activity and cannot penetrate protein rich materials." [13] If WaterScope decides that a disinfectant would be enough, rather than complete sterilisation being required, we would recommend ethanol for its ease of use and procurement.

The anaerobic combustion of methanol to form formaldehyde can also be used for sterilisation, however, methanol can be very difficult to acquire in many countries, so would be unsuitable for practical field sterilisation in unspecified countries.

B. Silver Nanoparticles

Silver nanoparticles that are less than 15nm in size have good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms, although their efficacy towards gram-positive bacteria may be slightly lower [14] Their mechanism is still being debated, but it is thought that they interact with the outer membrane of bacteria, causing degradation and eventually cell death. They are widely used in medical devices as well as relatively low-cost items such as socks. Coating a surface with silver nanoparticles may mean the surface is permanently antimicrobial, vastly simplifying the sterilisation procedure saving time and increasing ease of use.

However, the efficacy is known to be affected by shape and size of the nanoparticles, so the literature would have to be carefully examined to work out the ideal fabrication technique for this. The technique will also heavily depend on the nature of the surface we need to sterilise.

Specifying a time for sterilisation may be an issue due to the unknown amount of time it takes to kill bacteria. For example, with copper coatings, bacteria take up to 2 hours to kill, meaning the surface would have to be protected from recontamination for up to 2 hours before use (although nanoparticles are more effective than simple coatings). No literature can be currently found regarding a timescale, so this would have to be found experimentally. Regarding the fabrication, nanosynthesis in oil and in synthetic polymers [15] were considered alongside plasma spraying to achieve this coating. Without access to the facilities to explore this properly, it was decided that other methods would be concentrated on.

Reviewing the literature also suggested that ion leaching occurs. If the water sample is then contaminated with silver ions, killing the sample bacteria, this would cause false positives on the water test result. Therefore, this risk should be avoided for WaterScope's product.

C. Gas

Gas sterilisation has the advantage that it can be done at low temperatures (some even at room temperature) and in contrast to UV, it can reach cavities in the product. Ethylene oxide is a widely used gas for sterilising surgical equipment, but since it is flammable and explosive [13] and requires sourcing at the country where it is used, it is not considered further.

Ozone is a favourable candidate because it kills bacteria rapidly (under 30s for many bacteria) [16] It can be produced from air and does not leave bacteria-toxic residue that can affect water sample bacteria. However, this idea was abandoned because use of ozone in sterilising surgical equipment is not a mature and widespread technology and using it on WaterScope could potentially make certification difficult.

The use of nitrogen dioxide as a gas sterilant in a low resource environment was commercialised in around 2014-15 by the company Eniware. The process does not require electrical power or water [17] but has the disadvantage that disposal cartridges of nitrogen dioxide and scrubbers to absorb the gas have to be purchased and are unlikely to be locally available.

V. Experiments and Results

A. Microbiological and Image processing procedures

A protocol for contamination and plating was developed in order to ensure consistency across all experiments.

Non-pathogenic Escherichia Coli (E. coli) was used due to its safety and availability. Metal blocks of dimensions 40x40x15mm and metal sheets of dimensions 40x40x1mm, both made of stainless steel, were manufactured for the tests. For contamination of the blocks and sheets, we used a Gilson pipette to place 10 microlitres of $1x10^8$ bacteria/ml solution onto the surface and used a sterile spreader to spread this uniformly over the surface. Any sterilisation tests were performed, then a 'swab and rinse' technique [18] was performed to remove bacteria from the surface. Fig. 3 shows the pattern used each time to ensure the whole surface was swabbed. It also demonstrates the rinse technique for ensuring as much bacteria as possible

is removed from the swab. A weighing balance was used to ensure that the pressure applied to the surface was approximately the same each time.

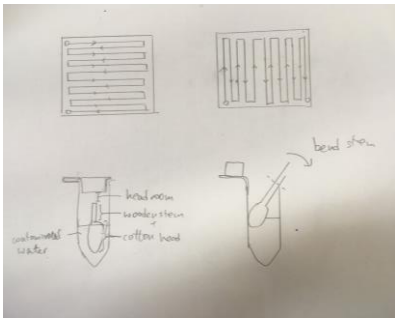

Fig. 3. Waterscope sample pot and 'cartridge'

To ensure maximum efficacy in this contamination, swabbing and rinsing process, lysogeny broth (LB) was used rather than water, to minimise the number of bacteria which are killed in the process. Also, the swab was saturated with LB before swabbing and the rinse solution containing the swab head was vortexed and left on ice for a fixed time, to ensure maximum, but consistent transferral. Finally, 50 microlitres of the rinse solution was transferred to a petri dish containing agar jelly and incubated overnight so that visible colonies could be counted. A streaking technique and a spreading technique were both tried (see Appendix 1, Fig. 1-3), with the spreading technique appearing to give better results.

Thus, in the end, theoretically $1x10^5$ bacteria should have been transferred to the petri dish to grow. Control studies showed that between 7100 and 11000 bacteria were actually transferred, depending on the surface (see section B). Maximum variation in number transferred to the same type of surface was 946 colonies. These errors are relatively minor considering that the $\log_{10}$ values are used for analysis.

A solution of $1x10^5$ bacteria/ml was chosen for further tests, as a lower concentration would not survive the protocol. However, any higher means that the counting becomes less accurate, as colonies become smaller and merge into each other.

ImageJ software was used to process the images to count cells (See Appendix 1, Fig. 4-6) We used automated cell counting using the "analyse particles feature", but this had a large dependence on thresholding and other parameters used and gave very inaccurate results for large numbers of colonies. For control dishes, the image processing software was very poor at counting (out by a factor of 5.7), so the 'Cell Counter' tool was used, which aided counting by eye instead. This was very labour intensive, but worthwhile for more accurate results.

The main limitation of this protocol is the fact that each method described for sterilisation should be tested with its most resistant organism, not just E. coli [18]. For example, ethanol kills E. coli very well, but would not kill bacterial spores. Relying on our protocol which only uses E. coli would mean that ethanol appears to be effective even though it is actually unsuitable. Therefore, it should be recommended that any conclusion reached by this experiment be further tested with other bacteria.

B. Consistency of the swab and rinse protocol

Taking into account the various control samples done where 10^6 bacteria were plated onto either a metal block or a metal sheet, with the swab and rinse technique applied immediately to test how many bacteria were moved into the rinse solution, it was concluded that when LB was used, 11% of bacteria on the metal sheets could be consistently transferred into the rinse solution, and about 6.8% from the metal block. The calculations done to arrive at these figures are detailed Appendix 2 table 4.2.

TABLE II
CONSISTENCY OF THE SWAB AND RINSE PROTOCOL: EXPERIMENTAL RESULTS

Test number	Solution	Block/ Sheet	Number of bacteria on petri dish (by counting), a	Bacteria transferred to the rinse solution, $b=a*10$	Recovery rate, $d = b/10^6$
1C3	Water	Block	2330	23300	2.3%
1C4	Water	Block	3280	32800	3.3%
1C5	Water	Block	3200	32000	3.2%
1T1	LB	Sheet	11200	112000	11.2%
1UV1	LB	Sheet	11120	111200	11.1%
1UV6	LB	Sheet	11930	111930	11.2%
1T6	LB	Sheet	7110	71100	7.1%
1H9	LB	Sheet	6470	64700	6.5%

C. UV Experimental Setup

Figs. 4a and 4b show the UV enclosure. The spectrum and intensity of the LEDs given by WaterScope were measured with a spectrometer at the Cambridge Graphene Centre.

Two different LEDs were tested initially, LED1 and LED2. LED1, provided by WaterScope, represented the cheapest option and was ordered online without a datasheet. LED2 was a more expensive option with a shorter wavelength.

The voltages set across LED1 and LED2 were 12V $\mp$0.1V and 7.4$\mp$0.1V respectively, and the identical stainless steel sheets were placed under the LED for different amounts of time. Both were mounted at 25mm from the samples.

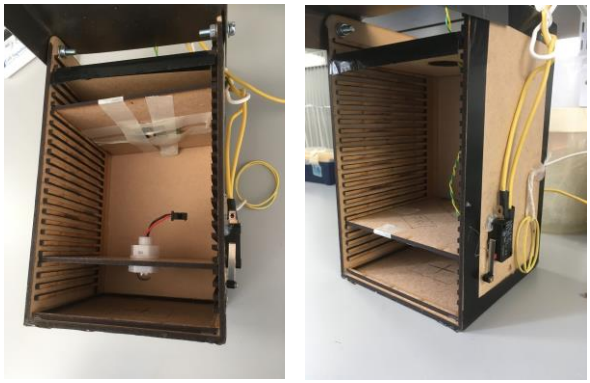

Fig. 4a and 4b UV test rig

D. UV Experimental Procedure

First, the metal sheet was plated with 10^6 bacteria, then transferred to the bottom of the enclosure at the marked position directly beneath the LED. After a set amount of time, the stainless steel sample was removed and swabbed. The swab was then rinsed and 10% of the rinse solution pipetted onto the petri dish for incubation.

To investigate how the intensity of the UV varied with viewing angle (ie radial distance on the sample surface), petri dishes full of bacteria were directly placed 18mm under the LEDs for 5 minutes and then incubated. The distribution of remaining bacteria would indicate the area effectively irradiated by the UV source.

E. UV Results and Discussions

Fig. 5 shows the percentage of bacteria remaining on the stainless steel samples after varied irradiation times. (Calculations from raw data can be found in Appendix 2 table 4.4.)

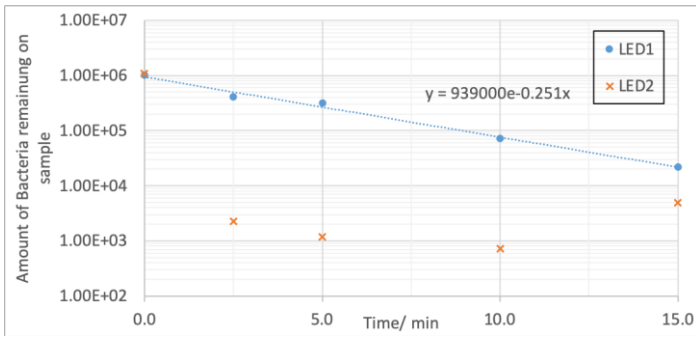

Fig. 5. Bacteria remaining vs time

The spectrum of LED1 is shown in Fig. 6, with a peak at around 470nm. LED2 had not yet been acquired at time of testing, but the datasheet claims a wavelength of 365nm.

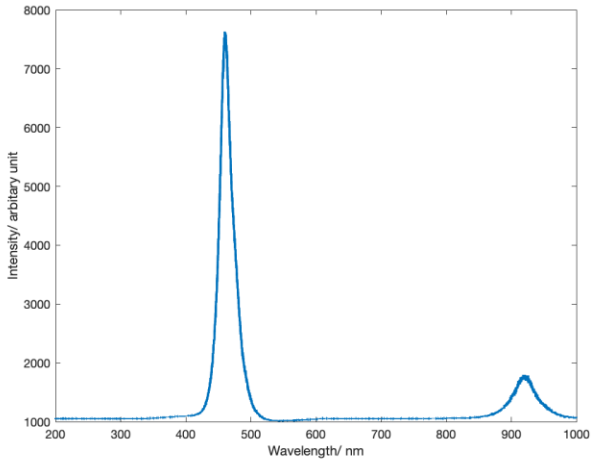

Fig. 6. LED1 spectrum

Pictures of the effective area tests on petri dishes are presented in Appendix 1 (Fig. 7-9) and it can be seen that that LED2 was more effective.

1) Dependence on Time

From Fig. 5, there seems to be an exponential relationship between the time under LED1 and the number of bacteria remaining. However, after 15min, there was less than a 2 orders of magnitude reduction in the number of bacteria, which was much less than the 6 order of magnitude needed. Thus, a single LED1 would not serve the purpose.

For LED2, there was an almost 3-orders-of-magnitude reduction in the number of bacteria within 2.5 minutes and extending the exposure time to 10 minutes only further reduced the number of bacteria by a factor of 3. The high result for 15min is likely to be anomalous and should be retested. The 3 orders of magnitude reduction for a single LED suggests that there may be potential for effective sterilisation with multiple LEDs within the time.

2) Dependence on wavelength

Given LED1 had no spectrum in the UV range, it was surprising it had any germicidal effect at all.

LED2 (with a claimed wavelength of 365nm) was considerably more effective than LED1. The CGC will be able to confirm this value on a date after the submission of the report, however out initial tests suggest that wavelengths around 365nm are germicidal.

3) Viewing angle

The images of petri dishes (Appendix 1, Figs. 7-9) show a distinct area within which bacteria are killed, and little sterilisation outside of it. As expected, this shows that distance from source and viewing angle will have a

significant effect on affected area and should be calculated for any given setup.

G. Heat Experimental Setup

The dry heat oven in the veterinary school was used to heat sample blocks of stainless steel to certain temperatures for certain times. The microbiological procedure described earlier in this section was used. The blocks were placed in Pyrex beakers and covered in tin foil during heating and transport, for safety. This will have affected the heat transference of the blocks.

H. Heat Experimental Procedure

The sterilisation procedure was simply to place the contaminated stainless-steel block into the oven for the prescribed time. The oven was given sufficient time to get to temperature, however, when the door was opened, the temperature usually fell by around 20 ° C, taking 5 minutes to return to temperature which was the timescale of the experiment. This meant that our average temperatures were around 10 ° C lower than expected. The oven also did not reach 200 ° C, so the range of dependent variables was smaller than we designed for.

The blocks were left to cool for 20 minutes after they had been removed from the oven. As blocks could also be left for up to an hour between contamination and sterilisation, a control was done to see whether a significant number of bacteria died in this waiting time. Fig. 7 shows the results of this and shows that for metal blocks, no significant decrease was seen in the timeframe of this experiment (time point 90 minutes is anomalous). It should be noted that no bacteria survived the control left in air for 36 hours.

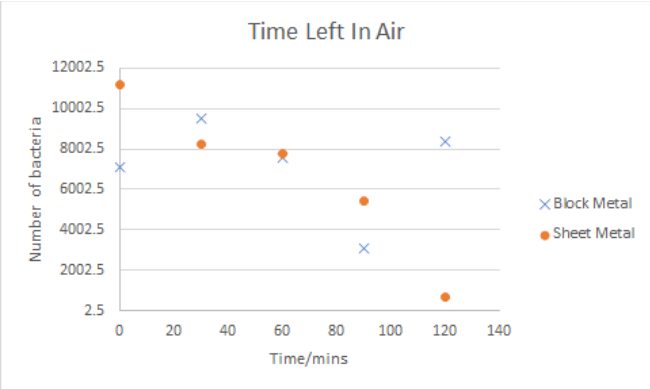

Fig. 7. Remaining bacteria vs Time left in air

I. Heat Results and Discussions

1) Dependence on Temperature and time

The experimental results for heat as a method of sterilisation were very promising, showing no bacteria on the petri dishes for all time periods and temperatures (the shortest time period and lowest temperature being 6 minutes and 130°C respectively). This suggests a maximum of 100 bacteria on the metal block and thus there was at least a 4-order-magnitude reduction in number of bacteria, since the metal blocks were each plated with 10^6 bacteria initially. The maximum of 100 bacteria was because only 8.6% (and for purpose of this estimation take this to be 10%) of bacteria on the metal block could be transferred to the rinse solution using the swab and rinse technique, and 10% of the rinse solution was transferred on the petri dish. Thus, with the block having fewer than 100 bacteria, fewer than 10 would be transferred to the rinse solution and fewer than 1 to the petri dish, thus no bacteria is expected to be seen on the petri dish. The surprisingly promising results were contrary to the research performed, which indicated that higher temperatures and longer times would be necessary. It is possible that this is because the research may have included significant factors of safety and was designed for larger components which take longer to sterilise. Another possible factor is that the E. coli bacteria used to test with are not necessarily the most difficult to kill. However, taking the results at face value, this provides proof of concept for heat as a sterilisation method.

TABLE III
BACTERIAL REMOVAL RATE FROM METAL BLOCKS WITH VARYING TIME AND TEMPERATURES

TEST NUMBER	TIME IN OVEN (MIN)	AVERAGE TEMPERATURE IN OVEN FOR THE PREVIOUS 5 OR 6 MINUTES (°C)*	AMOUNT OF BACTERIA ON PETRI DISH
1H1	6	130	0
1H2	11	150	0
1H3	16	150	0
1H4	21	150	0
1H5	6	150	0
1H6	11	175	0
1H7	16	175	0
1H8	21	175	0
1H9	0	N/A	6470

*The time between the last sample being taken out and this sample being taken out

VI. Social Aspects

A. Summarize the Results and Discuss the Social Impact

The goal of WaterScope's project is to devise a quicker, cheaper, easier to use method of conducting water samples, which improves accessibility of testing to communities that need it. If the kit can be made available to individual communities to use regularly and independently without extensive professional help, the process tends towards WHO's ideal standards for testing availability and frequency. This could have a significant impact in areas where people do not have improved water sources that are guaranteed consistently safe. The current cost of components, size and ease of use of the methods we are investigating prove promising for meeting this ethos of WaterScope's project.

2. How would you mass manufacture?

A benefit of the dry heat and UV methods is that the testing rigs do not use any extraordinary parts. A UV prototype would require an enclosure made from sheet material, a simple electrical circuit, and UV sources, likely LEDs which can be ordered from standard electronics suppliers. The most complex parts of the heat rig are the fabricated metal lid and the machined cartridge 'socket' - they must be hand-made for now but would be feasible for small batches in their current form (although the design would be adapted for mass manufactured prototypes anyway). There is also the issue of sealing the heating element into the socket with molten metal - a task more difficult to do in a standard workshop but could be negated with a precise press-fit for example in more detailed design iterations.

3. Summarize the Economics and Logistics

For these UV and dry heat methods, the only consumable is energy. This both reduces running cost of buying chemicals and removes the logistical issue of transporting chemicals to countries where there may be legal restrictions. Analysis of the energy used per sterilisation is needed to work out cost per cycle, but it is likely to be very low cost. (Specification states <£250 for development, and <15-50p for each run). The most expensive components to buy may well be the UV LEDs, at a cost of as much as £15 each, but with lifetimes of 5000+ hours corresponding to at least 15000 cycles, this is a negligible cost per cycle.

VII. Future scope

Given that the heat tests provided much more conclusive results over short timescales, we will be restructuring our team to focus development on a prototype for heat sterilisation, with minor efforts continuing to test higher power UV sources. The next important step is to prove that the heat-based sterilisation can be specialised for WaterScope's needs within the scope of their requirements.

Before the end of the project, we must begin to analyse the finer details of the method we recommend, including how much energy is used per sterilisation, how a prototype will be developed, and how well this will apply to WaterScope's situation. We should ensure that all of the information we have collected so far is well documented to present as a final report, that will be informative to WaterScope. Similarly, we should consider a cost analysis of our sterilisation methods against using disposable products, in case it is more cost effective to dispose rather than sterilise.

We should also look to carefully manage the time remaining in the project to ensure that the prototype production is feasible and that the remaining time spent will yield the most useful results for Waterscope.

VIII. Conclusion

Our team has delved deeper into the dry heat treatment and UV irradiation methods that originally were thought to be able to meet the requirements set by WaterScope – cheap, simple and quick sterilisation. Other methods considered, like IPA spraying, were not further pursued for the reasons aforementioned. Considerable thought and application of engineering knowledge was put into designing and manufacturing the existing prototypes that were tested. The experimental results for the dry heat were promising as they give at least a 4-orders-of-magnitude reduction of bacteria at low temperatures and short time. In contrast, UV LEDs were not as effective, as not all bacteria were eliminated within the time frame required by WaterScope. Consequently, more focus and time will be put into applying the existing heat prototype to WaterScope's cartridge. However, given that UV has shown some small potential, we will attempt to test whether the LEDs can be used effectively for the sample cups, with simpler geometry. Lastly, we are confident as a team that our contribution can potentially have a positive effect into tackling the problem of inaccessibility of clean water.

IX. Individual contributions

All individual contributions were equal in effort and importance, with different responsibilities divided among the team members. Report and presentation duties were split equally between the team, and time and project management were agreed together at team meetings.

A. Calvin Chow

Member of UV team responsible for lab testing. Also, microbiology protocol development, lab work and image processing. Investigated gas sterilant as a potential method.

B. Becky Donaldson

Member of Heat team responsible for lab testing. Also, microbiology protocol development, lab work and image processing. Investigated alcohols and silver nanoparticles as potential methods.

C. Will Honeyman

Member of Heat sub-team. Researched possible heating elements and conducted experiments to verify the properties of the heat element provided by Waterscope and designed the prototype. Also researched hydrophobic coating as a possible alternative method of sterilisation.

D. Renos Lyssiotis

Member of the Heat sub-team. Conducted research on the various heat treatment methods and drafted the heat treatment experimental procedure and lab protocol to be followed by the lab team. Designed and manufactured all the prototype components depicted in Fig. 2.

E. Bradley Sawyer

Member of UV team responsible for electrical design and building of testing rig. Conducted the risk assessment for safe use of UV, ensuring the testing rig meets the safety requirements and the team is briefed on its safe use.

F. Charlie Spicer

Member of UV team responsible for the SolidWorks design and laser cutting of test the rig, and the design and manufacture of all the specimens required for lab testing. Conducted initial research and feasibility maths on UV. Helped Calvin test the wavelength of donated bulbs.

X. References

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WaterScope Sterilisation—Appendix 1

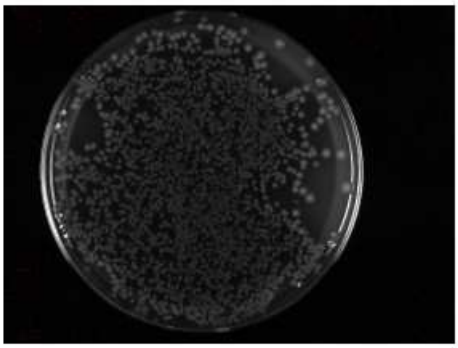


Figure 1 - Results of control procedure for contamination, rinse and swab and plating. Note that this first control used water rather than LB, so had a lower efficacy than the subsequent controls. 3 of these were done to find the range of transference as 946 colonies.

Figure 2 - The streaking procedure used with water (rather than LB). The colonies are more dense and thus harder to count.

Figure 3 - Complete procedure used using an equivalent amount of water rather than E.Coli bacteria to ensure false positives were not occurring.

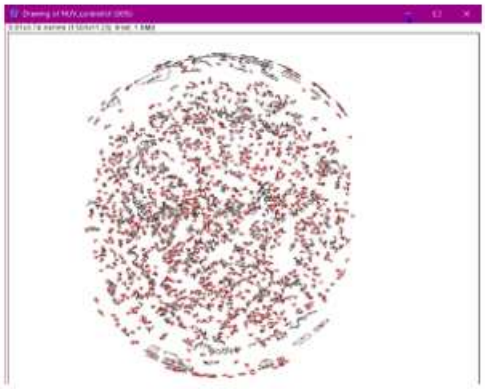


Figure 4 – The outlines of cells counted using ImageJ. The software counts the outside of the dish as cells, so a procedure was found for removing this error.

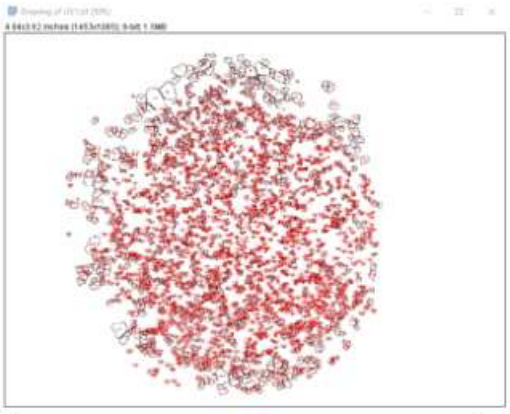


Figure 5 – Refined process for counting colonies that removes the errors of the side of the petri dish and manages to correctly identify more colonies.

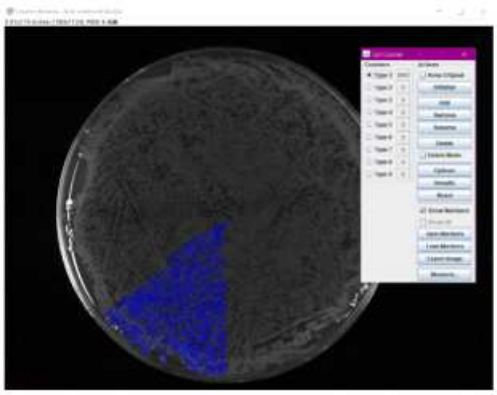


Figure 6 – Hand counting was done to test the accuracy of the automated software. It was found that the software was extremely inaccurate, so hand counting was done for all the results.

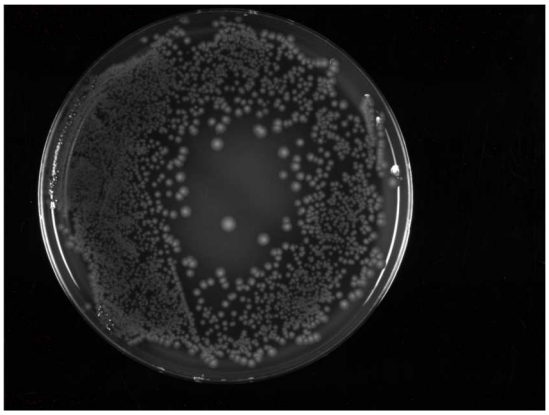


Figure 7 - Petri dish which was placed under LED1 for 5 mins at slit level 3. A clear kill radius can be seen.

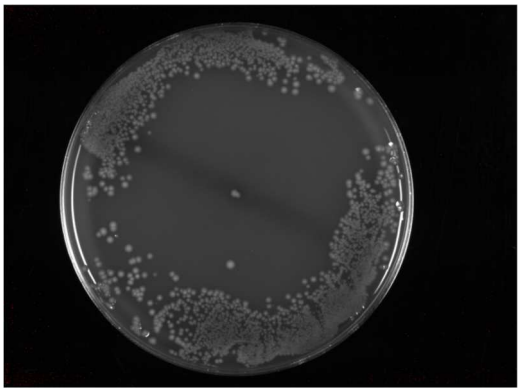


Figure 8- Petri dish which was placed under LED2 for 5 mins at slit level 3. A clear kill radius can be seen which is larger than that with LED1.

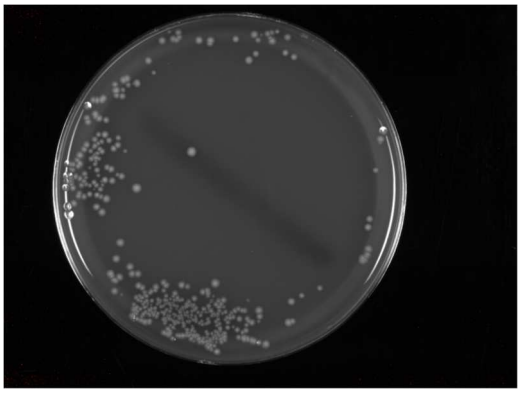


Figure 9- Petri dish which was placed under LED2 for 5 mins at slit level 5. A clear kill radius can be seen which is larger than that with LED1 and LED2 with slit level 3.

Procedures, Raw Data and Calculations – Appendix 2

1. Introductions and Objectives:

The experiments that were performed could be divided according to the following purposes:

1.1. To test the effectiveness and consistency of the swab and rinse technique on metal blocks (Mon 20th May) - except water was used

1.2. To test the survival rate of the bacteria as a function of time left on the metal sheet and metal blocks (Thu 23rd)

1.3. To test the effectiveness of dry heat on bacteria with varying time and temperatures (Wed 22nd)

1.4. To test the effectiveness of 2 types of LED bulbs given by varying the time of treatment (Wed 22nd for 1 type and Thu 23rd for the second type)

1.5. To determine the wavelengths present in one of the LEDs given and in the filament bulb given by WaterScope (Wed 22nd).

2. Procedures

2.1. To test the effectiveness and consistency of the swab and rinse technique on metal blocks using water

2.1.1. For the first block, labelled 'test', 10^6 bacteria were placed onto a face of the block using techniques described in experimental protocol: swab and rinse technique. A swab (cotton bud) drew 2 horizontal lines on the surface and then drew lines on petri dish, which was labelled 'streaky'. Then a new swab was rolled on the surface in S shape, drawing out 12 horizontal lines. Then this swab was dipped into 500μl of distilled water, was shaken a bit in the water, then taken out. 50μl of this water was then put on a petri dish labelled 'B'.

2.1.2. For the next 6 blocks (labelled 1-6), the swap and rinse protocol was followed. Block 1-3 had 10^6 bacteria put on the surface, block 4-6 had 10^4, using different dilutions of the bacteria solution. In all 6 cases, 10μl of bacterial solution was pipetted onto the metal blocks. Swab was used to wipe the surface, then 50μl of contaminated water was transferred to petri dishes, labelled '1' to '6', corresponding to the block the sample was taken from. Procedure followed same as described in the 'experimental protocol' except for the following as this was the initial test: different concentration of bacterial solution for block 4-6; no balance was used to ensure constant pressure; no vertical movement of swap was made when wiping the blocks' faces; in all except 1 of the 6 microfuges with cotton head, the wooden stem was very close to the cap so the cotton head might not have

moved much when the microfuges were vortexed, probably reducing the amount of bacteria leaching into the water in the microfuges.

2.1.3. The last block, labelled 'water', acted as a control. The same procedure described in '*experimental protocol*' was applied except rather than bacteria solution, 10μl of distilled water was put on the block. The 50μl of 'contaminated' water was placed on petri dish labeled 'water'.

2.1.4. Block 'water' and block '6' was disinfected with ethanol, then renamed 'AC' and 'Not AC'. They were each infected with 10^6 bacteria. Then the AC block was autoclaved after 24h and left further for 24h; and the 'Not AC' was left in a box at room temperature for 48h. After 48h, on Wednesday 22nd, the swap and rinse technique was followed and 50μl of contaminated water transferred to petri dishes labelled 'AC' and 'Not AC'.

2.2. To test the survival rate of the bacteria used in the test as a function of time left on the metal sheet and metal blocks

2.2.1. 5 metal sheets and 5 metal blocks were plated with 10^6 bacteria.

2.2.2. Then swab and rinse technique was used to determine how many bacteria remained after 3 min (allowed the bacterial solution to dry), 30 min, 1h, 1.5h and 2h.

2.3. To test the effectiveness of dry heat on bacteria with varying time and temperatures

2.3.1. 10^6 bacteria were transferred to each of the 8 metal blocks.

2.3.2. The 4 metal blocks labeled heat_1 to heat_4 went into the oven together as a batch when the oven was set to 150^{o}C, and was taken out at different times; the other 4 metal blocks labeled heat_5 to heat_8 went into the oven together as another batch when the oven was set to 200^{o}C, and was taken out at different times.

2.3.3. The oven's temperature fluctuated for about ∓20OC every time the door was open.

2.3.4. There was up to 1.5h delay in the plating the metal block and them going into the oven due to logistics of the experiments.

2.4. To test the effectiveness of 2 types of LED bulbs given by varying the time of treatment

2.4.1. Transfer 10^6 bacteria onto the metal sheet.

2.4.2. Place the metal sheet under the LED, which was mounted at fixed distance (slit level 4 for LED1 and slit level 3 for LED2), for different amount of time. The voltage used across LED1 and LED2 were 12V ∓0.1V and 7.4∓0.1V

2.4.3. Then the bacteria left on the metal sheet were transferred by swab and rinse technique onto the agar plate for incubation.

2.4.4. To find the viewing angle, a petri dish with bacteria was put under the LEDs for 5 min. To prepare one of those petri dishes, 10^6 bacteria were transferred onto the metal plate, then swap and rinse technique used to transfer those bacteria onto the petri dish.

2.4.5. Dimensions of the slit levels, height of LED from mount and height of bacteria from bottom of enclosure are given in figure 2.4.

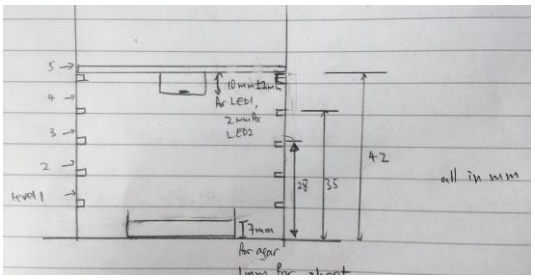


Figure 2.4 The dimensions and set up of tests conducted in the UV enclosure.

3. Results

3.1. To test the effectiveness and consistency of the swab and rinse technique on metal blocks

Test number	Block label	Petri dish label	Original amount of bacteria on block	Amount of bacteria on petri dish (by counting), a	Inferred amount of bacteria transferred to the 500μl of water containing the cotton head, $b = a^*10$	Expected amount of bacteria on block when swabbed, c	Recovery rate in %, $d = b/c$
1C1	test	demonstration	10^6	First test of physically doing the procedure, not consistent enough for statistical observation			
1C2	test	streaky	10^6				
1C3	1	1	10^6	2330	23,300	10^6	2.3%
1C4	2	2	10^6	3280	32,800	10^6	3.3%
1C5	3	3	10^6	3200	32,000	10^6	3.2%
1C6	4	4	10^4	0	0	10^4	0
1C7	5	5	10^4	0	0	10^4	0
1C8	6	6	10^4	0	0	10^4	0
1C9	water	water	0	0	0	0	n/a
1C10	AC	AC	10^6	0	0	0	n/a
1C11	Not AC	Not AC	10^6	0	0	10^6	0

Table 3.1: Initial tests using the rinse and swab technique with water as medium. Recovery rate is defined as the number of bacteria contained in the rinse solution as a proportion of bacteria plated onto the sample

3.2. To test the bacterial survival rate bacteria as a function of time left on the metal specimen

Test number	Sheet or block	Petri dish label	Time left before sampling	Amount of bacteria on petri dish (by counting)
1T1	sheet	time_1	3min	11200
1T2	sheet	time_2	30min	8220
1T3	sheet	time_3	1h	7800
1T4	sheet	time_4	1.5h	5400
1T5	sheet	time_5	2h	700
1T6	block	time_6	3min	7110
1T7	block	time_7	30min	9490
1T8	block	time_8	1h	7570
1T9	block	time_9	1.5h	3090
1T10	block	time_10	2h	8400

Table 3.2: The number of bacteria found of petri dishes corresponding to metal blocks/ sheets left in fume cupboard for varying amount of time.

3.3. To test the effectiveness of dry heat on bacteria with varying time and temperatures

Test number	Petri dish label	Time in oven (min)	Average temp in oven for the previous 5 or 6 min * (^{o}C)	Amount of bacteria on petri dish (by counting)
1H1	heat_1	6	130	0
1H2	heat_2	11	150	0
1H3	heat_3	16	150	0
1H4	heat_4	21	150	0
1H5	heat_5	6	150	0
1H6	heat_6	11	175	0
1H7	heat_7	16	175	0
1H8	heat_8	21	175	0
1H9	heat_control	0	n/a	6470

* (i.e. for time between the last sample was taken out and this sample (the sample in the same row) being taken out)

Table 3.3: The amount of bacteria found of petri dishes corresponding to metal blocks left in the dry heat oven for varying temperatures and time.

3.4. To test the effectiveness of 2 types of LED bulbs given by varying the time of treatment

Test number	Test date	Petri dish label	Bulb	Time (min)	Distance from LED to bacteria surface (mm); slit level of LED	Amount of bacteria counted on petri dish, a
1UV1	Wed 22/5	UV1	No bulb, no sterilisation (control)	0	*n/a*	11120
1UV2	Wed 22/5	UV2	LED1	2.5	*24; Slit 4*	4500
1UV3	Wed 22/5	UV3	LED1	5	*24; Slit 4*	3470
1UV4	Wed 22/5	UV4	LED1	10	*24; Slit 4*	800
1UV5	Wed 22/5	UV5	LED1	15	*24; Slit 4*	240
1UV6	Thu 23/5	NUV_control	No bulb, no sterilisation (control)	0	*n/a*	11930
1UV7	Thu 23/5	NUV_2	LED2	2.5	*25; Slit 3*	25
1UV8	Thu 23/5	NUV_3	LED2	5	*25; Slit 3*	13
1UV9	Thu 23/5	NUV_4	LED2	10	*25; Slit 3*	8
1UV10	Thu 23/5	NUV_5	LED2	15	*25; Slit 3*	54

Table 3.4.1: The amount of bacteria found of petri dishes corresponding to metal sheets that were put under 2 types of LED for varying amount of time.

Diameter of petri dish: 87mm						
Test number	Test date	Petri dish label	Bulb	Time (min)	Distance from bulb to bacteria surface (mm); slit level of LED	Diameter of circle on petri dish mostly free of bacteria (mm)
1UV11	Wed 22/5	Petri radius UV	LED1	5	*17; Slit 4*	*22*
1UV12	Thu 23/5	NUV_P1	LED2	5	*18; Slit 3*	*55*
1UV13	Thu 23/5	NUV_P2	LED2	5	*33; Slit 5*	*65*

Table 3.4.2: The amount of bacteria found of petri dishes that were put under 2 types of LED at varying height from the LEDs for constant time.

4. Calculations and analysis

4.1. To test the effectiveness and consistency of the swab and rinse technique

Table 4.1 collates the different tests that acted as control tests where the bacteria plated onto metal block/ sheet underwent no sterilisation treatment and was immediately transferred to the rinse solution using the rinse and swab technique. It could be seen that the swab and rinse technique was applied consistently for sheet (1T1, 1UV1, 1UV6) and for block (1C3, 1C4, 1C5).

For initial number of bacteria of 10^6, and using LB, the recovery rate taken for calculations for sheet was 11% (2sf) and for block was 6.8% (2sf) (mean of 7.1% and 6.5%). The main reason why the protocol changed from using water to LB was that the recovery rate of about 2-3% for water was too low. Water might have bursted bacteria due to difference in salt concentration inside cell and the surrounding water environment, whereas LB provides salt balance.

Test number	Petri dish label	Solution	Block/ sheet	Amount of bacteria on petri dish (by counting), a	Inferred amount of bacteria transferred to the 500μl of water containing the cotton head, b = a* 10	Recovery rate in %, d = b/10^6
1C3	1	water	Block	2330	23,300	2.3%
1C4	2	water	Block	3280	32,800	3.3%
1C5	3	water	Block	3200	32,000	3.2%
1T1	time_1	LB	sheet	11200	112,000	11.2%
1UV1	UV1	LB	sheet	11120	111,200	11.1%
1UV6	NUV_control	LB	sheet	11930	111,930	11.2%
1T6	time_6	LB	block	7110	71,100	7.1%
1H9	heat_control	LB	block	6470	64,700	6.5%

Table 4.1: Summary of different control tests that simply involved transferring 10^6 bacteria onto the sample (metal sheet/ block), then using swab and rinse technique to transfer the bacteria into rinse solution. These tested the consistency of the swab and rinse technique.

4.2. To test the survival rate of the bacteria used in the test as a function of time left on the metal sheet and metal blocks (Thu 23rd)

Test number	Metal sheet/ block	Time/ min	Number on petri dish, a	Number in rinse solution, $b = a*10$	Number on sample, c = b/(recovery rate=0.11 for sheet and 0.068 for block)	Number on sample in log_10, log c	Removal rate (fraction of bacteria removed), d = 1-c/10^6
1T1	sheet	3	11,200	112,000	1,018,182	6.01	-0.0182
1T2	sheet	30	8,220	82,200	747,273	5.87	0.2527
1T3	sheet	60	7,800	78,000	709,091	5.85	0.2909
1T4	sheet	90	5,400	54,000	490,909	5.69	0.5091
1T5	sheet	120	700	7,000	63,636	4.80	0.9364
1T6	block	3	7,110	71,100	1,045,588	6.02	-0.0456
1T7	block	30	9,490	94,900	1,395,588	6.14	-0.3956
1T8	block	60	7,570	75,700	1,113,235	6.05	-0.1132
1T9	block	90	3,090	30,900	454,412	5.66	0.5456
1T10	block	120	8,400	84,000	1,235,294	6.09	-0.2353

Table 4.2 Analysis of the results for the sheets and blocks left inside fume cupboard for varying amount of time.

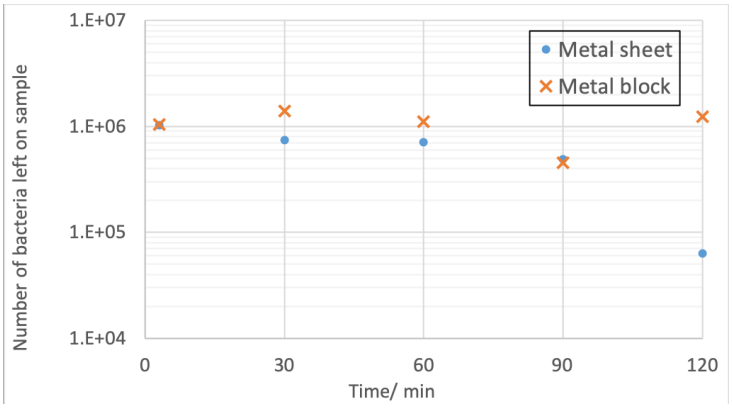


Figure 4.2: Graph showing results in table 4.2.

4.3. To test the effectiveness of dry heat on bacteria with varying time and temperatures (Wed 22nd)
All bacteria were killed. See section 3.3

4.4. To test the effectiveness of 2 types of LED bulbs given by varying the time of treatment (Wed 22nd for 1 type and Thu 23rd for the second type)

Test number	LED used	Time/ min	Number on petri dish, a	Number in rinse solution, $b = a*10$	Number on sample, $c = b/$(recovery rate=0.11 for sheet)	Number on sample in log_10, log c	Removal rate (fraction of bacteria removed), d = 1-c/10^6
1UV1	No bulb, no sterilisation (control)	0.0	11,120	111,200	1,010,909	6.00	-0.0109
1UV2	LED1	2.5	4,500	45,000	409,091	5.61	0.5909
1UV3	LED1	5.0	3,470	34,700	315,455	5.50	0.6845
1UV4	LED1	10.0	800	8,000	72,727	4.86	0.9273
1UV5	LED1	15.0	240	2,400	21,818	4.34	0.9782
1UV6	No bulb, no sterilisation (control)	0.0	11,930	119,300	1,084,545	6.04	-0.0845
1UV7	LED2	2.5	25	250	2,273	3.36	0.9977
1UV8	LED2	5.0	13	130	1,182	3.07	0.9988
1UV9	LED2	10.0	8	80	727	2.86	0.9993
1UV10	LED2	15.0	54	540	4,909	3.69	0.9951

Table 4.4: Analysis of the results of metal plates placed under 2 different LEDs for varying amounts of time.

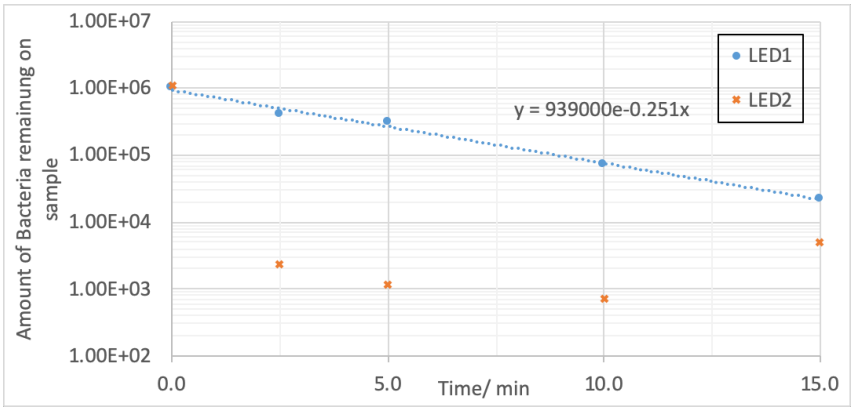


Figure 4.4: Graph showing results of table 4.4.

4.5. To determine the wavelengths present in one of the LEDs given and in the filament bulb.

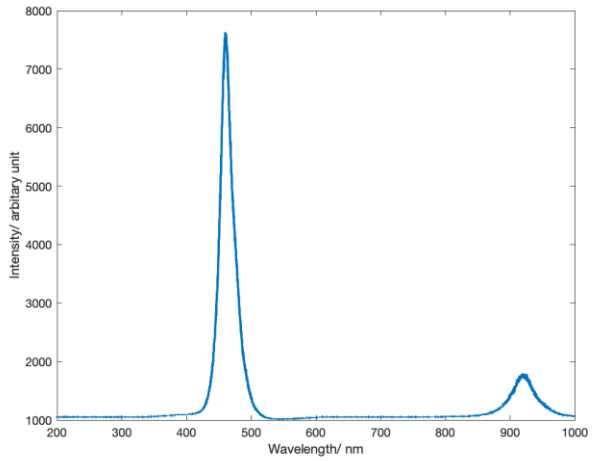


Figure 4.5.1: Spectrum of LED1, which shows no component in the UV spectrum.

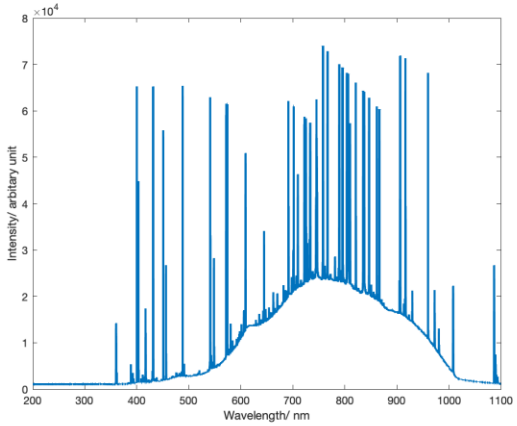


Figure 4.5.2: Spectrum of the filament bulb given by WaterScope (whose germicidal effectiveness was not tested), showing the majority of the bulb's energy is in the visible spectrum (380-700nm, https://science.nasa.gov/ems/09_visiblelight) and IR, with one peak in the UV spectrum at about 370nm.

Experimental Protocol: Swab and Rinse Technique - Appendix 3

1. Objective

This document outlines the procedures that would be followed to ensure consistency in measuring the number of bacteria present on a standardized-geometry samples (square blocks and sheets). The procedure is based on the swap and rinse technique, which is widely used in literature to test for levels of contamination, but with modifications to allow for materials availability in the lab.

2. Procedure

2.1 Work space preparations:

1. Put on lab coat, gloves.
2. Spray the gloves and fume cupboard with ethanol. Turn on extraction system for fume cupboard, wait for 3 min for system to warm up as stated on the fume cupboard display. Spray anything going into the fume cupboard with ethanol.
3. Collect a bottle of distilled water, a self-standing tube of Lysogeny broth (LB) medium, put them into fume cupboard.
4. Collect 1ml, 200μl and 10μl pipettes, and corresponding pipette tips. Collect a pair of scissors and a microfuge rack and put them into fume cupboard.
5. Collect electronic balance and place it in fume cupboard.
6. Collect a polystyrene box and put ice on it.

2.2 Contaminating the standardized sample with known amount of bacteria

1. Collect bacteria sample from the freezer, and put it in the ice. The bacteria sample is a 1ml bacteria solution at concentration of 4 x 10^8 bacteria per ml. Vortex the cryovial containing the bacteria (cryogenic vial, i.e. small containers that withstand very low temperatures).
2. Dilute the bacteria solution into 4 microfuges, each containing a 1ml bacteria solution at 10^8 bacteria per ml. This is done by pipetting 250μl of bacteria solution from cryovial and 750μl of LB into each microfuge. Then vortex the microfuges. Keep the microfuges in ice to prevent bacteria from multiplying.
3. Pipette 10μl of the diluted bacteria solution on the square block/ sheet. This will contaminate it with 10^6 bacteria.
4. Spread the bacteria out evenly on the square block, covering the whole face of the block, using a sterile plastic spreader, (don't worry about residue of bacteria that are on the spreader). Allow the block to dry. Then proceed to the sterilisation technique.

2.3 Measuring the number of bacteria remaining on the square block after the sterilisation technique

Swap and Rinse Technique

1. Pipette 50μl of LB into a microfuge, leave on rack to later receive contaminated cotton bud.
2. Dip a sterile swab (cotton bud) into the tube of LB.
3. Put the contaminated square block on an electronic balance after damping the balance surface with ethanol, with the contaminated face facing up, tare the balance (make it show 0 g on the display when the square block is on).
4. Roll it with constant pressure* in an S shape manner to draw out 12 horizontal lines (6 left to right, intertwined with 6 right to left).
5. Then roll the swap with constant pressure* in an S shape manner to draw out 12 vertical lines (6 bottom to top, intertwined with top to bottom).
   * To ensure constant pressure, make sure the balance reads 180-220g during the whole of the rolling process.
6. Dip the contaminated cotton head into 500μl LB microfuge. Break the wooden stem of the cotton bud (with aid of scissors if necessary) so that the cotton head is submerged in the LB in the microfuge and that there is sufficient headroom between the top of the wooden stem remaining and the top of the microfuge when its cap is closed.
7. Disinfect the balance surface by wiping tissue paper damped with ethanol.
8. Vortex the microfuge with cotton head for 10 seconds. Then leave for 2 minutes. Then vortex it again for 10 seconds, then leave for another 2 minutes.
9. Then vortex the microfuge with cotton head for 10 seconds again, and then pipette 50μl of the contaminated LB from the microfuge and transfer to agar jelly.
10. Use sterile spreader to spread the 50μl of contaminated LB evenly on the surface of the agar jelly, by moving the spreader from side to side of the dish with one hand and rotating the dish with the other hand.
11. Incubate the petri dish at 37^{o}C for 24h.
12. Count the number of bacteria on the jelly.

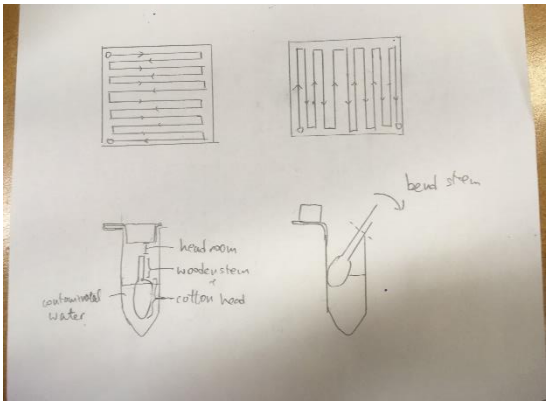
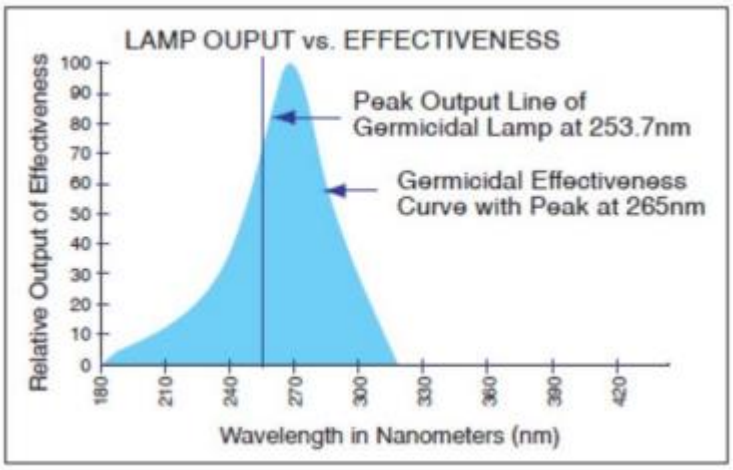


Figure 1: Showing the swabbing motion and the way to put the cotton swab into the rinse solution.

UV Feasibility Research – Appendix 4



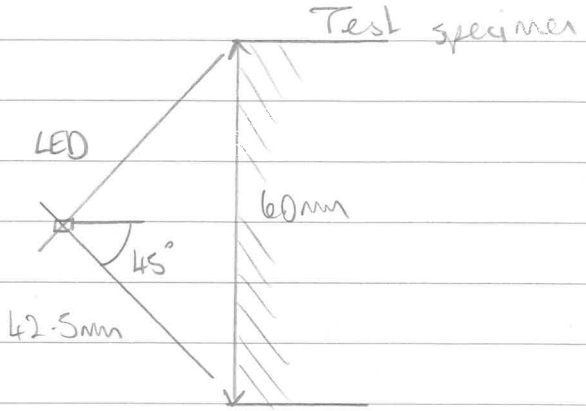
The germicidal effectiveness of different UV wavelengths
https://www.clordisys.com/pdfs/misc/UV%20Data%20Sheet.pdf

Ultraviolet Dosage Required For 99.9% Destruction of Various Organisms
(μW-s/cm² at 254 nanometer)

Bacteria		Mold Spores	
Bacillus anthracis	8,700	*Aspergillus flavus*	99,000
B. enteritidis	7,600	*Aspergillus glaucus*	88,000
B. Megatherium sp. (vegatative)	2,500	*Aspergillus niger*	330,000
B. Megatherium sp. (spores)	52,000	*Mucor racemosus A*	35,200
B. paratyphosus	6,100	*Mucor racemosus B*	35,200
B. subtilis (vegatative)	11,000	*Oospora lactis*	11,000
B. subtilis (spores)	58,000	*Penicillium digitatum*	88,000
Clostridium tetani	22,000	*Penicillium expansum*	22,000
Corynebacterium diphtheria	6,500	*Penicillium roqueforti*	26,400
Eberthella typhosa	4,100	*Rhizopus nigricans*	220,000
Escherichia coli	7,000		
Leptospira interrogans	6,000		
Micrococcus candidus	12,300	**Algae / Protozoa**	
Micrococcus sphaeroides	15,400	*Chlorella vulgaris* (algae)	22,000
Mycobacterium tuberculosis	10,000	Nematode eggs	92,000
Neisseria catarrhalis	8,500	Paramecium	200,000
Phytomonas tumefaciens	8,500		
Proteus vulgaris	6,600		
Pseudomonas aeruginosa	10,500	**Virus**	
Pseudomonas fluorescens	6,600	Bacteriophage (*E. coli*)	6,600
Salmonella enteritidis	7,600	Hepatitis virus	8,000
Salmonella paratyphi	6,100	Influenza virus	6,600
Salmonella typhimurium	15,200	Polio virus	6,000
Salmonella typhosa (Typhoid)	6,000	Rotavirus	24,000
Sarcina lutea	26,400	Tobacco mosaic	440,000
Serratia marcescens	6,200		
Shigella dysenteriae (Dysentery)	4,200		
Shigella paradysenteriae	3,400		
Spirillum rubrum	6,160	**Yeast**	
Staphylococcus albus	5,720	Baker's yeast	8,800
Staphylococcus aureus	6,600	Brewer's yeast	6,600
Streptococcus hemolyticus	5,500	Common yeast cake	13,200
Streptococcus lactis	8,800	*Saccharomyces cerevisiae*	13,200
Streptococcus viridans	3,800	*Saccharomyces ellipsoideus*	13,200
Vibrio cholerae	6,500	*Saccharomyces sp.*	17,600

UV dosage required for 99.9% destruction of various organisms
https://www.clordisys.com/pdfs/misc/UV%20Data%20Sheet.pdf

LED 7 LUEVA – 278 nm – 2mW max



1) output of at least 80% max intensity within 90° viewing angle

$$\text{flux} = \frac{\text{power}}{\text{area}}$$

$$= \frac{2\times10^{-3} \times 0.8}{4\pi \times (4.25^2)}$$

$$= 7.05 \mu W/cm^2$$

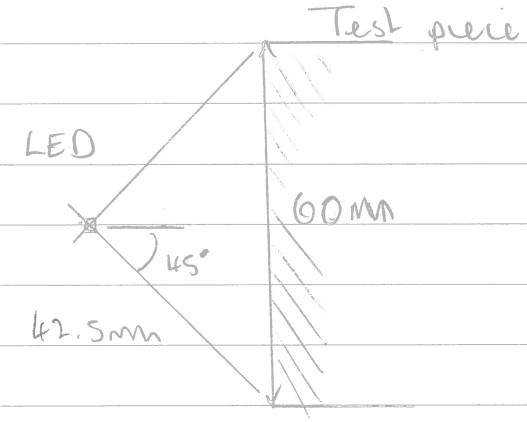
2) assume 440000 $\mu S/cm^2$ required for log 3 reduction

$$\text{time} = \frac{\text{dose needed}}{\text{flux}}$$

$$= \frac{440000}{7.05}$$

$$= 17\text{hr } 20\text{ mins}$$

LED 2 VISHAY - 370nm - 20mW max



1) output of at least 90% of max intensity with 90° viewing angle

$$\text{flux} = \frac{\text{power}}{\text{area}}$$

$$= \frac{20 \times 10^{-3} \times 0.9}{4\pi \times (4.25^2)}$$

$$= 79.3\ \mu W/cm^2$$

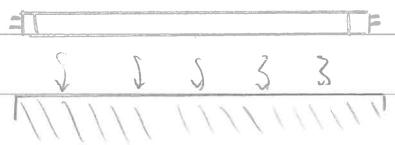
2) assume 440000 required for log 3 reduction
- ignore - fact that this wavelength may not be as effective

$$\text{time} = \frac{\text{dose needed}}{\text{flux}}$$

$$= \frac{440000}{79.3}$$

$$= 1\text{hr } 33\text{mins}$$

BULB 1 LAWTRONICS - 253.7nm



1) flux of 4000μ W/cm^2 according to datasheet - but - do not know distance above test piece

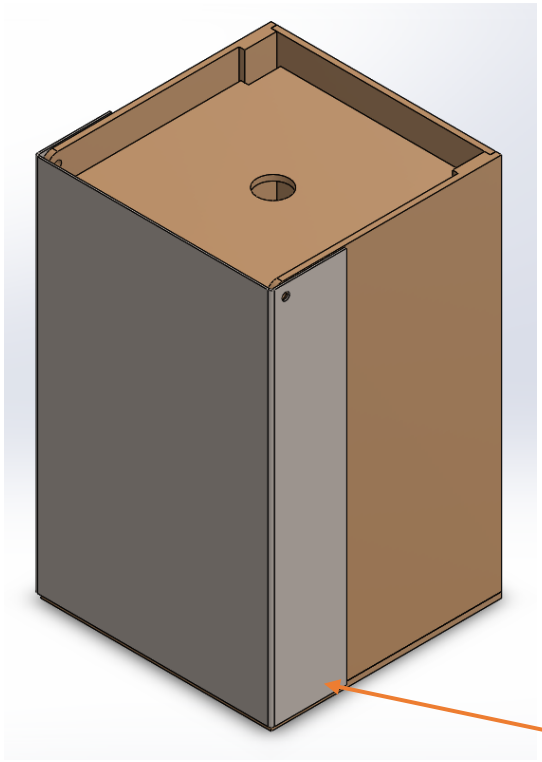
2) assume 440000 required for log 3 reduction

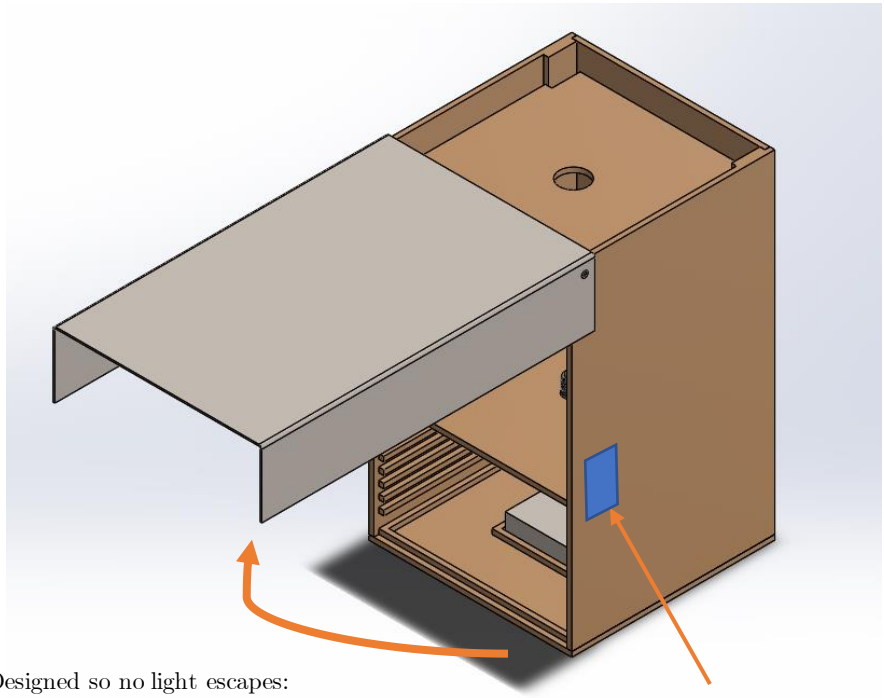
time = dose needed / flux

= 440 000 / 4000

= 2mins

UV Test Rig Design – Appendix 5

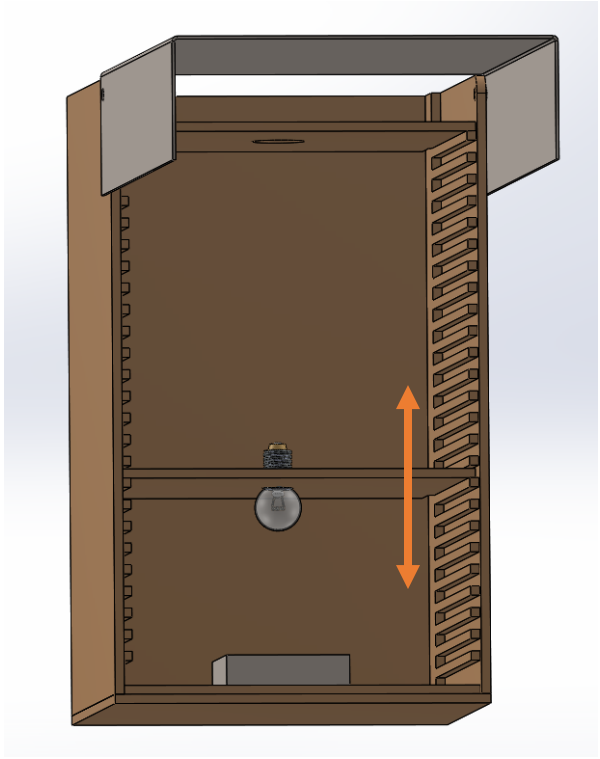




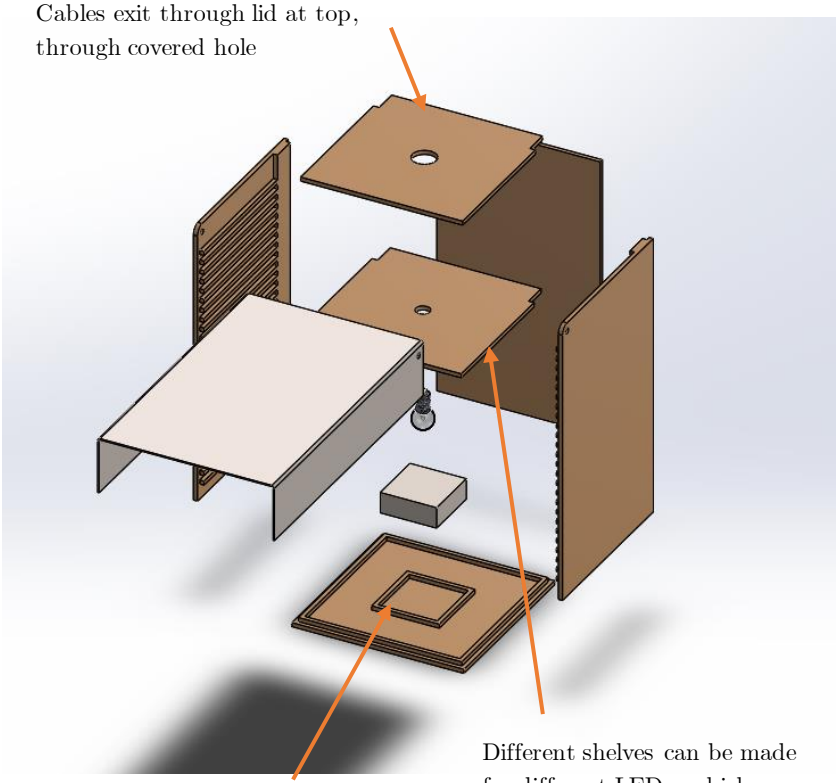
Designed so no light escapes:

- Door has flanges
- Edges are taped
- Cables exit through lid at top, through covered hole

Microswitch ensures box can only operate when door is closed



Shelves can be raises or lowered to allow for different LED sizes.



Test pieces placed into centring square

Different shelves can be made for different LEDs, which are sealed into the shelf