SASTA OLIPHANT SCIENCE AWARDS

SOUTH AUSTRALIAN SCIENCE TEACHERS ASSOCIATION

Prize Winner

Scientific Inquiry

Year 9-10

Liana Walters

Brighton Secondary School

Science Journal

Note: While doing this experiment, planning stage and report, I was recovering from a surgery I had on my back from a few weeks prior. As a result, I wasn't at school and wasn't able to start this until later. There were some days where I wasn't able to check the bacteria in the incubator. That is why my Journal seems very spread out. Sorry for the inconvenience.

8/6/21

Today I came up with ideas for what I should do an experiment on and what interested me to see if there was something I could do that I liked. I came up with ideas about doing a experiment on bones and bone structures but I didn't come far with the restrictions there were at school and where I would be able to get materials from. I thought about doing something that had to do with chemistry as I like chemsity and am interested by it. One of my ideas was to do something with fire and changing colours but I didn't like it. Then I had an idea to do an experiment with cells and repairing cells. I did like the idea but when I asked my teacher, I couldn't do it with the time we had. Also while coming up with ideas I didn't really know what direction I wanted to do in and turned down most of my thoguhts as they were to out landish. I asked my teacher for some help about what topic I should do and she told me of past students projects. I was intrigued by this one project were a girl measured the different strenghs of handsanitiser on different strains of bacteria. I was keen on doing my own version of it but my mind still thought of possible ideas so I didn't choose that idea.

10/6/21

Today I was basically doing the same things I did two days ago and decided to try and do something that involves space and the planets. I didn't really do much though as I was trying to figure out what to do and how I would do it.

17/6/21

Today I was still looking at different experiments I could do and still hadn't made up my mind on what I should do for the report. After a lot of thinking I decided on expanding the effect of handsanitiser on bacteria, to come up with my own question. I kept thinking to myself that I was copying this girl's idea but I was simply inspired by it and wanted to make my own variation of it with a different goal in mind. With a final idea, I came up with possible questions I could base my research on such as, the effects of mutliple types of hand sanitiser brands on one strain of common hand bacteria or the effect of one hand sanitiser on multiple types of hand bacteria. I chose the second option "The effect of one hand sanitiser on multiple hand bacteria strians".

21/6/21

Today I researched different types of bacteria that is found on the hand but all of the ones I found were not safe to be used in a school setting. I also looked at different ways I could do this experiment online. I found a website about university students doing the same thing that I intend to do and looked through it. I saw that they put there hands on different things like shopping trollies and bathroom doors as well as having some wash their hands before putting it on the petri dish and someone not washing their hands at all. I found this very interesting and looked more into it and found out that the students were able to tell what types of bacteria were growing which I thought was really cool. Apart from that I didn't really do much. I also learned what Gram-Negative and Gram-Postive bacteria means.

Gram-Negative bacteria is when a bacteria has a cell membrane around it to protect itself from the white blood cells so they won't kill it allowing the bacteria to grow and cause infections such as pneumonia. While Gram-Postive bacteria don't have a cell membrane allowing white blood cells the abilitiy to destroy them. Gram-Negative bacteria goes red when it has the chemical process Gram, applied to it. Whereas Gram-Positive bacteria stains blue when it goes through the same process. Bothe Gram-Negative and Gram-Positive Bacteria are treated with antibiotics suited for the type of bacteria and if its Gram-Negative or not. They also have different types of infection with Gram-Negative bacteria being more dangerous.

The websites I used to look for different bacteria:

2021. *A comparison of the bacteria found on the hands of 'homemakers' and neonatal intensive care unit nurses.* [online] Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2062569/> [Accessed 21 June 2021].

n.d. *Normal bacterial flora on hands*. [online] Available at: <https://www.ncbi.nlm.nih.gov/books/NBK144001/> [Accessed 21 June 2021].
n.d. *Pseudomonas putida and Pseudomonas fluorescens Species Group Recovery from Human Homes Varies Seasonally and by Environment*. [online] Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4449118/> [Accessed 21 June 2021].
n.d. *Staphylococcus warneri: Skin Commensal and a Rare Cause of Urinary Tract Infection*. [online] Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7263002/> [Accessed 21 June 2021].
n.d. *Enterobacter aerogenes and Enterobacter cloacae; versatile bacterial pathogens confronting antibiotic treatment*. [online] Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4435039/> [Accessed 21 June 2021].
Cdc.gov. n.d. *Klebsiella pneumoniae in Healthcare Settings | HAI | CDC*. [online] Available at: <https://www.cdc.gov/hai/organisms/klebsiella/klebsiella.html> [Accessed 21 June 2021].
Betterhealth.vic.gov.au. n.d. *Staphylococcus aureus - golden staph - Better Health Channel*. [online] Available at: <https://www.betterhealth.vic.gov.au/health/conditionsandtreatments/staphylococcus-aureus-golden-staph> [Accessed 21 June 2021].
MSD Manual Consumer Version. n.d. *Overview of Gram-Negative Bacteria - Infections - MSD Manual Consumer Version*. [online] Available at: <https://www.msdmanuals.com/en-au/home/infections/bacterial-infections-gram-negative-bacteria/overview-of-gram-negative-bacteria> [Accessed 21 June 2021].

The website where I found the Univeristy Investigation:
CNA. n.d. *In pictures: The bacteria living on your hands right now*. [online] Available at: <https://www.channelnewsasia.com/news/singapore/bacteria-living-on-your-hands-10067062> [Accessed 21 June 2021].

22/6/21

Today I told my teacher about all the research I did and she said that all the bacteria I looked at wasn't safe to do an experiment with. She then said she would ask my schools lab supervisor, if there was any bacteria available for me to use. That was also when she wrote down the risk assessment. I decided on that I would do four different types of bacteria if my school had them and if not, I had to research what types of safe bacteria lived on the hands and tell my teacher so she could order them in.

24/6/21

Today I was given the all go for the experiment and was given a list of all of the types of bacteria strains my school had. These strains were:

- Bacillus subtilis
- Staphylococcus epidermidis
- Escherichia coli K12 strain
- Micrococcus luteus

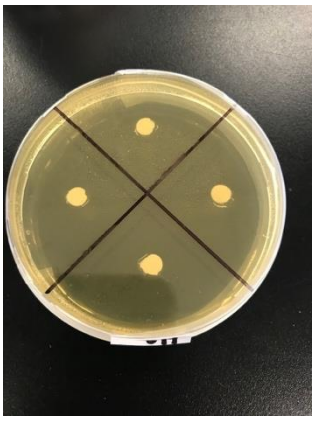
I was also told to research them when I got home so that when I was doing the experiment I knew what the types of bacteria were. When I got home that night, I lost the stickynote that had the types of bacteria on it so I didn't know what to research. I also forgot to ask my teacher for the types of bacteria the school had via email.

28/6/21

Today was the day I did the experiment, when I got to class, my teacher gave me a run down on what to do and how to do it. Then I put on a lab coat and safety glasses and washed my hands before I touched any of the equipment I was using. I did wear gloves for some of it but then I asked my teacher if I could take them off and she said it was fine, as long as I was really careful.

The first thing I did was cut up a bunch of little discs from the fitler paper that would go in the petri dishes with the bacteria. I put the little discs into a plastic bowl and put another bowl ontop of that when I was done, to minimise the contact the discs had with the air. I then put a bit of hand sanitiser into one bowl and distilled water into another one. Seeing as I didn't know how to put the bacteria into the petri dish, how to put the filter paper in and so forth, I had my teacher show me how to do it. I tried doing it myself and made a few testers. From doing the testers, I was able to come to a decision of putting black lines on the backside of the petri dish so that I would know where to place the filter paper instead of putting them anywhere.

Here are photos of plates with lines and without:



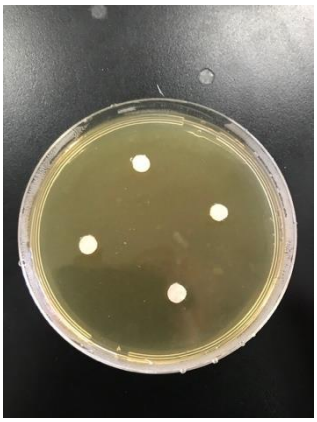


Figure 1: I found it easier to place the filter paper discs into the right places with the lines spliting the petri dish into quadrants instead of estimating where I should place them.

I also came to the decision to use 0.5ml f the bacteria instead of 1ml as I found that it was too much and made the filter paper discs to move around. Once I was confident with making the samples, I repeated what I had done with testers and tried my best to make the filter paper be placed in the middle of one of the four quadrants. I did two hand sanitiser petri dishes and one control with distilled water. The bacteria I used for the first dishes was Micrococcus luteus.

I wasn't able to do all of the petri dishes today as I didn't have enough time to do them inbetween my lunch and next lesson so I put the ones I had done in the incubator and decided I would do the rest of them tomorrow.

When I got home that night I did some research on the bacteria I was using:

Bacillus subtilis – An aerobic Gram-Positive, Rod shaped bacteria that is spore forming and found in soil and vegetation. The optimal temperature for Bacillus subtilis to thrive is from 25-35 degress celcius and only takes around 48h for it to grow. The B. subtilis produces numerous amounts of emzymes that deteriorate a variety of substances, allwoing the bacteria to thrive and prosper in an ever changing enviroment.

Staphylococcus epidermidis – This bacterium is a Gram-Positive, member of the Staphylococcus family and is commonly found on the skin on the face. Staphylococcus epidermidis doesn't produce any harmful toxins but can work as a conductor for Staphylococcus aureus, enhancing its resistance to antibiotics and pathogenic success making it more dangerous than it originally was.

Escherichia coli K12 strain – A Rob shaped bacteria that has no nuclear membrane because it is a prokryate. This is a rapidly growing bacterium and can start to grow within an hour and reproduces by binary fission creating two cells within a short span of time. Though Escherichia coli is a Gram-Positive bacteria, it possess the characteristics of a Gram-Negative bacteria as it is enveloped by two membranes.

Micrococcus luteus – A spheriacl, Gram-Positive bacteria that isn't usually pathogenic and are common inhabitants of the human skin and body. Micrococcus luteus has even been said to be a key part in keeping balance between Gram-Positive and Gram-Negative bacteria that lives on the skin. As well as other types of viruses.

The websites I used:

Microchemlab.com. n.d. *Bacillus subtilis | Microchem Laboratory*. [online] Available at: <https://microchemlab.com/microorganisms/bacillus-subtilis> [Accessed 28 June 2021].
Microbial Cell Factories. n.d. *Bacillus subtilis: a universal cell factory for industry, agriculture, biomaterials and medicine*. [online] Available at: <https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-020-01436-8> [Accessed 28 June 2021].
PLOS PATHOGENS. n.d. *Staphylococcus epidermidis — the 'accidental' pathogen*. [online] Available at: <https://www.nature.com/articles/nrmicro2182> [Accessed 28 June 2021].
PLOS PATHOGENS. n.d. *Staphylococcus epidermidis—Skin friend or foe?*. [online] Available at: <https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1009026> [Accessed 28 June 2021]

Uniprot.org. n.d. *Escherichia coli (strain K12)*. [online] Available at: <https://www.uniprot.org/proteomes/UP000000625> [Accessed 28 June 2021].
Sciencedirect.com. n.d. *Escherichia Coli K 12 - an overview | ScienceDirect Topics*. [online] Available at: <https://www.sciencedirect.com/topics/medicine-and-dentistry/escherichia-coli-k-12> [Accessed 28 June 2021].
Encyclopedia Britannica. n.d. *Micrococcus | bacteria genus*. [online] Available at: <https://www.britannica.com/science/Micrococcus> [Accessed 28 June 2021].

29/6/21

Today I used the same method of making the test dish I did yesterday with the rest of the bacterial strains I had. While doing each petri dish, I realised that when I placed the coated filter paper onto the agar, I touched the end of my tweezers on the bacteria. Once I realised this, I had already done 5 plates and I couldn't change the plates so I decided that I would be more careful when putting the filter paper on the bacteria coated agar. I was also told by my teacher that I should clean the tweezers by using the flame of a bunsen burner and that's what I did. I was also given more tweezers so I could use them as I waited for the other tweezers to cool down. Once I finished doing all of the bacteria and used up all of the petri dishes, I put them all on the bottom shelf of the incubator so that I know those plates were put in the day after the first ones I did.

Today was also the day I took photos of the plates to see the if there was any growth. When I was looking at the Micrococcus luteus petri dishes, I realised that when I picked them up to take a photo of the growth, the bacteria liquid would move and get into the filter paper. This was human error and it resulted in some of the samples to not show any results.

The photos I took:

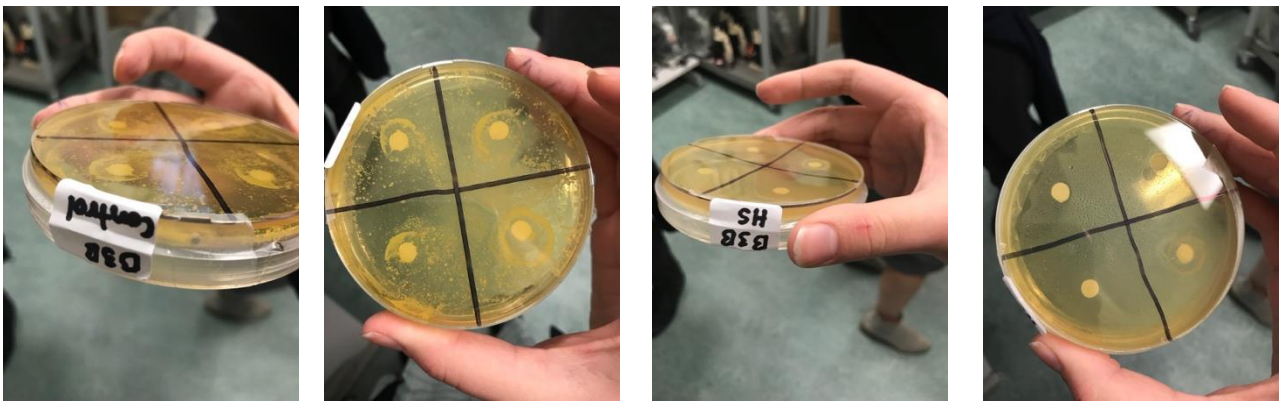


Figure 2: The 2 photos to the left are the control for Micrococcus luteus and the two on the right are the hand sanitiser sample.

Figure 3: This is how I set up the incubator, Mondays batch of petri dishes are on the first shelf and Tuesdays batch of petri dishes are on the bottom.

30/6/21

Today I took photos again and saw some growth on some of the plates but there wasn't a lot all together. There was like a cloudy coating in some areas which I believe is the bacteria growing. Also I noticed the same problem I had noticed the day before, everytime I picked up a petri dish to look at the growth on the agar, the bacteria liquid would move around and get on the coated filter paper. It didn't happen to all of the petri dishes which is lucky but I was still disappointed that it happened to some of them. When I got home I realised I didn't take photo's of the Staphylococcus Epidermis which was a pretty big mistake.

Here are the photos I took:
Bacillus subtilis (Tuesday):

Figure 4: The photo on the left are the control and the two photos on the right are the hand sanitiser samples.

Escherichia coli K12 strain (Tuesday):

Figure 5: The two photos on the left are the control and the two photos on the right are the hand sanitiser samples.

Micrococcus luteus (Monday):

Figure 6: The two photos on the left are the control and the two photos on the right are the hand sanitiser samples.

2/7/21

Today I was sick and couldn't go to school so I had my teacher take photos of the petri dishes for me and I was going to measure the inhibition zone but I wanted to wait until I went back to school to do it as I could get a better reading that way. Also my teacher told me that she put the petri dishes in the fridge to stop the bacteria growing so that I could see them when I got back from holidays.
Inhibition zone – An area around the filter cirlces that has no bacteria growing.

19/7/21

Today I measured the inhibition zone. I did this by finding the closest point the bacteria was to the filter paper, then I drew a line between the center of the filter paper and the bacteria and multiplied it by two to get the diameter. Some of the samples didn't show an inhibition zone so I just left them a zero. Some of the samples were hard to tell were the inhibition zone was but when the plates were placed against a light, they were visiable.

Here are some photos I took of the inhibition zone:

Figure 7: Both of these photos are testers I did with Micrococcus luteus, I did this so I could understand how to spread the bacteria and how to place the filter paper discs on the agar. There was 1ml of bacteria on the plates but was changed to 0.5ml because there was to much excess bacteria liquid left on the plate. This caused the filter circles to move around.

Risk Assessment Form

RISK ASSESSMENT **Brighton Secondary School**

Investigating bacteria

Written by: Maria Galouzis **Commenced on:** 24 Jun 2021 **Expires:** 24 Sep 2022

Classes for which experiment is required

Teacher: Maria Galouzis (training code 1) **Year Group:** 9 STEM

Room	Period	Date
74	lunchtime	Mon 28/6/21
74	lunchtime-lesson 5	Tue 29/6/21

Liana Walters Science Oliphant Entry (Science Inquiry)

Items to be prepared by laboratory technician (training code 1)

agar plates (20)
bacterial cultures - all four that we have Michelle
sterile swabs
spreaders
parafilm
hand sanitiser
beakers (50mL)
filter paper
hole punch
pipettes
distilled water

Procedure or reference, including variations

Liana Walters Science Oliphant Entry (Science Inquiry).

Equipment to be used

glass beaker, 200 mL or less

Potential hazards
Breakage of beaker. Cuts from chipped rims.

Standard handling procedures
Inspect and discard any chipped or cracked beakers, no matter how small the damage. Sweep up broken glass with brush and dustpan; do not use fingers.

hole puncher

inoculation spreader, disposable plastic (L-shaped spreader)

Potential hazards
Disposable plastic spreader should not be heated in flame since already sterile and will burn, producing toxic fumes.

Standard handling procedures
Dispose of appropriately. Do not inoculate unknown organisms, since they may be pathogenic.

parafilm

Potential hazards
Organic solvents may affect parafilm, causing leaks. May cause burns if molten. Irritating fumes are released if heated to high temperatures.

Standard handling procedures
Avoid use of organic solvents or heating the parafilm.

pipette bulb (pipet bulb)

Standard handling procedures
Dispose of old bulb if the rubber becomes cracked.

sterile swab

Standard handling procedures
Dispose of used swab appropriately if used medically.

nutrient agar plate

Potential hazards
Agar is harmless, but bacteria or fungi grown on agar may be pathogenic. Knowledge of microbiology and aseptic techniques is required to minimise risks to staff, students and the environment.

Standard handling procedures
It is generally not recommended to incubate agar at temperatures around 37°C, since this increases the likelihood of pathogenic organisms growing. Risk group 1 organisms, as described in AS/NZS 2243.3:2010, are

	generally regarded as suitable for supervised school experiments. Check the policy of your school authority.

filter paper

Potential hazards	*Standard handling procedures*
Flammable. Used filter paper may contain harmful residues.	After use, dispose of residue and filter paper appropriately.

Biologicals and food to be used

Bacillus subtilis (B. subtilis)

Potential hazards	*Standard handling procedures*
Not pathogenic, but may be mixed in the wild with other bacteria that are pathogenic.	Some school authorities ban the culturing of unknown micro-organisms in experiments, due to the possibility of culturing pathogens. Commercially obtained pure non-pathogenic strains may be bought from reputable suppliers. Your school authority may allow these strains of the bacteria to be subcultured from one plate to another, provided the appropriate safeguards are followed. Risk group 1 organisms, as described in AS/NZS 2243.3:2010, are generally regarded as suitable for supervised school experiments. Check the policy of your school authority.

Escherichia coli (E. coli)

Potential hazards	*Standard handling procedures*
Possibility of infection during experiments with E. coli. Some strains are highly pathogenic.	Some school authorities ban the culturing of unknown micro-organisms in experiments, due to the possibility of culturing pathogens. Commercially obtained pure non-pathogenic strains may be bought from reputable suppliers. Many school authorities do not permit sub-culturing of bacteria from wild cultures. Your school authority may allow commercially obtained pure strains of non pathogenic bacteria to be subcultured from one plate to another, provided the appropriate safeguards are followed. Risk group 1 organisms, as described in AS/NZS 2243.3:2010, are generally regarded as suitable for supervised school experiments. Check the policy of your school authority.

Staphylococcus epidermidis (S. epidermidis)

Potential hazards	*Standard handling procedures*
May cause infections in individuals who are immunocompromised or debilitated.	Some school authorities ban the culturing of unknown micro-organisms in experiments, due to the possibility of culturing pathogens. Commercially obtained pure non-pathogenic strains may be bought from reputable suppliers. Many school authorities do not permit sub-culturing of bacteria from wild cultures. Your school authority may allow commercially obtained pure strains of non pathogenic bacteria to be subcultured from one plate to another, provided the appropriate safeguards are followed. Risk group 1 organisms, as described in AS/NZS 2243.3:2010, are generally regarded as suitable for supervised school experiments. Check the policy of your school authority.

Others

distilled water
hand sanitiser

Knowledge

I have read and understood the potential hazards and standard handling procedures of all the equipment, chemicals and biological items, including living organisms.
I have read and understood the Safety Data Sheets for all hazardous chemicals used in the experiment.

I have copies of the Safety Data Sheets of all the hazardous chemicals available in or near the laboratory.

Risk assessment

I have considered the risks of:

fire or explosion	breakage of equipment	exposure to pathogens	waste disposal
chemicals in eyes	injuries from equipment	injuries from animals	improper labelling/storage
inhalation of gas/dust	rotating equipment	intense light/lasers	inappropriate behaviour
chemicals on skin	electrical shock	UV, IR, nuclear radiation	communication issues
ingestion of chemicals	vibration or noise	pressure inside equipment	allergies
runaway reaction	sharp objects	heavy lifting	special needs
heat or cold	falling or flying objects	slipping, tripping, falling	other risks

Certification by Teacher

I have assessed the risks associated with performing this experiment in the classroom on the basis of likelihood and consequences using the School's risk matrix, according to International Organization for Standardization Standard ISO 31000:2018.

I consider the inherent level of risk (risk level without control measures) to be:

Low risk Medium risk High risk Extreme risk

Risks will therefore be managed by routine procedures in the classroom.

Electronic Signature: Maria Galouzis **Date:** 28 Jun 2021

You have provided an electronic signature which is the equivalent of signing your name with a pen and as such will constitute a legally binding agreement between the relevant parties. We can give no warranty in respect to fraud or security breach resulting from the use of an electronic signature.

Certification by Laboratory Technician

I have assessed the risks associated with preparing the equipment, chemicals and and biological items, including living organisms, for this experiment and subsequently cleaning up after the experiment and disposing of wastes, on the basis of likelihood and consequences using the School's risk matrix, according to International Organization for Standardization Standard ISO 31000:2018.

I consider the inherent level of risk (risk level without control measures) to be:

☐ Low risk ☐ Medium risk ☐ High risk ☐ Extreme risk

Where the risk level is "medium risk", "high risk" or "extreme risk", the following control measures will be employed:

Control measures (attach further pages as required):

☐ safety glasses ☐ gloves ☐ lab coat ☐ apron ☐ fume cupboard

With the specified control measures in place, I have found that all the risks are "low risk". Risks will therefore be managed by routine procedures in the laboratory, in combination with the specified control measures.

Name: ..... **Signature:** ..... **Date:** .....

Monitoring and review

This risk assessment will be monitored using comments below and will be reviewed within 15 months from the date of certification.

Attach further pages as required

How effective is hand sanitiser on bacteria found on hands?

Introduction

Each type of hand sanitiser have different levels of alcohol, the sanitisers that have higher percentages of ethanol can kill bacteria more efficiently. The SA Health Alcohol-Based Hand Rub fact sheet states that hand sanitisers should have an "achohol concerntration of 60 – 80%" to be deemed bacteria resistant to an extent. The fact sheet also states that both ethanol and isopropanol have showed to hold the ability to isolate the activity of bacteria, fungi and some types of viruses. Scientists have conducted numerous experiments for the effectiveness of different ratios of alcohol in sanitisers and found that isopropanol has stronger effects against bacteria than ethanol. Hand Sanitiser that contains 60% isopropanol holds the similar *in-vitro* activity against bacterium as hand sanitiser containing 77% ethanol. That doesn't necessarily mean that isopropanol is stronger than ethanol with all bacteria strains.

For hand sanitiser to effectively disrupt and kill bacteria cells, the ethanol and isopropanol attach themselves onto the water-based membrane that surrounds the bacteria cell. The alcohol is then able to breakdown the membrane as the molecules in the proteins and a membrane of the cell easily bond with the molecules of the alcohol, tearing the molecules away from one another. The alcohol in hand sanitiser is an amphiphile chemical compound, meaning it possess both hydrophilic and lipophilic properties, make killing bacteria cells highly effective as the fatty amino acids and water are attracted to the alcohol molecules. Leaving the bacteria cells defenceless against hand sanitisers.

The hand sanitiser that was used in this experiment is a gel based sanitiser and has an alchohol concentration of 70%. This infers that this sanitiser is able to inhibit the growth of bacteria and holds the abiltiy to eradicate microorganisms, referred to as 'antimicrobial activity' in scientific terms. The bacteria that will be used and tested in this experiment are Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli K12 strain, Micrococcus luteus. All of which don't hold much resistance to hand sanitisers. Taking all of this into consideration, I believe that once the hand sanitiser is placed in the petri dish, the alcohol will break down the cell membrane of the bacteria and kill its core. This will inhibit the growth of the bacteria around the filter discs leaving a space were no bacteria has grown.

Aim

To investigate the effectiveness of hand sanitiser on bacteria that's commonly found on the surfaces of human hands.

Variables

Independent variable – The type of bacteria used throughout the experiment.

Dependant variable – The area of inhibition around the filter paper disc.

Controlled Variable	**Method of Control**
The hand sanitiser brand	The hand sanitiser that is used will be the same type on all of the bacteria.
The number of petri dishes for each strain	Each strain of bacteria was given a certain number of petri dishes as well as one controll.
The number of paper discs in each petri dish	The petri dish was split up into four different quadrants with the filter paper disc placed in the middle.
The amount of bacteria in the petri dish	The amount of bacteria in each petri dish was 0.5ml to keep it consistant.
The time the bacteria spends in the incubator	Once the petri dishes were placed in the incubator, the petri dishes were left in there for 4 days.
The temperature of the incubator	The temperature of the incubator was left at 35°c constantly.

The distance between each paper disc	The filter paperdiscs were placed in the center of each quadrant to keep the same distance between them all.

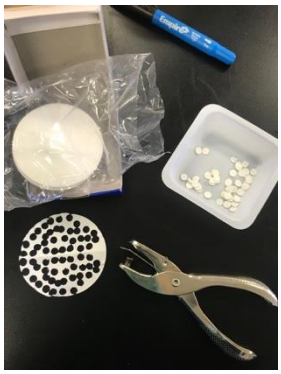
Materials/Equipment

- Bacillus subtilis (B5B)
- Staphylococcus epidermidis(S.albus) (B2)
- Escherichia coli K12 strain (B1)
- Micrococcus luteus(s.Lutea) (B3B)
- Filter Paper
- Hand sanitiser
- Petri dishes (25 including testers)
- Permanent marker
- Tweezers
- Plastic weigh trays
- Distilled water
- Teat Pippets
- Parafilm
- Hole punch
- Labels
- Spreader
- Ruler
- Scissors
- Gloves
- Bunsen Burner
- Heat proof mat
- Saftey Glasses
- Lab Coat
- Glass beaker
- Incubator
- Phone (to take photos)

Figure 8: The materials used in the experiment minus the busen burner, lab coat, safety glasses, incubator, phone, ruler and heat proof mat. ****The petri dishes were made the day before the experiment****

Method

1. 5mm in diameter discs were cut out of the filter paper with the hole punch and then placed into a plastic weigh tray. Another weigh tray was placed ontop to minimise he contact the filter paper had with the air.



2. Hand sanitiser and distilled water were added into seperate plastic weigh trays.

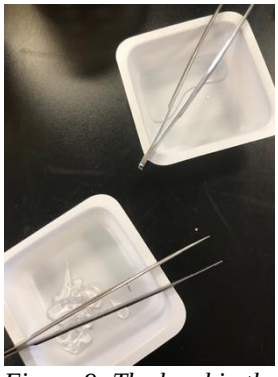
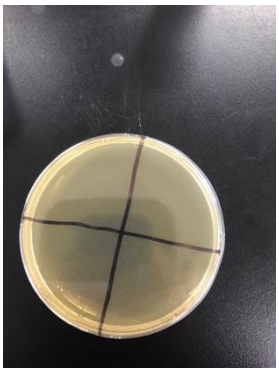


Figure 9: The bowl in the top right corner is distilled water and the bowl in the bottom left corner is the hand sanitiser.

3. Then lines were drawn on the underside of the petri dish to section off four quadrants. All the petri dishes had this done.




4. The pippet was used to extract 0.5 ml of the bacteria and then was placed on the petri dish as quickly as possible without rushing to minimise the contact it had to the air.


5. The bacteria was the spread in a circular motion with the sterile spreader to get an even coat on the petri dish. The spreader was then thrown out after each use.

6. One filter paper disc was picked up with the tweezers and placed into the hand sanitiser or distilled water. Then the discs were placed into a quadrant in the petri dish, with the discs as close to the centre.

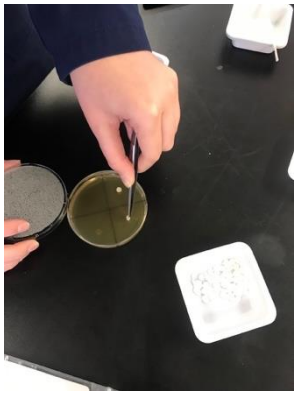
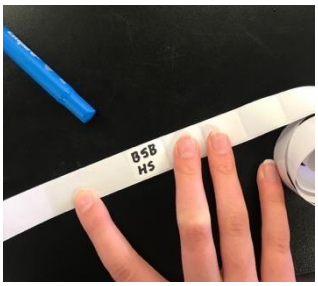


Figure 10: A new pair of tweezers were used to pick up the filter paper discs when dealing with a new strain of bacteria and when the tip of the tweezer would touch the agar plate covered in bacteria to stop it from spreading.

7. Once the lid was placed on the petri dish, the parafilm was wrapped around the edge to kept the petri dish together.

8. A label was written that had the strain classification and whether the dish was a control or had the handsanitiser on it (HS).



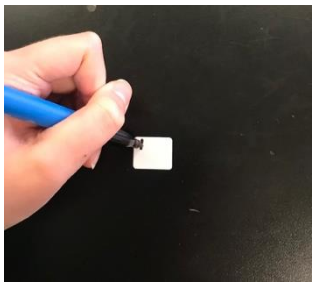


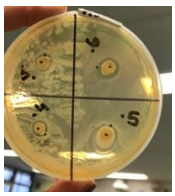
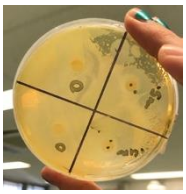
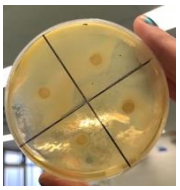
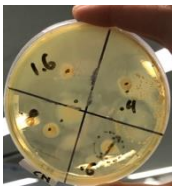
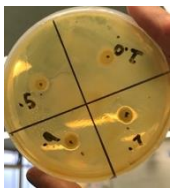
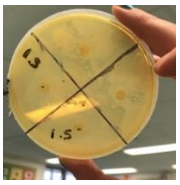
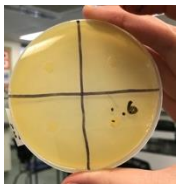
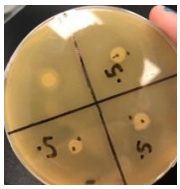
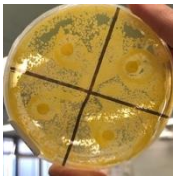
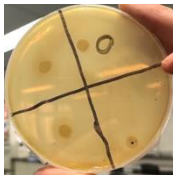
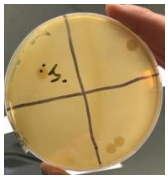
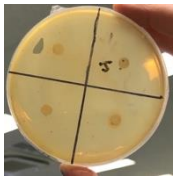
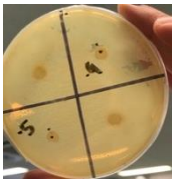
Figure 11: Each type of bacteria had 3 hand sanitiser plates, 1 plate of control and were done in bacteria specific groups to avoid confusion.

9. The finished group of petri dishes were placed into the incubator that had a set temperature of 35°c and were checked each day at the same time they were put in.


10. Once the tweezers were used, they were cleaned with the flame of a Bunsen burner and left to cool to be used again.

Results

Table 2: The resultant zones of inhibition for each replicate and average

Type of bacteria	Zone of inhibition (mm)			Zone of inhibition Average	Photo		
	Plate 1	Plate 2	Plate 3		Plate 1	Plate 2	Plate 3
Staphylococcus epidermidis (HS)	10 10 10 8	10 12 8 8	10 12 0 0	98 ÷ 12 = 8.17 Average = 8.17			
Staphylococcus epidermidis (Control)	0 0 0 0			0 ÷ 4 = 0 Average = 0			
Escherichia coli K12 strain (HS)	0 0 0 0	32 16 12 8	20 14 10 18	130 ÷ 12 = 10.83 Average = 10.83mm			
Escherichia coli K12 strain (Control)	26 30 0 0			56 ÷ 4 = 14 Average = 14mm			
Micrococcus luteus (HS)	12 0 0 0	8 10 10 10	10 10 10 0	80 ÷ 12 = 6.67 Average = 6.67mm			
Micrococcus luteus (Control)	0 0 0 0			0 ÷ 4 = 0 Average = 0mm			
Bacillus subtilis (HS)	0 0 0 0	8 0 0 0	10 0 0 0	18 ÷ 12 = 1.5 Average = 1.5mm			
Bacillus subtilis (Control)	10 8 0 0			18 ÷ 4 = 4.5 Average = 4.5mm			

Discussion

The results showed that 3 out of the 4 bacteria didn't grow around the hand sanitiser coated filter discs. However, the bacteria that continued to grow around the discs was Bacillus subtilis with only having results from 2 of the 12 filter discs. The zone of inhibition from the 2 discs were 8mm and 10mm, possessing the same data as the control sample of the same bacteria. The average zone of inhibition for Bacillus subtilis was 1.5mm and is the most resistant to a hand sanitiser that contains 70% alcohol compared to the other 3 bacteria.

Staphylococcus epidermidis, Escherichia coli and Micrococcus luteus all produced data that showed a range of zones of inhibition. Both Staphylococcus epidermidis and Micrococcus luteus, zones of inhibition across the 3 handsanitiser plates all ranged between 8mm and 12mm. These results inferred that these 2 types of bacteria weren't able to stop the hand sanitiser to break away the cell membrane that was protecting the bacteria cell. This is why the bacteria that surrounded the filter paper discs weren't able to thrive due to the alcohol in the sanitiser stopping the growth of the bacteria. The averages for these types of bacteria are 8.17mm and 6.67mm.

The bacteria that allowed the handsanitiser to destroy most of the bacteria cells around the paper discs was Escherichia coli. 1 of this bacteria's plates did not show any results because of an error that occurred while it was in the process of creation, this error had consistantly been shown to repeat itself on a few other agar plates over the experimenting period. However, the 2 other handsanitiser test plates showed some promising data by day four of incubation. The first plate had the zone of inhibition range between 8mm and 32mm, and the second plate had the zone of inhibition range between 10mm and 20mm. These ranges were the most spread out than the other 3 bacteria, and because of that, made this bacteria the lest resistant towards this type of bacteria. This means that the cell membrane of the bacteria wasn't able to stop the alcohol from destroying it before the bacteria had the chance to reproduce.

The prediction that was made at the start of this experiment would be around 75% accurate because 3 of the 4 bacteria strains were able to produce results and the other 1 wasn't able to do just that.

The concept of human error occurs quite a few times throughout the experiment and is evident on the results. Some of the sample plates showed that the filter paper discs had moved from their intended quadrants, allowing bacteria to get on it and stop the hand sanitiser from working properly. Human error is also present on the filter discs that hadn't moved but still had the bacteria grow on it. This is because once the petri dishes were moved into the incubator and when they were observed inbetween each day, the excess bacteria moved across the agar and coated the circles. Resulting in bacteria to form on top of some of the filter papers. Another human error occurred when the petri dishes were being made, once the coated discs were placed onto the layer of bacteria, the tweezers would touch the bacteria. Allowing it to travel onto the end of the tweezers and tranferrer onto the paper discs in the plastic weigh tray when a new disc was needed.

Evaluation

After observing all the potential errors that had occurred while I was doing this experiment, I now know how I can avoid them the best I can to make this experiment better and show a higher quality set of results. Such as letting the plates grow for the full amount of time, instead of checking on them every day, which avoids the possiblitiy of bacteria growing over the filter paper on the agar. There are still a few things I would need to work on to make this experiment run smoother but even so, the results that I managed to collate can be helpful in the field of Science. I say this because I was able to find out that Escherichia coli wasn't able to protect its cells from the alcohol content in the hand sanitiser used.

Questions that can be made off of this experiment are 'The effects of different strenghted hand sanitiser on Escherichia coli?' or something along those lines. That question can also be used for the other 3 types of bacteria giving researcher the opportunity to discover what percentage of alcohol in hand sanitiser can disintergrate the cell membrane allowing the alcohol molecules to breakdown the bacteria's cell structure before it can reproduce and cause harm to the human body.

Conclusion

This experiment produced results that showed that Escherichia coli wasn't able to hold strong defences against the alcohol as its cells were being destroyed. Whereas, Bacillus subtilis was able to stop the hand sanitiser from breaking it's cell membrane, giving the bacteria enough time to reproduce and overpower the hand sanitiser. These result's demonstrated the effectiveness of hand sanitiser against 4 types of hand bacteria and allowed for potential research and data to be accumulated. This data has the abiltiy to help researchers develop more accurate views and ideas about each of the bacteria used and tested in this experiment.

The hypothesis stated at the beginning of this report had been consistently proven over the course of the testing and recording phases of this experiment. An area of inhibition had appeared around the filter paper discs in the petri discs, some might not have been the biggest but there were areas where no bacteria had formed. There was a few petri dishes that had bacteria grow around the filter paper discs but was the result of human error not because the hand sanitiser wasn't able to kill the bacteria around it. Overall this experiment had produced promising results that were in alignment with the hypothesis and aim intened, deeming this experminet successful with the occasional human error.

Report Word count: 1,778

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