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Logbook Science Fair

By: Hanish Dokuparti 9C

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Logbook

Name: Hanish Dokuparti

Grade: 9

Project: Are the effects of different antibiotics identical on both gram-positive and gram-negative bacteria?

Project type: Experimental

Date	Notes:
Thursday, October 5, 2023	Met with Ms. Bretner **(Science Fair lead at STEM)** and had question approved. Question: **Are the effects of different antibiotics identical on both gram-positive and gram-negative bacteria?** Type of Project: **Experimental**
Sunday, October 29, 2023	*Today, I will be scratching the shell of my problem and will create an overview of what I need. This will be a framework for me to continue the project and look back to on what to research in detail. Before doing so though, I will be looking at the experimental project rubric to see what components I need to include!* - Problem: Are the effects of different antibiotics identical on both gram-positive and gram-negative bacteria? *(What is gram-positive and gram-negative bacteria? What are some common antibiotics? How are you going to execute this to be an experimental project? How are you going to get the bacteria? How are you going to test?)* Today's basic procedure idea: • *I have gone onto my main search engine—google—and I went to research this project to get an overview and resource to help me throughout my experiment.* • *Using this website, I am going to have root work for where I get my information to deliver the experiment, and I will be giving major credits to the website.* ▪ With 4 distinct types of common antibiotics, we will see if their effect on gram-positive and gram-negative bacteria is the same. ▪ For the bacteria, I will buy live cultures online or grow them myself. I will do a process called gram-staining on a glass disc or petri-dish (preferably) and use chemicals provided in the kit for their colors. The distinct colors help determine whether the bacteria is gram-positive or negative.

	▪ The project has 2 different points of data. In other words, the project method has 2 steps. One step is the gram-staining process, and the other is the data collection steps. ▪ The gram-staining process (still need to read to master understanding) and data collection are 2 big parts of the method. For data collection, we will look for diameter measurements like cm where the bacteria are inhibited by the antibiotics to see if it is the same. Another part is qualitative data, such as observations I see of the bacteria reaction to the eye. ▪ Gram-staining is creating a small film of the bacteria culture on the petri-dish and then using colorizing chemicals on it to see which is gram-positive and which are gram-negative. Certain bacteria will retain the color, while some will not, and some will absorb additional chemicals changing their color, helping tell which are gram-positive and which are gram-negative. Links: https://stemia.schoology.com/group/6912913417/materials#/group/6912913417/materials?f=312138664 https://learning-center.homesciencetools.com/article/gram-stain-antibiotics-project/ Bibliography: Bretner, J. (2023, September 8). *CYSF Rubrics*. Schoology. https://stemia.schoology.com/group/6912913417/materials#/group/6912913417/materials?f=312138664 HSTL. (Unknown year, Unknown month, Unknown day). *Learn About Bacteria with Gram Staining & Antibiotics*. Home Science Tools. https://learning-center.homesciencetools.com/article/gram-stain-antibiotics-project/
Friday, November 3, 2023	*Today, I will be diving further into the project title and find meanings for the unfamiliar parts. I will also look at what materials I need and see their availability. I will also, if possible, create a detailed method of what I must do. Another optional thing is to further investigate gram-staining.* • Problem: Are the effects of different antibiotics the same on gram-negative and gram-positive bacteria? ▪ *What are antibiotics?* (Background research) → Antibiotics are drugs prescribed by doctors to kill infections that take place in your body that are caused by bacteria. → Antibiotics directly translate to “against life.” They either directly kill bacteria or stop them from reproducing/replicating. → Antibiotics can only kill bacteria. They do not affect viruses or anything that is viral. → They can cure infections like some ear infections, kidney & bladder infections, dental infections, etc.

	→ A lot of the bacteria in you resides in your intestines—guts. Some side effects of antibiotics include vomiting, nausea, diarrhea, etc. → There are some distinct types of anti-biotics, which include ▪ *What are gram-negative and gram-positive bacteria? Characteristics? Differences? Effects? Any additional information.* ▪ *What materials would you need for this project? Provide links to materials and find their costs!* Links: https://www.webmd.com/a-to-z-guides/what-are-antibiotics Bibliography: WebMD Editorial Contributors. (2021, November 3). *What are antibiotics?* WebMD. https://www.webmd.com/a-to-z-guides/what-are-antibiotics
Tuesday, November 7, 2023	*Today, I will be continuing what I originally planned to do the last entry:* • What are antibiotics? (Background research) → There are 2 distinct types of antibiotics: Broad-spectrum and narrow spectrum. Broad-spectrum antibiotics affect and work on many diverse types of bacteria. Narrow-spectrum bacteria only target specific types of bacteria. • What are gram-negative and gram-positive bacteria? Characteristics? Differences? Effects? Any additional information. (Background research) → Gram-negative and gram-positive bacteria are the most common ways bacteria are categorized. Classifying the bacteria is crucial to determining which antibiotics should be used or how the bacterial infection should be treated. → Gram-negative and gram-positive bacteria differ in their structure. Gram-positive bacteria have thicker cell walls and are known as monoderm—single membrane organisms. → Gram-negative bacteria have far thinner cell membrane walls, but they have an additional cell membrane wall outside. They are known as diderm—2 membrane organisms. **Sidetrack: Went to research how to do the project execution such as growing own cultures and testing with antibiotics. Also did little bit of clarification by looking at material contents and looks.* • What materials would you need for this project? Provide links to materials and find their costs! (Materials) Links:

	https://www.technologynetworks.com/immunology/articles/gram-positive-vs-gram-negative-323007 https://www.webmd.com/a-to-z-guides/what-are-antibiotics https://learning-center.homesciencetools.com/article/gram-stain-antibiotics-project/ https://en.wiktionary.org/wiki/monoderm#:~:text=monoderm%20(not%20comparable),a%20thick%20layer%20of%20peptidoglycan https://www.google.com/search?q=how+to+grow+actual+bacteria+culture&sca_esv=580369605&tbm=vid&source=lnms&sa=X&ved=2ahUKEwibzNPWwbOCAxXNCTQIHcmmB8cQ_AUoAnoECAUQBA&biw=1470&bih=764&dpr=2#fpstate=ive&vld=cid:59917b5c,vid:Bwjxi3vS0K4,st:0 https://learning-center.homesciencetools.com/article/bacteria-experiment-guide/
Friday, November 10, 2023	*Today, I will be continuing my background research for my project. I will add some more questions to provide a framework for my project so I know more and can educate more about it. Some of this framework will also include some applications and future applications.* • What are gram-negative and gram-positive bacteria? Characteristics? Differences? Effects? Any additional information. (Background research) → Gram-positive bacteria have a much thicker cell membrane that easily absorbs substances. There are diverse types of gram-positive bacteria: Resident flora lives freely in your body without harming you or causing any problems. Some include bacteria on your skin and in your mouth and nose. They also include the 'good bacteria' in your body; Pathogenic bacteria harms you and causes disease in people. Some include anthrax and diphtheria. Gram-positive bacteria can easily be treated with → Gram-negative bacteria have a much thinner membrane, but 2 of them, one that is on the outer side of the bacteria. The membranes are much stronger and do not absorb substances easily. When gram-negative bacteria's cell wall is disturbed, it releases endotoxins which worsen your symptoms. → Gram-negative bacteria are so much harder to kill then gram-positive bacteria because it has a much harder membrane that does not absorb substances easily. → Currently, there is a problem regarding antibiotic resistance. This is caused by too many antibiotics being used to kill bacteria at a time, and bacteria start getting used to it and resist them. We can stop the spread of antibiotic resistant bacteria by only taking the prescribed amount each day. Do not take any more! • Where would you normally find gram-positive and gram-negative bacteria in a household? (Background research)

	→ You would normally find gram-negative bacteria in damp places or wet places, like your kitchen sink or refrigerator. → You would normally find gram-positive bacteria in dry places, like on your skin or dry counters. • What materials would you need for this project? (Materials) → Agar – nutrient liquid for bacteria to safely grow in a controlled environment. → Petri-dishes x 8 (x10) 90 x 15 mm – to grow the bacteria in and to experiment in for 3 trials. → Inoculating needle – to transfer the bacteria from petri-dish to slide and antibiotic slide. → Gram stain kit (has crystal violet stain, Gram iodine stain, ethyl alcohol solvent, Safranin O counterstain, plain microscope slides, medicine dropper, coverslips) → Wash bottle – to gently wash bacteria specimen after gram-staining. → Compound microscope – to examine bacteria after gram-staining to see which is gram-positive and gram-negative. → 24 antibiotic discs (Penicillin, Ampicillin, Neomycin, Erythromycin – Most common antibiotics used to treat common bacterial infections for 3 trials) "Must be refrigerated" → Ruler – to measure the diameter of bacterial growth inhibited by antibiotic discs. → Cotton swabs – to distribute bacilli to agar-filled petri-dish. → Candle(s) - to heat fix the bacilli specimen to the microscope slide. → Stopwatch – to time the length of each stain. → Tweezers – to put/transfer certain things like the antibiotic discs. Links: https://www.webmd.com/a-to-z-guides/difference-between-gram-positive-bacillus-gram-negative-bacillus https://learning-center.homesciencetools.com/article/gram-stain-antibiotics-project/ https://www.usmslab.com/gram-negative-bacteria/#:~:text=In%20ISO%2Dclassified%20areas%2C%20the,other%20sources%20of%20standing%20water. https://www.healthline.com/health/gram-positive#:~:text=Most%20of%20these%20bacteria%20are,can%20cause%20serious%20medical%20conditions.
Tuesday, November 14, 2023	*Today, I will wrap up with the background research.* • How do antibiotics kill bacteria? (Background research) → Some antibiotics like penicillin kill bacteria by destroying the bacteria's cell wall.

	→ Some antibiotics like tetracycline interfere with the bacteria's ability to reproduce and copy itself. • What is Penicillin, Ampicillin, Neomycin, and Erythromycin? How are they different and what are their effects? (Background research) → Penicillin, Ampicillin, Neomycin, and Erythromycin are common antibiotics used in the medical industry to treat bacterial infections. → Penicillin is effective against only gram-positive bacteria. → Ampicillin is effective against both gram-negative and gram-positive bacteria. → Neomycin is effective against variety of gram-negative bacteria. → Erythromycin is effective against gram-positive and some gram-negative bacteria. • *I came across the term bacilli significantly while researching.* What are bacilli? (Background research) → Bacilli is the plural term for bacillus, meaning disease-causing bacterium. Links: ❑ https://www.healthychildren.org/English/health-issues/conditions/treatments/Pages/How-Do-Antibiotics-Work.aspx#:~:text=Some%2C%20such%20as%20penicillin%2C%20kill,nutrients%20they%20need%20to%20survive ❑ https://chat.openai.com/c/79edec9f-5219-4294-aa90-9310b25875db ❑ https://www.google.com/search?sca_esv=582393838&sxsrf=AM9HkKmw2BVLhWvz7qUD-CDK739dIjjnLg:1699996264443&q=bacilli&si=ALGXSlZCBshTM3a3nPTSW0d1OmQeLsPCMAUICQClim3BPnviAcvMht1lQi2kqobyIs7YZ1lay7Us0csS1_Wes3FVrR6wj7GE6w%3D%3D&expnd=1&sa=X&sqi=2&ved=2ahUKEwj6q9jLs8SCAxUYMTQIHb1SAYQQ2v4IegQIGhAR&biw=1633&bih=892&dpr=1.8
Monday, November 20, 2023	*Today, I will do hypothesis, variables, and come up with a method for the project.* • Form a prediction on what you are going to expect in the experiment. It should form an answer to your problem. (Hypothesis) → I think that the effects of different antibiotics are not identical on gram-negative and gram-positive bacteria because gram-negative bacteria is much harder to kill due to it's thinner, stronger, double membrane, while gram-positive bacteria is easier to kill due to it's one thick membrane that easily absorbs substances. The effects will also not be identical because the different antibiotics are effective

against different types of bacteria; penicillin best with gram-positive, ampicillin with both, neomycin with gram-negative, and cethromycin with gram-positive and some gram-negative.

- What are your 3 variables (manipulated, responding, controlled)? For controlled, what are they, why they should be controlled, and how you are going to control them. (Variables)

→ Manipulated: The manipulated variable is the antibiotics being used to test on the gram-negative & gram-positive bacteria.

"This is the thing being changed throughout the experiment to see if similar results of the inhibition of bacterial growth is achieved."

→ Responded: The responded variable(s) is the diameter of the inhibition of bacterial growth. (Discs are circular). This will be measured in cm with a 30 cm ruler.

- Another responding variable can be qualitative observations. Such as color, anything else!

*(Dependent variable is the effects on the gram-negative and gram-positive bacteria. It is dependent on the antibiotics used).

Controlled variables Variables that need to be maintained throughout the experiment for best results.	Condition: What should be controlled throughout the project?	Why: Why does that condition need to be maintained?	How: How are you going to control that condition?
	Temperature	Being exposed to different types of temperature can affect bacterial growth. Some excel while some die, affecting results.	Control heaters in the house so same temperature is maintained throughout home.
	Testing environment	Some environments have more bacteria in the air than others. This can affect how much the bacterial petri-dish can get contaminated when exposed to testing.	Only test and perform the experiment in one room of the house. Do not keep opening the room and keep it closed.
	Concentration of antibiotics	If one bacterium had a higher concentration of antibiotic than another, it may have more inhibitions. If	Only use one type of antibiotic disc without changing. If not using disc,

		concentration of the antibiotic differs in the specimens, the results will vary and not be accurate.	only use set number of pills or measure with spoon before using.

Method:
"*Method will be split into 3 big parts. One is the bacterial growth process, gram-staining process, and the antibiotic testing."*

<u>Bacterial-growth process:</u>

1. Make sure you have all the materials necessary for the staining process; 2 petri-dishes, liquid agar, sharpie or marker & tape, cotton swabs, and a camera. To prepare the agar, follow the instructions on the back of its packaging.
2. With the prepared agar, pour it into the 2 petri-dishes. Fill it to just until it covers the bottom of the dish. Quickly put the lid back on to avoid contamination from the air. Let it cool for about an hour, when it turns solid.
3. Recall that gram-positive bacteria normally like dry places and gram-negative likes damp places. With one cotton swab, rub it on your skin or non-clean counter and rub it in a zig zag pattern across one petri-dish. Turn the dish and do it again for maximum distribution, and quickly put the lid back on. Label where you got the bacteria from on the dish with sharpie. Take a picture for future comparison.
4. Perform step 3, doing the cotton swab collection from a damp place such as a kitchen sink.
5. Wait for a few days while the bacteria grow. Take a picture of each petri-dish every 24 hours for future reference/comparison. You should start using the bacteria after 3-5 days of growth or until it looks clearly matured.

<u>Gram-staining process</u>

1. Sterilize the inoculating needle by passing it through a candle flame a few times. Let it cool for about 5-7 seconds.
2. With the inoculating needle, transfer some of the bacteria from one dish onto the microscope slide. With another microscope slide's edge, scrape or press on the specimen smear, making it very thin. Let the specimen air dry, and quickly pass it through a candle flame to heat fix it. Don't stop, and don't let it get too hot.
3. Cover the specimen with 1—2 drops of the Crystal Violet stain for 60 seconds, and gently wash off with slow running water. Cover the specimen with a few drops of Gram's iodine for 60 seconds and then gently wash off like for the crystal violet stain.
4. Tilt the slide a bit and drop the ethyl alchohol drop by drop in a way it covers the whole specimen. Drop until the running alchohol is no longer colored. Then, gently wash the specimen with running water.

	5. Then, cover with safranin stain for 60 seconds, and then gently wash with water. Put a paper towel gently on the bacteria so it absorbs the water, and then put a cover slip on it. 6. Now, look under the microscope. Gram-positive cells would be purple; the retained the color. Gram-negative cells would be pink or red; the purple stain was washed away by the alchohol and replaced by the safranin stain. Label the petri-dish it was taken from. 7. Repeat the whole gram-staining process for the other petri-dish of bacteria. <u>Antibiotic testing</u> 1. Prepare the agar according to the instructions on the packaging. Pour until the bottom of one dish is covered and quickly put the lid back on. Let it harden. 2. Sterilize the inoculating needle. Then take some of one type of bacteria and lightly zigzag on the petri-dish. 3. Put one type of each disc in the bacteria at a good distance away from each other. 4. Repeat for the other dish and bacteria. 5. Take a picture every 24 hours. Each day, measure the diameter of the circle the antibiotic disc has inhibited. Take down any qualitative observations. 6. To dispose, put some bleach in it (powder or liquid), cover and seal, put it in a plastic bag, and throw away. Links: https://learning-center.homesciencetools.com/article/gram-stain-antibiotics-project/
Wednesday, November 22, 20234	*I am refining the methods to describe the actual experimental design by having 3 controlled dishes to compare the antibiotic tested bacteria with:* <u>Bacterial-growth process:</u> 1. Make sure you have all the materials necessary for the staining process; 2 petri-dishes, liquid agar, sharpie or marker & tape, cotton swabs, and a camera. To prepare the agar, follow the instructions on the back of its packaging. 2. With the prepared agar, pour it into the 2 petri-dishes. Fill it to just until it covers the bottom of the dish. Quickly put the lid back on to

avoid contamination from the air. Let it cool for about an hour, when it turns solid.

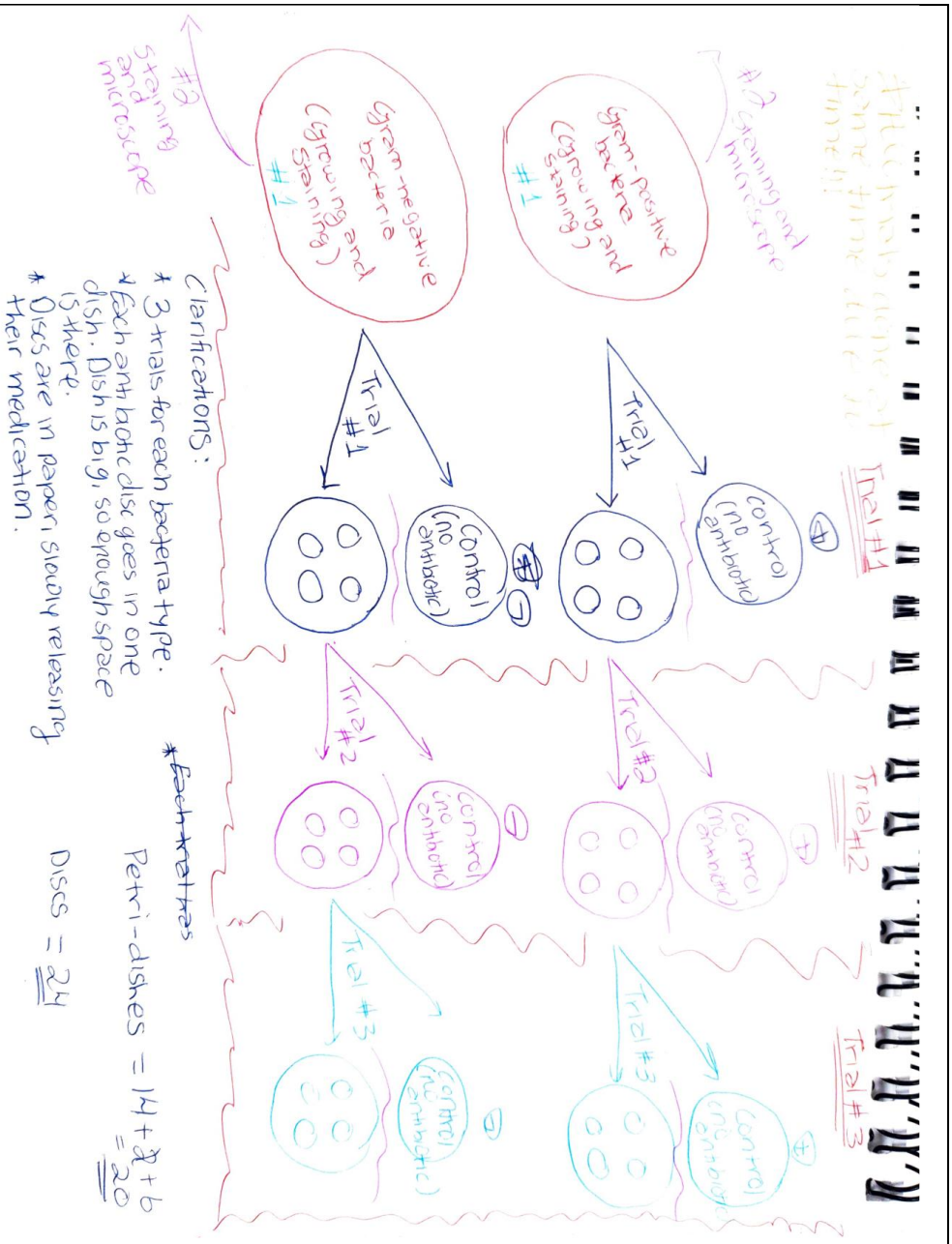
3. Recall that gram-positive bacteria normally like dry places and gram-negative likes damp places. With one cotton swab, rub it on your skin or non-clean counter and rub it in a zig zag pattern across one petri-dish. Turn the dish and do it again for maximum distribution, and quickly put the lid back on. Label where you got the bacteria from on the dish with sharpie. Take a picture for future comparison.
4. Perform step 3, doing the cotton swab collection from a damp place such as a kitchen sink.
5. Wait for a few days while the bacteria grow. Take a picture of each petri-dish every 24 hours for future reference/comparison. You should start using the bacteria after 3-5 days of growth or until it looks clearly matured.

<u>Gram-staining process</u>

1. Sterilize the inoculating needle by passing it through a candle flame a few times. Let it cool for about 5-7 seconds.
2. Put 1 drop of distilled water on microscope slide. With the inoculating needle, transfer some of the bacteria from one dish onto the microscope slide. With another microscope slide's edge, scrape or press on the specimen smear, making it very thin. Let the specimen air dry, and quickly pass it through a candle flame to heat fix it. Don't stop, and don't let it get too hot. Do it until the water evaporates.
3. Put the microscope slide on a few layers of paper towels. Cover the specimen with 1—2 drops of the Crystal Violet stain for 60 seconds, and gently wash off with slow running water. Cover the specimen with a few drops of Gram's iodine for 60 seconds and then gently wash off like for the crystal violet stain.
4. Tilt the slide a bit and drop the ethyl alcohol drop by drop in a way it covers the whole specimen. Drop until the running alcohol is no longer colored. Then, gently wash the specimen with running water.
5. Then, cover with safranin stain for 60 seconds, and then gently wash with water. Put a paper towel gently on the bacteria so it absorbs the water, and then put a cover slip on it.
6. Now, look under the microscope. Gram-positive cells would be purple; the retained the color. Gram-negative cells would be pink or red; the purple stain was washed away by the alcohol and replaced by the safranin stain. Label the petri-dish it was taken from.
7. Repeat the whole gram-staining process for the other petri-dish of bacteria.

<u>Antibiotic testing</u>

	1. Prepare the agar according to the instructions on the packaging. Pour until the bottom of 2 dishes are covered and quickly put the lids back on. Let them harden. 2. Sterilize the inoculating needle. Then take some of one gram-positive bacteria and lightly zigzag on both petri-dishes. 3. Put one type of each disc in the bacteria at a good distance away from each other in one petri-dish. Label this dish A1+. Label the dish without the antibiotics C1+. This is going to be your trial one controlled dish to compare with the trial one gram-positive trial one. 4. Repeat for the trial one gram-negative bacteria, replacing the plus signs with a minus sign. 5. Take a picture every 24 hours. Each day, measure the diameter of the circle the antibiotic disc has inhibited. Take down any qualitative observations. Compare the consecutive controlled dishes and note down similarities/differences. This is crucial for differences and explaining the effects of the antibiotics. 6. Repeat steps 1-5 of antibiotic testing for Trials 2-3 for both gram-negative and positive bacteria. Remember to replace the 1 with 2 and 3 for their consecutive trial #, and the positive/negative signs for gram-positive and negative bacteria. Don't forget the controlled slides of each trial. 7. After doing each trial and noting down observations for each, think and write final analysis concluding your results, and then do your conclusion with the answer to the problem, referring to your data doing so. 8. To dispose, put some bleach in it (powder or liquid), cover and seal, put it in a plastic bag, and throw away. This is a picture on how your trials should be like:

	
Sunday, November 26, 2023	*Today, I will be reviewing all the materials I will need before ordering. Ordering may be today, but most likely tomorrow on Monday, November 26, 2023. I will also propose data charts/tables for data collection.* Materials needed for the experiment: - Nutrient agar: For the bacterial growth. - Inoculating needle: Transplanting bacteria. - Cotton swabs: Collecting bacilli for growth. - Petri-dishes (10-20) (90x15mm): For testing.

- Growth kit: Ethyl alcohol, crystal violet stain, gram's iodine stain, safranin counter stain, dropper.
- Microscope slides
- Cover slips
- Antibiotic discs: Penicillin, Ampicillin, Neomycin, Ethromycin (24)
- Sharpie: Labeling Petri dishes
- Ruler: Measuring bacterial inhibition
- Tape: Sealing Petri dish lids
- Compound microscope: To see which are gram-negative and positive bacilli.
- Tweezers: To transplant the antibiotic discs
- Candle: To heat fix the specimen and sterilize inoculating needle.

This is the table for bacterial inhibition data.

	Trials	Trial 1	Trial 2	Trial 3	Controlled
Gram-positive bacilli (Bacterial growth inhibition in (cm))	Penicillin				
	Ampicillin				
	Neomycin				
	Erythromycin				
Gram-negative bacilli (Bacterial growth inhibition in (cm))	Penicillin				
	Ampicillin				
	Neomycin				
	Erythromycin				

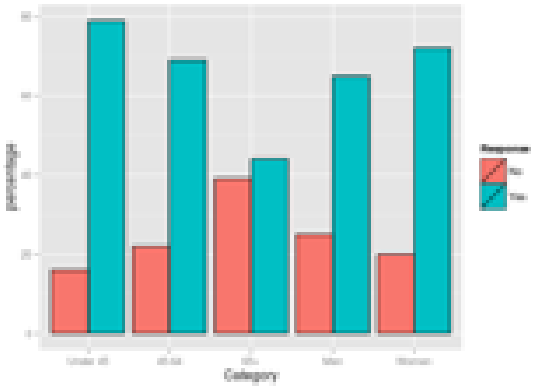
This is the exact same table, but for qualitative observations.

	Trials	Trial 1	Trial 2	Trial 3	Controlled

Gram-positive bacilli (Qualitative observations)	Penicillin				
	Ampicillin				
	Neomycin				
	Erythromycin				
Gram-negative bacilli (Qualitative observations)	Penicillin				
	Ampicillin				
	Neomycin				
	Erythromycin				

**For the quantitative observations (diameter of inhibition growth), I will represent it in a double bar graph. For the qualitative observations, I will keep it in a table like the ones above.*

Similar bar graph I will use for the quantitative observations.



**Remember, I will also have pictures of the bacterial growth and staining process!*

<table>
<tr>
<td>Monday, November 27, 2023</td>
<td>
Today, I will review materials needed for final order (today or tomorrow), off amazon and home science tools. I will also create tables for observations for the experiment.

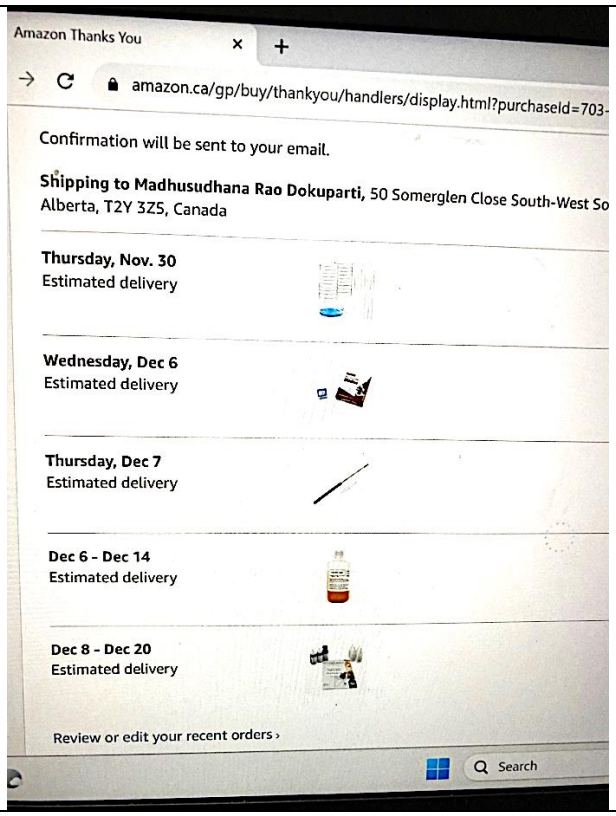
<u>Observations:</u>

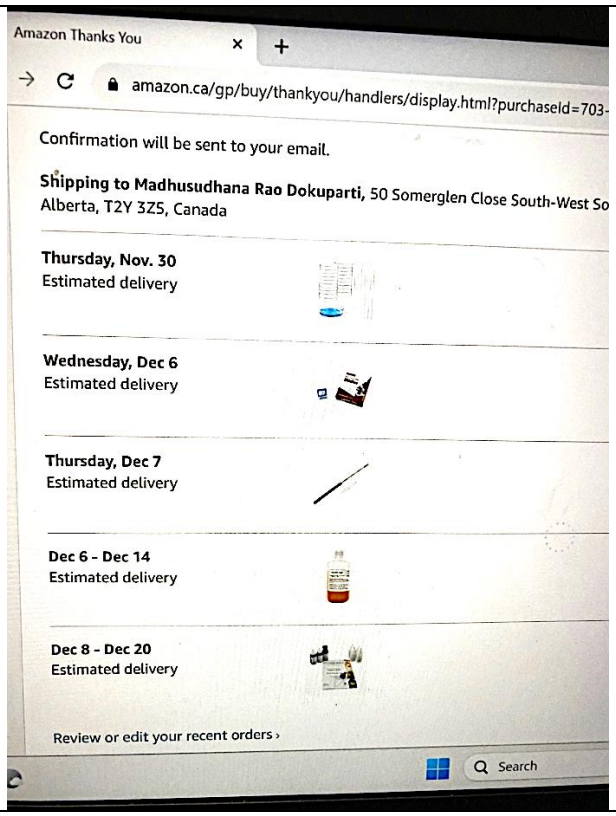
Observations table of substances
(Before the staining and experimenting)

<table>
<tr><td rowspan="6">Observations of the substances before experiment</td><td>Gram-positive bacteria</td><td></td></tr>
<tr><td>Gram-negative bacteria</td><td></td></tr>
<tr><td>Crystal Violet Stain</td><td></td></tr>
<tr><td>Gram’s Iodine</td><td></td></tr>
<tr><td>Safranin Counter Stain</td><td></td></tr>
<tr><td>Ethyl Alcohol</td><td></td></tr>
</table>

Observations of the close-up bacteria (microscope)
(During the staining process)

<table>
<tr><td rowspan="2">Observations of microscope bacteria</td><td>Gram-positive bacteria</td><td></td></tr>
<tr><td>Gram-negative bacteria</td><td></td></tr>
</table>
</td>
</tr>
<tr>
<td>Tuesday, November 28, 2023</td>
<td>Today, the materials (not including the antibiotic discs) were ordered of amazon:</td>
</tr>
</table>

	
Thursday, November 30, 2023	*Today, the Petri dishes came.*
Saturday, December 2, 2023	*Today, we will be going to local pharmacies to see if we can find antibiotic disc sets. This is because the shipping from U.S. costs so much with conversion fees, shipping fees internationally, etc.* *We came back after 2 hours, and we could not find the antibiotic disc sets anywhere at the pharmacies. Now, we are planning to see if our family doctor can write us a prescription.*
Sunday, December 3, 2023	*We realized that we cannot get the antibiotics without a medical use and prescription. So, we just went forth with the U.S. website and ordered it. It is to come at the latest of January 3, 2023.* *Here are dates for the other materials that I got of amazon:*

	
Monday, December 4, 2023	***Today, the microscope slides with the cover slips came!***
Tuesday, December 5, 2023	*Today, the inoculating needle and gram-stain kit came. I have opened the kit, and then saw the safety data sheets. Skimming and scamming, gram's iodine is corrosive, safranin and crystal violet stain are general storage, and ethyl alcohol is flammable.* - *To store the chemicals, I will put the bottles in a glass bottle to contain the chemicals for safety, and then put them in a box with small holes for protection from light and ventilation in the basement to keep them cool.*
Sunday, December 10, 2023	*Today, I will be searching and writing down the SDS and statements for all the chemicals I will be using from the Gram's Stain Kit. Any additional information to clarify somethings or make them clearer, I will do so as well.* *SDS for chemicals below:* *Crystal Violet Stain* | *SDS Statements* | *SDS Symbols*

	(General Storage)	+ May be harmful if swallowed. + Causes eye irritation. + Suspected of causing cancer. + Toxic to aquatic life. + Toxic to aquatic life with long lasting effects.	
	Gram's Iodine (Corrosive storage)	*SDS Statements* + Harmful in contact with skin. + Harmful if inhaled. + Very toxic to aquatic life.	*SDS Symbols* 
	Ethyl Alcohol (Flammable storage)	*SDS Statements* + Highly flammable liquid and vapor. + Toxic if swallowed. + Toxic in contact with skin. + Causes serious eye irritation. + Toxic if inhaled. + May cause drowsiness or dizziness. + May cause damage to organs.	*SDS Symbols* 
	Safranin 0 Counter Stain (General Storage)	*SDS Statements* + Causes eye irritation.	*SDS Symbols* *None assigned.

<table>
<tr><td></td><td>
<table>
<tr><td></td><td></td><td>*I assigned this for irritation:
  </td></tr>
</table>

Those chemicals that damage organs can also damage reproductive organs, meaning you can’t have kids.
</td></tr>
<tr><td>December 16, 2023</td><td>Today, the antibiotic disks arrived.</td></tr>
<tr><td>December 29, 2023</td><td>Today, I am starting the collection of the bacterial growth. Before I do so though, I fear the chemicals, so I will go to the MSDS that came in paper copy with the chemicals to see how it should be disposed.

*The MSDS does not show disposal methods. I will research this with federal regulations online.

<table>
<tr><th>Chemicals/Substances</th><th>How to Dispose:</th></tr>
<tr><td>Ethyl Alcohol</td><td>Cannot be diluted and poured down the drain. Sealed and labeled in leak-proof container and disposed through hazardous waste disposal program.</td></tr>
<tr><td>Crystal Violet Stain</td><td>Sealed and labeled in leak-proof container and disposed through hazardous waste disposal program.</td></tr>
<tr><td>Safranin 0 Counter.</td><td>Sealed and labeled in leak-proof container and disposed through hazardous waste disposal program.</td></tr>
<tr><td>Gram’s Iodine</td><td>Sealed and labeled in leak-proof container and disposed through hazardous waste disposal program.</td></tr>
<tr><td>Mixed Nutrient Agar</td><td>Garbage after neutralized with bleach because it would have bacteria on it.</td></tr>
</table>

I am getting the Gram-Positive bacteria from a mix of my skin and the dry kitchen counters. I am getting the Gram-Negative bacteria from the 2 kitchen sinks. [Gram-Positive thrive in drier, more nutrient-rich places. Gram-Negative thrive in more damp, dirty places.]

Here are the pictures of Day 1 for the bacterial growth. I am also creating a new data table to collect qualitative observations of each bacterial growth from Day’s 1-3 because that is average time bacteria that needs to be tested</td></tr>
</table>

<table>
<tr>
<td></td>
<td>
takes to grow. There are 4 days in total; one of them is for the day I start the bacteria collection.
<table>
<tr><th>Bacteria:</th><th>Days #:</th><th>Qualitative Observations:</th></tr>
<tr><td rowspan="4">Kitchen Counter and Skin</td><td>Day 1</td><td></td></tr>
<tr><td>Day 2</td><td></td></tr>
<tr><td>Day 3</td><td></td></tr>
<tr><td>Day 4</td><td></td></tr>
<tr><td rowspan="4">2 Kitchen Sinks</td><td>Day 1</td><td></td></tr>
<tr><td>Day 2</td><td></td></tr>
<tr><td>Day 3</td><td></td></tr>
<tr><td>Day 4</td><td></td></tr>
</table>
<table>
<tr><th>Bacteria</th><th>Pictures</th></tr>
<tr><td>Day 1 – KC & S</td><td></td></tr>
<tr><td>Day 1 – 2KS</td><td></td></tr>
<tr><td>Day 2 – KC & S</td><td></td></tr>
<tr><td>Day 2 – 2KS</td><td></td></tr>
<tr><td>Day 3 – KC & S</td><td></td></tr>
<tr><td>Day 3 - 2KS</td><td></td></tr>
<tr><td>Day 4 – KC & S</td><td></td></tr>
<tr><td>Day 4 – 2KS</td><td></td></tr>
</table>
Links:
https://ehs.stanford.edu/wp-content/uploads/19-157_Ethanol-Fact-Sheet_Final.pdf
</td>
</tr>
<tr>
<td>December 30, 2023</td>
<td>
Today, for the extra extension, I will give physical and chemical properties of the chemicals!
As I was researching on how to dispose chemicals, I came across the hazardous waste disposal program. I will explain my findings for the current regulations in Calgary.

The “Hazardous Waste Disposal Program” is a program where citizens can come to drop of hazardous waste. There are different drop-off locations situated in Calgary. They are mostly fire stations.
When dropping off the hazardous waste, they should be in compatible containers—ones that don’t break to the substance’s properties—and should be clearly labeled on what it is, in legible handwriting. All these safety measures are taken to protect the workers, families, other humans, and the environment.

</td>
</tr>
</table>

- After the hazardous waste is dropped off, the workers categorize the chemicals and send them to bigger waste disposers in Canada and the United States who treat or recycle the chemicals.

    - I must get rid of the waste chemicals after the gram-staining to these hazardous waste disposal programs because it is illegal and dangerous to pour them down the drain.
    - Even if I were to dilute the chemicals to very low concentrations, it would still be illegal, so it is essential I give it to the waste disposal programs.
    - I would dilute the waste chemicals and send it to them because it would be safer to handle with lower risks of putting lives in danger.

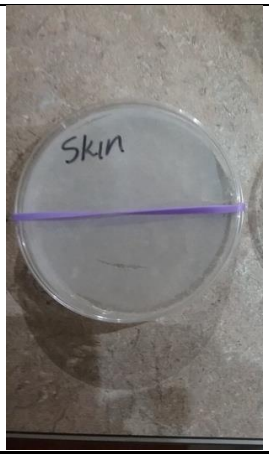
- One of the chemicals I have, Ethanol, is an accepted chemical by the disposal program. It is a photographic chemical, having history with photography, such as for its cleaning properties.
- The other 3 chemicals were neither one that are accepted or not accepted, so I assume it would be accepted because there is also a section for corrosive, flammable, or irritant chemicals to go.

Chemicals:	Properties:
95% Denatured Ethanol	Physical: Melt/Freeze – (-114 °C) Boiling – (74-80 °C)
	Chemical: Flash point – 5 °C pH – not available data
Crystal Violet Stain	Physical: Melt/Freeze – (0 °C) Boiling – (100 °C)
	Chemical: Flash point – none pH – data not available
Gram's Iodine	Physical: Melt/Freeze – (-0°C) Boiling – (-100°C)
	Chemical: pH – data not available Flash point – not flammable
Safranin 0 Counterstain	Physical: Melt/Freeze – (- 0°C) Boiling – (-100°C)
	Chemical: pH – data not available Flash point – data not available

- pH of a substance can be determined by using universal indicator. If the color is red, orange, or yellow, it is acidic. If it is green, it is neutral. If it is blue, or purple/pink, it is a base.

I am going to organize all my data tables so I can see them all at once:

Bacteria:	**Days #:**	**Qualitative Observations:**
Kitchen Counter and Skin	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
	Day 2	
	Day 3	
	Day 4	
2 Kitchen Sinks	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
	Day 2	
	Day 3	
	Day 4	

Bacteria	**Pictures**
Day 1 – KC & S	

Day 1 – 2KS	
Day 2 – KC & S	
Day 2 – 2KS	
Day 3 – KC & S	
Day 3 - 2KS	
Day 4 – KC & S	
Day 4 – 2KS	

	Trials	Trial 1 (diameter of inhibition in cm)	Trial 2 (diameter of inhibition in cm)	Trial 3 (diameter of inhibition in cm)	Controlled
Gram-positive bacilli (Bacterial growth inhibition in (cm))	Penicillin				
	Ampicillin				
	Neomycin				
	Erythromycin				
Gram-negative bacilli (Bacterial growth inhibition in (cm))	Penicillin				
	Ampicillin				
	Neomycin				
	Erythromycin				

	Trials	Trial 1 (qualitative observations)	Trial 2 (qualitative observations)	Trial 3 (qualitative observations)	Controlled (qualitative observations)
Gram-positive bacilli (Qualitative observations)	Penicillin				
	Ampicillin				
	Neomycin				
	Erythromycin				
Gram-negative bacilli (Qualitative observations)	Penicillin				
	Ampicillin				
	Neomycin				
	Erythromycin				

Observations of the substances before experiment	Gram-positive bacteria	
	Gram-negative bacteria	
	Crystal Violet Stain	
	Gram's Iodine	
	Safranin Counter Stain	
	Ethyl Alcohol	
	Solid agar	Foggy, transluscent solid with a little bit of liquid running on top.

		Light yellow with a few chunks of brown here and there. Odor could not be detected.
	Liquid/melted agar. [Hot water bath method]	Thick, viscous liquid with a lot of bubbles within. Richer yellow that is more translucent. Hardening starts within 1 minute. Took 30 mins to fully melt. Odor could not be detected.

Observations of microscope bacteria after gram-staining	Gram-positive bacteria	
	Gram-negative bacteria	

Today, I have melted the agar and poured it into the Petri dishes. I wore all my protective gear, and I melted it using the hot water bath method, which is putting it on a pan on a stove and bringing the water to a boil. I stood for 30 mins for it to fully melt, and some safety measures I took were using an exhaust fan so the fumes would not go around the whole house, lab goggles, so the liquid could not accidently get into my eyes, and gloves, to protect my skin from heat or any accidental spills. When pouring in the agar, I made sure it covered just the bottom and immediately put the lid back on to avoid contamination from unwanted bacteria in the air.

{I am creating a separate book/document to hold my evidence!}

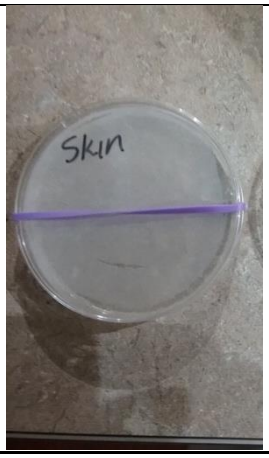
I also collected the bacteria samples and swabbed them on the Petri dishes that have the hardened liquid agar. I collected one from both kitchen sinks and the drainers which were dirty, while the another was from the human skin. I collected it from my family, such as their backs and hands. Now, I will wait for a few days for it to grow.

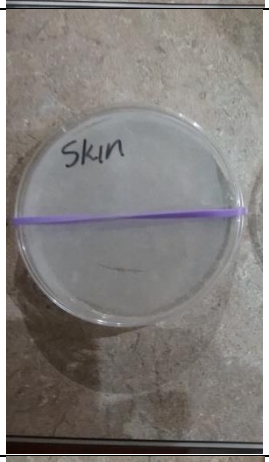
Links:
https://www.calgary.ca/waste/residential/household-hazardous-waste-drop-off-program.html
https://www.calgary.ca/waste/residential/household-hazardous-waste-accepted-materials.html
https://chat.openai.com/c/69f08bf4-8749-4bc5-95c9-104adbe537c5

December 31, 2023

Today, I will be continuing my observations on the bacterial growth.

Bacteria:	**Days #:**	**Qualitative Observations:**
Kitchen Counter and Skin	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
	Day 2	Clearer, translucent agar seen. No signs of bacterial growth yet, though some very faint, light green spots seen.
	Day 3	
	Day 4	
2 Kitchen Sinks	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
	Day 2	Clearer, translucent agar seen. No signs of bacterial growth.
	Day 3	
	Day 4	

Bacteria	**Pictures**
Day 1 – KC & S	

	Day 1 – 2KS	
	Day 2 – KC & S	
	Day 2 – 2KS	
	Day 3 – KC & S	
	Day 3 - 2KS	
	Day 4 – KC & S	
	Day 4 – 2KS	

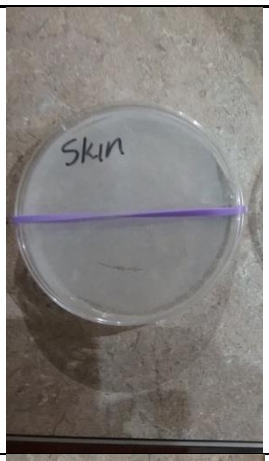
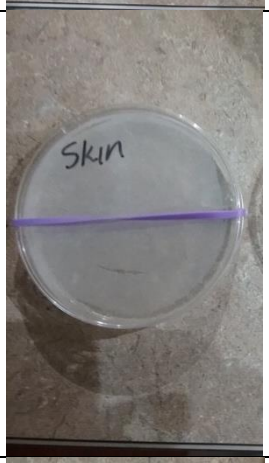
January 1, 2024

Today, I will continue my observations on bacterial growth. Heads up: Just to make sure I am doing right entries on right days; I am re-pasting the data tables for bacterial growth again and again for new data.

Bacteria:	**Days #:**	**Qualitative Observations:**
Kitchen Counter and Skin	Day 1	Just clear, light yellow, translucent

		agar seen. No bacteria yet.
Day 2	Clearer, translucent agar seen. No signs of bacterial growth yet, though some very faint, light green spots seen.	
Day 3	A little bit of white spots growing on agar. Agar is still translucent, with a fainter light-yellow tint. Small signs of bacterial growth; not dense bacteria yet.	
Day 4		
2 Kitchen Sinks	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
Day 2	Clearer, translucent agar seen. No signs of bacterial growth.	
Day 3	Lots of formed small, white bumps on agar. Agar is now no longer translucent, but closer to opaque, a bit foggier. Light yellow tint gone; more dense white bumps seen.	
Day 4		

Bacteria	**Pictures**

	Day 1 – KC & S		
	Day 1 – 2KS		
	Day 2 – KC & S		
	Day 2 – 2KS		

	Day 3 – KC & S	
	Day 3 - 2KS	
	Day 4 – KC & S	
	Day 4 – 2KS	

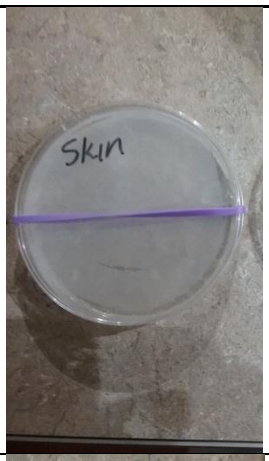
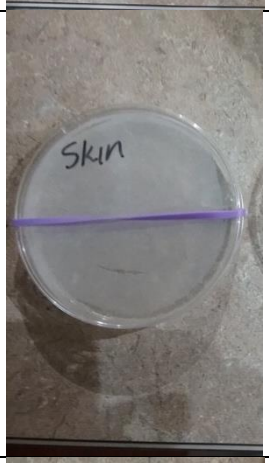
January 2, 2024

Again, I am continuing my bacterial growth data observations. I might need to add a few extra days to the data table for it. 2 days for now!

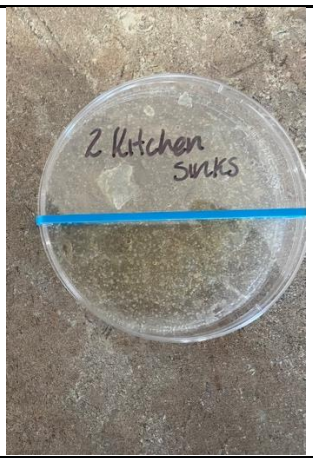
Bacteria:	**Days #:**	**Qualitative Observations:**
Kitchen Counter and Skin	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
	Day 2	Clearer, translucent agar seen. No signs of bacterial growth yet, though some very faint, light green spots seen.
	Day 3	A little bit of white spots growing on agar.

		Agar is still translucent, with a fainter light-yellow tint. Small signs of bacterial growth; not dense bacteria yet.
	Day 4	A little more dense white spots that are small on liquid agar. A little bit of faint, light yellow tint still present. More clear signs of bacterial growth.
	Day 5	
	Day 6	
2 Kitchen Sinks	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
	Day 2	Clearer, translucent agar seen. No signs of bacterial growth.
	Day 3	Lots of formed small, white bumps on agar. Agar is now no longer translucent, but closer to opaque, a bit foggier. Light yellow tint gone; more dense white bumps seen.
	Day 4	Even more dense white, small spots on agar. More opaque, very little translucence left. Small bacteria culture starting (indicating much more bacteria in the sink).
	Day 5	
	Day 6	

Bacteria	**Pictures**

	Day 1 – KC & S		
	Day 1 – 2KS		
	Day 2 – KC & S		
	Day 2 – 2KS		

	Day 3 – KC & S		
	Day 3 - 2KS		
	Day 4 – KC & S		

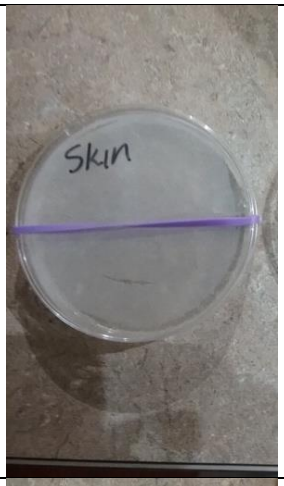
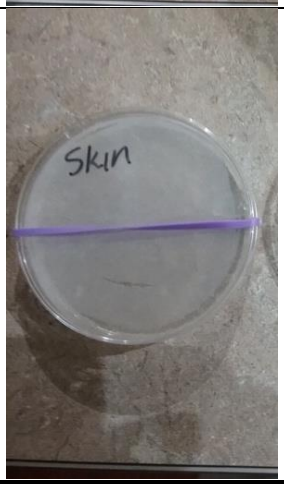
	Day 4 – 2KS	
	Day 5 – KC & S	
	Day 5 – 2KS	
	Day 6 – KC & S	
	Day 6 – 2KS	

Wednesday, January 3, 2024

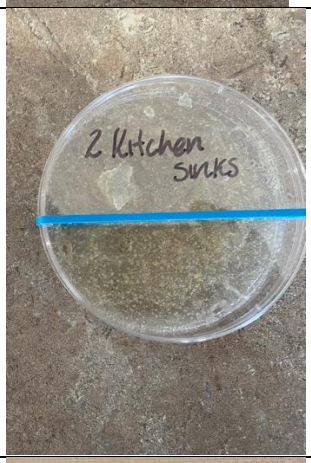
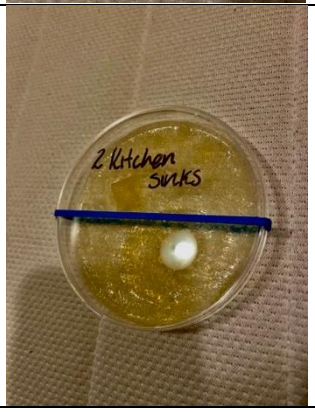
Today, I will be continuing my observations on the bacteria growth.

Bacteria:	**Days #:**	**Qualitative Observations:**
Kitchen Counter and Skin	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
	Day 2	Clearer, translucent agar seen. No signs of bacterial growth yet, though some very faint, light green spots seen.
	Day 3	A little bit of white spots growing on agar. Agar is still translucent, with a fainter light-yellow tint. Small signs of bacterial growth; not dense bacteria yet.
	Day 4	A little more dense white spots that are small on liquid agar. A little bit of faint, light yellow tint still present. More clear

			signs of bacterial growth.
		Day 5	Bacteria is growing, covering the whole inside of the Petri dish with bacteria. Color is changing to a light grey with a few green spots, and culture is starting to form. More bacteria than Day 4.
		Day 6	
	2 Kitchen Sinks	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
		Day 2	Clearer, translucent agar seen. No signs of bacterial growth.
		Day 3	Lots of formed small, white bumps on agar. Agar is now no longer translucent, but closer to opaque, a bit foggier. Light yellow tint gone; more dense white bumps seen.
		Day 4	Even more dense white, small spots on agar. More opaque, very little translucence left. Small bacteria culture starting (indicating much more bacteria in the sink).
		Day 5	Bacteria is denser as more bacteria is growing. More white spots indicating more bacterial growth. As culture is getting denser, the color is changing to a light green. Predicting fully grown culture will be a green color. More bacteria than Day 4.
		Day 6	

Bacteria	Pictures
Day 1 – KC & S	
Day 1 – 2KS	
Day 2 – KC & S	

	Day 2 – 2KS		
	Day 3 – KC & S		
	Day 3 - 2KS		

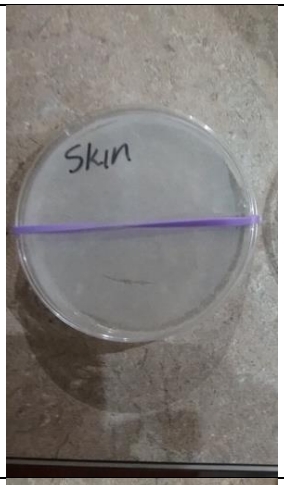
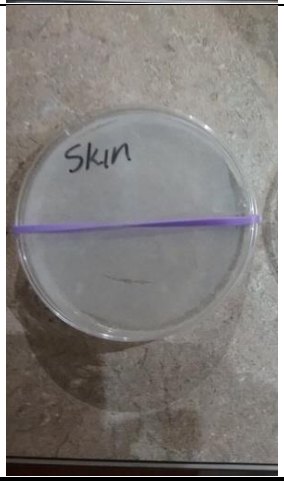
	Day 4 – KC & S	
	Day 4 – 2KS	
	Day 5 – KC & S	
	Day 5 – 2KS	
	Day 6 – KC & S	

	Day 6 – 2KS	
Thursday, January 4, 2024	*Today, I am doing more observations on my bacterial growth. I predict, before checking bacteria, that I might need 1 more day for it to grow.*	

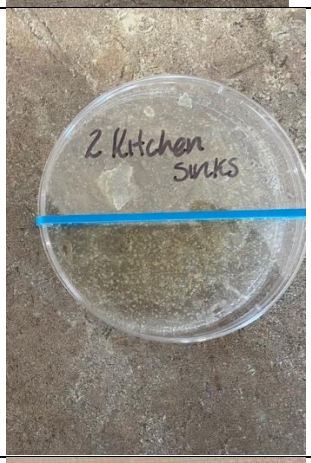
Bacteria:	**Days #:**	**Qualitative Observations:**
Kitchen Counter and Skin	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
	Day 2	Clearer, translucent agar seen. No signs of bacterial growth yet, though some very faint, light green spots seen.
	Day 3	A little bit of white spots growing on agar. Agar is still translucent, with a fainter light-yellow tint. Small signs of bacterial growth; not dense bacteria yet.
	Day 4	A little more dense white spots that are small on liquid agar. A little bit of faint, light yellow tint still present. More clear signs of bacterial growth.
	Day 5	Bacteria is growing, covering the whole inside of the Petri dish with bacteria. Color is changing to a light grey with a few green spots, and culture is starting to form. More bacteria than Day 4.
	Day 6	A few more new white spots spotted, meaning bacteria is growing well. Some white spots started to turn

			green, meaning bacteria culture is starting and close to fully grown. Little translucence is left. Predicting whole bacteria culture is going to be green.
		Day 7	
	2 Kitchen Sinks	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
		Day 2	Clearer, translucent agar seen. No signs of bacterial growth.
		Day 3	Lots of formed small, white bumps on agar. Agar is now no longer translucent, but closer to opaque, a bit foggier. Light yellow tint gone; more dense white bumps seen.
		Day 4	Even more dense white, small spots on agar. More opaque, very little translucence left. Small bacteria culture starting (indicating much more bacteria in the sink).
		Day 5	Bacteria is denser as more bacteria is growing. More white spots indicating more bacterial growth. As culture is getting denser, the color is changing to a light green. Predicting fully grown culture will be a green color. More bacteria than Day 4.
		Day 6	Hard to tell if new white spots were grown due to dense bacteria already

		starting. Some parts of the bacteria are turning light pea green—a color between green and yellow. Very little translucence is left.
	Day 7	

Bacteria	Pictures
Day 1 – KC & S	
Day 1 – 2KS	
Day 2 – KC & S	

	Day 2 – 2KS		
	Day 3 – KC & S		
	Day 3 - 2KS		

	Day 4 – KC & S		
	Day 4 – 2KS		
	Day 5 – KC & S		
	Day 5 – 2KS		

	Day 6 – KC & S	
	Day 6 – 2KS	
	Day 7 – KC & S	
	Day 7 – 2KS	

Friday, January 5, 2024

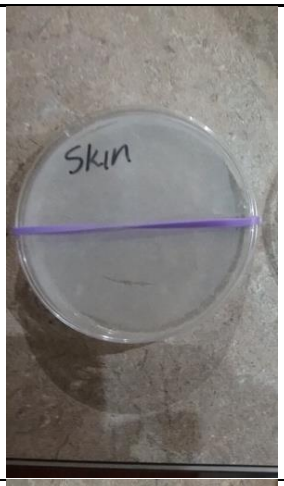
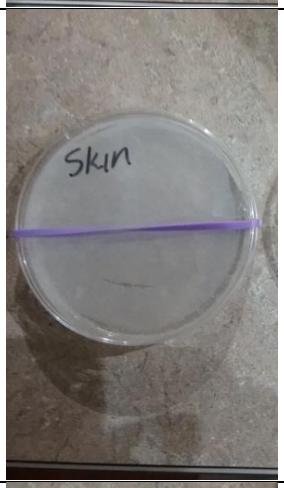
Today, I am continuing observations on my bacteria. I will add one more day for bacterial growth. I will do gram-staining on Sunday!

Bacteria:	**Days #:**	**Qualitative Observations:**
Kitchen Counter and Skin	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
	Day 2	Clearer, translucent agar seen. No signs of bacterial growth yet, though some very faint, light green spots seen.
	Day 3	A little bit of white spots growing on agar. Agar is still translucent, with a fainter light-yellow tint. Small signs of

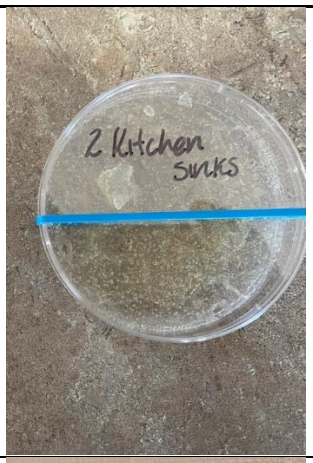
			bacterial growth; not dense bacteria yet.
		Day 4	A little more dense white spots that are small on liquid agar. A little bit of faint, light yellow tint still present. More clear signs of bacterial growth.
		Day 5	Bacteria is growing, covering the whole inside of the Petri dish with bacteria. Color is changing to a light grey with a few green spots, and culture is starting to form. More bacteria than Day 4.
		Day 6	A few more new white spots spotted, meaning bacteria is growing well. Some white spots started to turn green, meaning bacteria culture is starting and close to fully grown. Little translucence is left. Predicting whole bacteria culture is going to be green.
		Day 7	More bacteria is turning green, meaning the culture is maturing. More bacteria spotted, meaning conditions are good for bacterial growth.
	2 Kitchen Sinks	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
		Day 2	Clearer, translucent agar seen. No signs of bacterial growth.

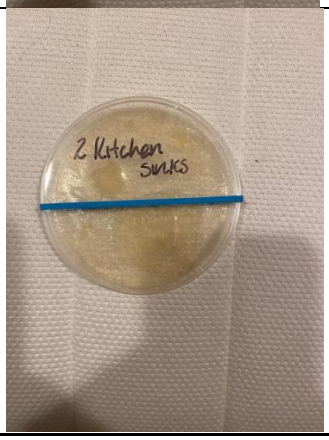
Day 3	Lots of formed small, white bumps on agar. Agar is now no longer translucent, but closer to opaque, a bit foggier. Light yellow tint gone; more dense white bumps seen.
Day 4	Even more dense white, small spots on agar. More opaque, very little translucence left. Small bacteria culture starting (indicating much more bacteria in the sink).
Day 5	Bacteria is denser as more bacteria is growing. More white spots indicating more bacterial growth. As culture is getting denser, the color is changing to a light green. Predicting fully grown culture will be a green color. More bacteria than Day 4.
Day 6	Hard to tell if new white spots were grown due to dense bacteria already starting. Some parts of the bacteria are turning light pea green—a color between green and yellow. Very little translucence is left.
Day 7	The bacteria looks the same as yesterday, although less color was seen. At this point, it looks like bacteria culture color is going to be light grey.

Bacteria	**Pictures**

	Day 1 – KC & S		
	Day 1 – 2KS		
	Day 2 – KC & S		
	Day 2 – 2KS		

	Day 3 – KC & S			
	Day 3 - 2KS			
	Day 4 – KC & S			

	Day 4 – 2KS		
	Day 5 – KC & S		
	Day 5 – 2KS		
	Day 6 – KC & S		

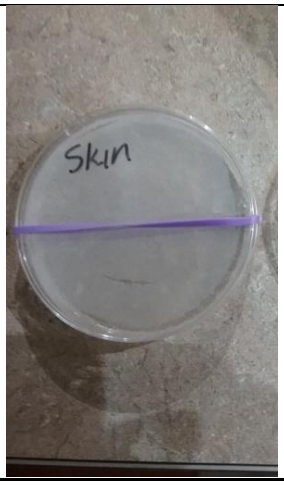
	Day 6 – 2KS		
	Day 7 – KC & S		
	Day 7 – 2KS		
Saturday, January 6, 2024	*Today, I am continuing bacterial growth observations!*		

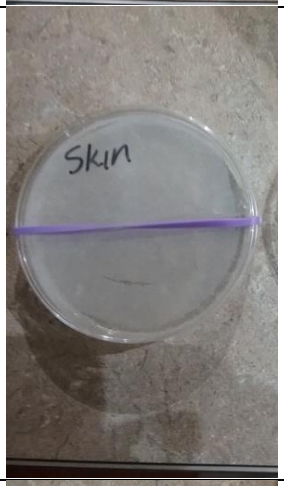
Bacteria:	**Days #:**	**Qualitative Observations:**
Kitchen Counter and Skin	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
	Day 2	Clearer, translucent agar seen. No signs of bacterial growth yet, though some very

			faint, light green spots seen.
		Day 3	A little bit of white spots growing on agar. Agar is still translucent, with a fainter light-yellow tint. Small signs of bacterial growth; not dense bacteria yet.
		Day 4	A little more dense white spots that are small on liquid agar. A little bit of faint, light yellow tint still present. More clear signs of bacterial growth.
		Day 5	Bacteria is growing, covering the whole inside of the Petri dish with bacteria. Color is changing to a light grey with a few green spots, and culture is starting to form. More bacteria than Day 4.
		Day 6	A few more new white spots spotted, meaning bacteria is growing well. Some white spots started to turn green, meaning bacteria culture is starting and close to fully grown. Little translucence is left. Predicting whole bacteria culture is going to be green.
		Day 7	More bacteria is turning green, meaning the culture is maturing. More bacteria spotted, meaning conditions are good for bacterial growth.

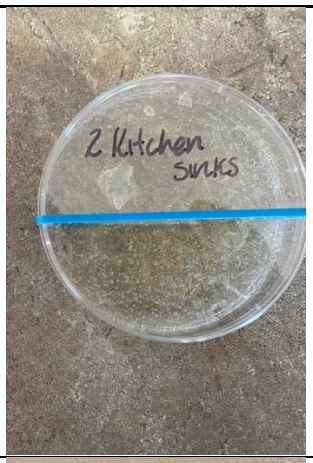
		Day 8	More green bacteria spotted. Simultaneously, while green bacteria was forming, more younger, white bacteria spots were seen! Bacteria growth is doing very well!
	2 Kitchen Sinks	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
		Day 2	Clearer, translucent agar seen. No signs of bacterial growth.
		Day 3	Lots of formed small, white bumps on agar. Agar is now no longer translucent, but closer to opaque, a bit foggier. Light yellow tint gone; more dense white bumps seen.
		Day 4	Even more dense white, small spots on agar. More opaque, very little translucence left. Small bacteria culture starting (indicating much more bacteria in the sink).
		Day 5	Bacteria is denser as more bacteria is growing. More white spots indicating more bacterial growth. As culture is getting denser, the color is changing to a light green. Predicting fully grown culture will be a green color. More bacteria than Day 4.
		Day 6	Hard to tell if new white spots were grown due to dense bacteria already

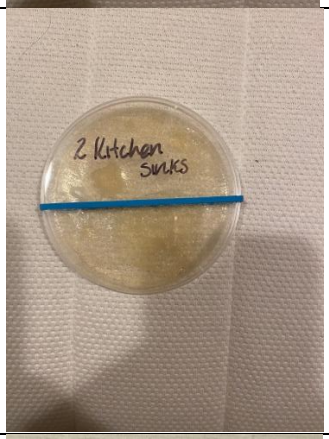
		starting. Some parts of the bacteria are turning light pea green—a color between green and yellow. Very little translucence is left.
	Day 7	The bacteria looks the same as yesterday, although less color was seen. At this point, it looks like bacteria culture color is going to be light grey.
	Day 8	The bacteria is small, white, but dense. Today, the bacteria looked very populated! This means bacteria is doing well! A small area on the petri dish also looked very green again today!

Bacteria	**Pictures**
Day 1 – KC & S	

	Day 1 – 2KS		
	Day 2 – KC & S		
	Day 2 – 2KS		

	Day 3 – KC & S			
	Day 3 - 2KS			
	Day 4 – KC & S			

	Day 4 – 2KS		
	Day 5 – KC & S		
	Day 5 – 2KS		
	Day 6 – KC & S		

	Day 6 – 2KS		
	Day 7 – KC & S		
	Day 7 – 2KS		
	Day 8 – KC & S		

	Day 8 – 2KS	

Sunday, January 7, 2024

Today, I am continuing bacterial growth observations! I will do the gram-staining process today as well!

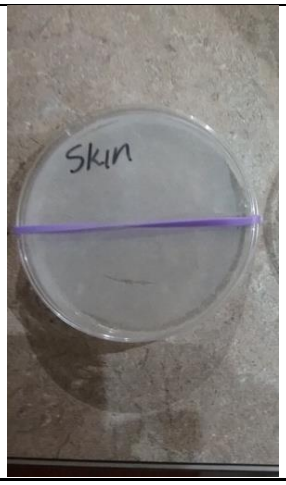
- Today, I found out why I need to do gram-staining.
- Bacteria from the same environment can vary hugely on species, and therefore, their size and other factors are affected.
- Because of this, it is important to do gram-staining

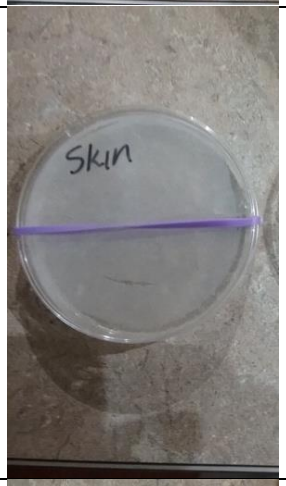
Bacteria:	**Days #:**	**Qualitative Observations:**
Kitchen Counter and Skin	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
	Day 2	Clearer, translucent agar seen. No signs of bacterial growth yet, though some very faint, light green spots seen.
	Day 3	A little bit of white spots growing on agar. Agar is still translucent, with a fainter light-yellow tint. Small signs of bacterial growth; not dense bacteria yet.
	Day 4	A little more dense white spots that are small on liquid agar. A little bit of faint, light yellow tint still present. More clear

			signs of bacterial growth.
		Day 5	Bacteria is growing, covering the whole inside of the Petri dish with bacteria. Color is changing to a light grey with a few green spots, and culture is starting to form. More bacteria than Day 4.
		Day 6	A few more new white spots spotted, meaning bacteria is growing well. Some white spots started to turn green, meaning bacteria culture is starting and close to fully grown. Little translucence is left. Predicting whole bacteria culture is going to be green.
		Day 7	More bacteria is turning green, meaning the culture is maturing. More bacteria spotted, meaning conditions are good for bacterial growth.
		Day 8	More green bacteria spotted. Simultaneously, while green bacteria was forming, more younger, white bacteria spots were seen! Bacteria growth is doing very well!
		Day 9	Again, more green bacteria were seen. Today, some of the green was starting to grow taller. It was thicker. It is looking

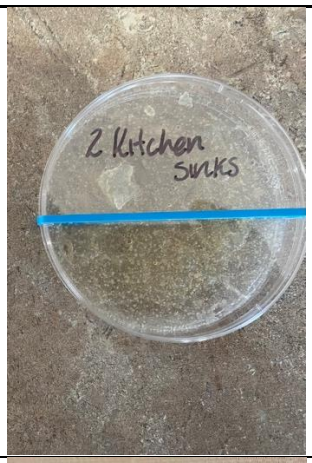
			like the bacteria is going to be green.
	2 Kitchen Sinks	Day 1	Just clear, light yellow, translucent agar seen. No bacteria yet.
		Day 2	Clearer, translucent agar seen. No signs of bacterial growth.
		Day 3	Lots of formed small, white bumps on agar. Agar is now no longer translucent, but closer to opaque, a bit foggier. Light yellow tint gone; more dense white bumps seen.
		Day 4	Even more dense white, small spots on agar. More opaque, very little translucence left. Small bacteria culture starting (indicating much more bacteria in the sink).
		Day 5	Bacteria is denser as more bacteria is growing. More white spots indicating more bacterial growth. As culture is getting denser, the color is changing to a light green. Predicting fully grown culture will be a green color. More bacteria than Day 4.
		Day 6	Hard to tell if new white spots were grown due to dense bacteria already starting. Some parts of the bacteria are turning light pea green—a color between green and yellow. Very little translucence is left.

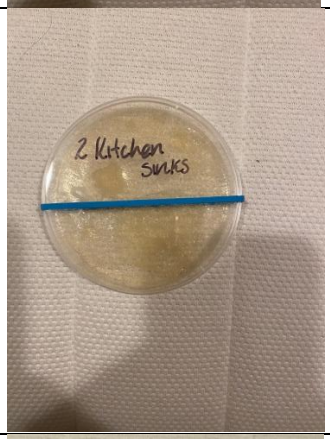
Day 7	The bacteria look the same as yesterday, although less color was seen. At this point, it looks like bacteria culture color is going to be light grey.
Day 8	The bacteria are small, white, but dense. Today, the bacteria looked very populated! This means bacteria is doing well! A small area on the petri dish also looked very green again today!
Day 9	The bacteria look the same as yesterday. Very small, grey/white, and dense/populating. Today, in the light, whole culture looked faint green.

Bacteria	**Pictures**
Day 1 – KC & S	

	Day 1 – 2KS			
	Day 2 – KC & S			
	Day 2 – 2KS			

	Day 3 – KC & S			
	Day 3 - 2KS			
	Day 4 – KC & S			

	Day 4 – 2KS		
	Day 5 – KC & S		
	Day 5 – 2KS		
	Day 6 – KC & S		

	Day 6 – 2KS		
	Day 7 – KC & S		
	Day 7 – 2KS		
	Day 8 – KC & S		

Day 8 – 2KS		
Day 9 – KC & S		
Day 9 – 2KS		

Observations of the substances before experiment	Gram-positive bacteria	Again, more green bacteria were seen. Today, some of the green was starting to grow taller. It was thicker. It is looking like the bacteria is going to be green.
	Gram-negative bacteria	The bacteria look the same as yesterday.

		Very small, grey/white, and dense/populating. Today, in the light, whole culture looked faint green.
	Crystal Violet Stain	Runny, purple liquid with a few small bubbles in. Very low viscosity.
	Gram's Iodine	Slightly low viscosity, light red/magenta liquid. Occasional bubbles seen.
	Safranin Counter Stain	Very viscous, thick dark-red liquid with a lot of bubbles in it.
	Ethyl Alcohol	Clear, colorless low viscosity liquid. Runny!
	Solid agar	Foggy, translucent solid with a little bit of liquid running on top. Light yellow with a few chunks of brown here and there. Odor could not be detected.
	Liquid/melted agar. [Hot water bath method]	Thick, viscous liquid with a lot of bubbles within. Richer yellow that is more translucent. Hardening starts within 1 minute. Took 30 mins to fully melt. Odor could not be detected.

Observations of microscope bacteria after gram-staining	Skin (Gram-positive)	Very faint light red/pink. Lots of blots of purple seen. This is the skin bacteria. Meandering, longer lines seen. Microscope not powerful enough to see all bacteria

			cells. This is gram-positive bacteria.
		2 Kitchen Sinks (Gram negative)	No faint purples. All light orange/pink and some small occasional blots of red. Small, black outlined lines seen. Could be bacteria. Microscope not powerful enough to see all bacteria cells. This is gram-negative bacteria.

The gram-staining process took a total of 1 hour 35 mins! It was very cold because I needed a ventilated area, so I did it in the garage with the garage door open in winter weather. I took precautions so chemicals can maintain their functions, such as using a heater beside my gram-staining process. Still, the bacteria froze a little and some liquid—fresh water—immediately froze on the table I was working on. Some more precautions I took were lab goggles, thick layers with full sleeves and closed toe shoes, splash resistant gloves, and a mask so I do not breath in the chemical vapors.

Monday, January 8, 2024

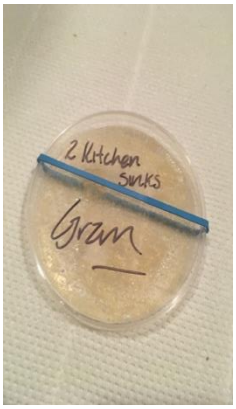
Today, I am doing more observations for the bacterial growth.

- Because I had to use the bacteria I grew for the gram-staining, I am letting it grow back a bit so I can do the next phase where I transplant some bacteria to other petri dishes.
- Also, because I did gram-staining for the first time, I had to use a bit more bacteria from the petri dishes due to me messing up. Especially in the cold.

Here are observations of the bacteria that is growing back from the gram-staining:

Gram-Positive Bacteria	Picture (Day 1 of regrowing)	Observations (Day 1 of regrowing)
		There are less bacteria due to gram-staining. But there are more bacteria than yesterday when I finished gram-staining, meaning bacteria is re-growing fast! A few new green spots were seen, which were thick, meaning bacteria culture is healthy and doing well.

Gram-Negative Bacteria	Picture (Day 1 of regrowing)	Observations (Day 1 of regrowing)

			Like gram-positive, there is less, but more than when I finished gram-staining yesterday, meaning bacteria is growing back fast. There are a lot of small, white/grey spots of bacteria. Other than seeing less, bacteria culture is doing well.

<table>
<tr><td></td><td>I finally got sent the pictures/evidence of myself doing the gram-staining. Here are the pictures:</td></tr>
<tr><td>January 21, 2024</td><td>Today, I found out the bacteria died because of the cold when I did the gram-staining. Unfortunately, I must do the bacterial growth once again. This time, I will not copy and paste the tables repeatedly, but just do part of the table at each entry.

Today, I will be melting the agar and doing bacterial collection again. I will also pre-prepare agar Petri dishes so I will not have to melt it again when I do the antibiotic transplants next week.

- I do not have enough petri dishes to do the entire plan again.
- So, I will be altering the plan a little bit. I will only have 1 control slide for gram-positive and gram-negative that will be used for all 3 trials.
- I must do all 3 trials at the same time due to time constraints.

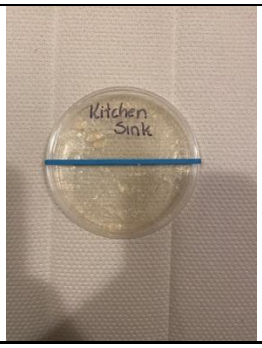
❑ 2 petri dishes for the initial gram-staining and bacterial growth.
❑ 2 controlled slides for gram-negative and gram-positive. 1 each.
❑ 3 Petri dishes for each trial. 6 in total for both trials.

I finished melting the agar and I poured it into the Petri dishes. I poured agar into petri dishes I did not need now, so I do not need to melt the agar again when I need them. I collected the bacteria and labeled the petri dishes.</td></tr>
<tr><td>January 22, 2024</td><td>Today, I am doing the bacteria growth observations. I will put all the bacteria observations in this entry here from now.

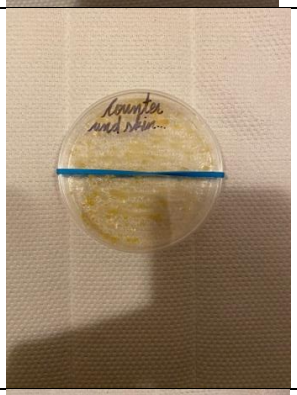
<table>
<tr><th>Bacteria:</th><th>Days #:</th><th>Qualitative Observations:</th></tr>
<tr><td rowspan="3">Kitchen Counter and Skin (Re-growth)</td><td>Day 1</td><td>Very small air bubbles, possible signs of bacterial growth seen. Small, white-grey spots seen.</td></tr>
<tr><td>Day 2</td><td>Very similar to yesterday. No signs of bacterial growth except for small, white-grey spots and a few additional bumps that looked clear.</td></tr>
<tr><td>Day 3</td><td>Small-big, white-grey bacteria spots seen. Starting to grow thicker and more visible bacteria.</td></tr>
</table>
</td></tr>
</table>

		Day 4	More small-big, white-grey bacteria spots seen. One or two spots are green.
		Day 5	Some more small-big white-grey bacteria spots seen. This time, some are green. Bacteria culture starting to form in green color.
		Day 6	More thick, small spots of bacteria seen in more rich-green color. Almost eery spot is green, and a little bit of small white spots cluster seen at some parts of the petri dish.
		Day 7	Not much difference from yesterday. More thick, small spots of bacteria seen in more rich-green color. Almost eery spot is green, and a little bit of small white spots cluster seen at some parts of the petri dish.
	2 Kitchen Sinks (Re-growth)	Day 1	Very small, white-grey spots seen. Possible air bubbles in the liquid agar.
		Day 2	Aggressive signs of bacterial growth seen. Many small, white spots in dense bunches.
		Day 3	Again, more aggressive signs of bacterial growth seen. Dense, small white spots seen in the cluster formation the cotton swab was rubbed against on the agar. Other than looking a little denser, no other changes.
		Day 4	Aggressive white/grey tiny spots of bacteria. In cluster formation in the way the swab was swabbed on pet

		 ri dish.
	Day 5	Aggressive white/grey tiny spots of bacteria. In cluster formation in the way the swab was swabbed on petri dish. A little denser and a little darker tint of grey-white than Day 4.
	Day 6	Not much difference from Day 5. Only a few more spots of bacteria seen and in the cluster formation.
	Day 7	Not much difference from Day 6. Only a few more spots of bacteria seen and in the cluster formation.

Bacteria	**Pictures**
Day 1 – KC & S	
Day 1 – 2KS	

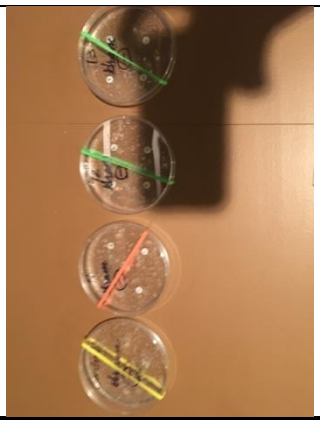
Day 2 – KC & S		
Day 2 – 2KS		
Day 3 – KC & S		
Day 3 - 2KS		
Day 4 – KC & S	[Picture not taken.]	
Day 4 – 2KS	[Picture not taken.]	

	Day 5 – KC & S		
	Day 5 – 2KS		
	Day 6 – KC & S		
	Day 6 – 2KS		

<table>
<tr><td></td><td>Day 7 – KC & S</td><td>  </td></tr>
<tr><td></td><td>Day 7 – 2KS</td><td>  </td></tr>
<tr><td>January 23, 2024</td><td colspan="2">Today, I am doing the bacteria re-growth observations.</td></tr>
<tr><td>January 24, 2024</td><td colspan="2">Today, I am doing the bacteria re-growth observations.</td></tr>
<tr><td>January 25, 2024</td><td colspan="2">Today, I am doing the bacteria re-growth observations.</td></tr>
<tr><td>January 26, 2024</td><td colspan="2">Today, I am doing the bacteria re-growth observations.</td></tr>
<tr><td>January 27, 2024</td><td colspan="2">Today, I am doing the bacteria re-growth observations.</td></tr>
<tr><td>January 28, 2024</td><td colspan="2">Today, I am doing the bacteria re-growth observations.</td></tr>
<tr><td>January 29, 2024</td><td colspan="2">Today, I have done the antibiotic transplant process/antibiotic testing phase. It was very difficult, especially with the inoculating loop design itself and the weak material it is normally made from. Here are the data tables I must use next:
<table>
<tr><th></th><th>Trials</th><th>Trial 1 (diameter of inhibition in cm)</th><th>Trial 2 (diameter of inhibition in cm)</th><th>Trial 3 (diameter of inhibition in cm)</th><th>Controlled</th></tr>
<tr><td rowspan="2">Gram-positive bacilli</td><td>Penicillin</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Ampicillin</td><td></td><td></td><td></td><td></td></tr>
</table></td></tr>
</table>

(Bacterial growth inhibition in (cm))					
	Neomycin				
	Erythromycin				
Gram-negative bacilli (Bacterial growth inhibition in (cm))	Penicillin				
	Ampicillin				
	Neomycin				
	Erythromycin				

	Trials	Trial 1 (qualitative observations)	Trial 2 (qualitative observations)	Trial 3 (qualitative observations)	Controlled (qualitative observations)
Gram-positive bacilli (Qualitative observations)	Penicillin				
	Ampicillin				
	Neomycin				
	Erythromycin				
Gram-negative bacilli (Qualitative observations)	Penicillin				
	Ampicillin				
	Neomycin				
	Erythromycin				

January 31, 2024	*Today, I am doing some antibiotic observations/pictures. I gave a day for the bacteria to grow. Now, I will start at Day 1.* *Day 1 (Gram-Positive)*  Not much bacterial growth around antibiotics. No signs of bacteria seen! *Day 1 (Gram-negative)*  Not much bacterial growth around antibiotics. No signs of bacteria seen!

February 4, 2024	*Today, I am measuring and recording bacterial inhibition data.*

	Trials	Trial 1 (diameter of inhibition in cm)	Trial 2 (diameter of inhibition in cm)	Trial 3 (diameter of inhibition in cm)	Controlled
Gram-positive bacilli (Bacterial growth inhibition in (cm))	Penicillin	1.5 cm	1.3 cm	1.4 cm	None
	Ampicillin	0.9 cm	1.0 cm	1.1 cm	None
	Neomycin	0.0 cm	0.0 cm	0.1 cm	None
	Erythromycin	1.1 cm	0.9 cm	1.0 cm	None

Gram-negative bacilli (Bacterial growth inhibition in (cm))	Penicillin	0.0 cm	0.0 cm	0.0 cm	None
	Ampicillin	0.6 cm	0.7 cm	1.2 cm	None
	Neomycin	1.2 cm	1.0 cm	1.1 cm	None
	Erythromycin	0.9 cm	0.8 cm	1.0 cm	None

	Trials	Trial 1 (qualitative observations)	Trial 2 (qualitative observations)	Trial 3 (qualitative observations)	Controlled (qualitative observations)
Gram-positive bacilli (Qualitative observations)	Penicillin	Around antibiotic, green bacteria is inhibited.	Around antibiotic, green bacteria is inhibited.	Around antibiotic, green bacteria is inhibited.	None
	Ampicillin				None
	Neomycin				None
	Erythromycin				None
Gram-negative bacilli (Qualitative observations)	Penicillin				None
	Ampicillin				None
	Neomycin				None
	Erythromycin				None

Fe	*Today, I am putting in the observations in the table in the entry above.*

bruary 5, 2024	
February 16, 2024	*Today, I am putting information into a PowerPoint to print. I am also going to do Analysis, Conclusion, Limitations/Improvements, and future applications (talk about Gram-Staining guy and how current antibiotic health care is the future application).*
February 17, 2024	*Here is the rest of my project. My anslysis, Conclusion, Limitations/Improvements:* The results match my prediction/hypothesis and research about what bacteria each antibiotic targets. The effects of the antibiotics changed as the type of bacteria changed: - Penicillin's effectiveness/effects changed when comparing the gram-positive bacteria with the gram-negative bacteria. It was better with the gram-positive, seen with the graph. - Ampicillin was relatively similar with both bacilli types, but slightly more efficient with gram-positive by 0.17 cm—proves how gram-positive is easier to kill with its cell membrane, further proving the hypothesis, as seen by the graph. - Neomycin was much more efficient with gram-negative; this was the bacteria it was designed to target, supporting the hypothesis—antibiotics working with designed pathogens. - Erythromycin, like ampicillin, had relatively similar effects with both bacilli, but was more efficient with gram-positive, when seen with the graph. Limitations | Possible improvements

- Temperature. Optimal temperature to encourage bacterial growth could not be achieved. Bacilli grew at lower concentrations and slower speeds. - Gram-staining temperature. While gram-staining, the environment was very cold, which killed the first round of bacilli as well. The gram-staining environment temperature may have affected accurate gram-stained bacilli observations, explaining some unwanted colors. - Antibiotic inhibition measurement. Inadequate inoculation of bacteria for antibiotic testing, making it harder to see more accurate measuring. - Bacilli under microscope after gram-staining could only be seen in blurs of colors. - Could not control temperature (controlled variable) well. - -	- Let bacteria grow in an area where temperature is stable. Possible buy or create a small area such like that. - Do gram-staining in an indoor area that is ventilated and safe instead of the garage. Maybe an unused bedroom. - Use a more efficient inoculating loop. So hard to use that I ripped agar off instead of just bacteria. Possibly watch tutorials to use properly or buy a more efficient one. - Use, rent, or borrow a more powerful microscope to properly see the bacilli. Or watch tutorials to use the current microscope to its full potential!

The goal of the experiment was to see and solve the problem if the effects of antibiotics are identical on both gram-positive and gram-negative bacteria and prove it with different antibiotics and several trials to achieve more accurate conclusions. Looking at the raw data and graphs, the hypothesis was correct; the effects of antibiotics did change as the bacteria they were tested on changed. Evidenced with the graphs, specifically the mean and standard deviation graph, if the bacteria being tested on changes, the effects of the antibiotics (the inhibition growth) changes. This is because different antibiotics were engineered to target specific kinds of pathogens, making their effects unpredictable/different with non-targeted bacteria. The experiment overall was successful, with raw data and graphs aligning with research, and the experiment data proving the hypothesis and presenting an accurate solution to the problem.

Future applications	Future research
- This experiment is already the foundation of our modern	- We can search more effective gram-staining processes to get quicker

healthcare. Antibiotics designed to kill bacterial diseases were originally found through the gram-staining and antibiotic testing process I have done. - In the future, if more bacterial diseases pop out, we can use the process I have done to help figure out effective antibiotics to design.	bacteria discovery. We could possibly integrate it with technology, helping save lives quicker. - We could also discover more effective strategies or innovative solutions to create effective antibiotics faster to speed up the entire process, helping save lives faster as well.

1) Sriram Dokuparti – helped at several parts of the project such as helping record data while I measured.
2) Friends and family – helped provide advice for several parts of the project, such as choosing the topic.
3) Teacher (Science Fair Lead) - helped be with advice at every single doubt I had (could not have done without).
4) ChatGPT – helped gain certain information which I could not directly find and helped with finding specific sources.
5) HomeScienceTools – gave me tools I needed last minute which I could not find anywhere else.

1. STEMIA. "STEMIA Group Materials." Schoology, https://stemia.schoology.com/group/6912913417/materials#/group/6912913417/materials?f=312138664.
2. "Gram Stain Antibiotics Project." Home Science Tools Learning Center, https://learning-center.homesciencetools.com/article/gram-stain-antibiotics-project/.
6. "What Are Antibiotics?" WebMD, https://www.webmd.com/a-to-z-guides/what-are-antibiotics.
7. "Gram-Positive vs. Gram-Negative." Technology Networks, https://www.technologynetworks.com/immunology/articles/gram-positive-vs-gram-negative-323007.
8. "monoderm." Wiktionary, https://en.wiktionary.org/wiki/monoderm#:~:text=monoderm%20(not%20comparable),a%20thick%20layer%20of%20peptidoglycan.
9. "How to Grow Actual Bacteria Culture." Google Search, https://www.google.com/search?q=how+to+grow+actual+bacteria+culture&sca_esv=580369605&tbm=vid&source=lnms&sa=X&ved=2ahUKEwibzNPWwbOCAxXNCTQIHcmmB8cQ_AUoAnoECAUQBA&biw=1470&bih=764&dpr=2#fpstate=ive&vld=cid:59917b5c,vid:Bwjxi3vS0K4,st:0.
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SCIENCE FAIR LOGBOOKS

Every science fair project **must include a logbook**, also sometimes called a research notebook, which is a complete, permanent record of how you did your experiment/research project; it shows what you did and thought every step along the way.

LOGBOOK POINTERS:

- write your logbook in a notebook
- make an entry every time you work on your project
- date each entry
- make your notes in point form
- don't worry about neatness; you do not need to re-copy your logbook to make it look "tidy"
- organize your logbook into sections such as: schedule, daily notes and ideas, background research, contacts and references, experimental procedure/method, data collection sheets, observations/results in tables and graphs, conclusions
- Write everything down, even if it seems insignificant at the time; the information may be useful later on
- Make sure that you describe things in enough detail that you and anyone else reading your logbook in the future will be able to understand your thoughts and repeat the entire experiment exactly like you did it in the first place, just using your logbook.
- You must create your logbook as you go; it is unacceptable to create your logbook on the computer after you have finished your project
- NOTE: The text that appears on your backboard/tri-fold is just a summary of what you write in your logbook; there is much more information in your logbook than what appears on your backboard/tri-fold.

LOGBOOK CONTENT:

- **Timetable :** Come up with a timetable for doing each of the steps of your project and try to stick to it
- **Choose a Topic**: make a list of topics that interest you, things that you are really curious about and that you want to find answers to; explain how you came up with your topic, why you decided to do it.
- **Background Research**: Record your background research about your topic from books, magazines, TV programs, the Internet (with supervision), people and companies. Keep a record about where you gathered your information for your bibliography/list of references and acknowledgements.
- **Testable Question/Purpose:** Based on your background research, write down your testable question/purpose

- **Hypothesis**: write down what you think the results of your experiment will be based on the research that you've done
- **Materials:** List everything that you will need to do your experiment, such as equipment, ingredients, quantities of ingredients, measuring tools etc. Be very specific – give lots of details
- **Procedure**: List the steps you will go through to do your experiment. If you make any changes to the procedure after you start your experiment, describe them in your logbook with an explanation about why you made the change(s) and if the change(s) will affect the results collected prior to the change.
- **Variables**: list the controlled variables, the manipulated variable, and the responding variable
- **Data:** record all of your measurements/raw data that you collected on data sheets in your logbook
- **Results:** record your collected data in charts, tables, graphs, pictures and use these to help you explain what happened in your testing; describe any problems you might have had while you were testing , any changes that you had to make to your original plans, and whether those changes would affect the results collected before you made the changes
- **Conclusions:** write down your conclusions, whether or not your hypothesis was correct and why. It is OK if your results do not support your hypothesis - the information you collected still supports science.
- **Recommendations/Applications**: Make recommendations for improving your project, for further study, and applications I can make from my research