

Microbiology Lab

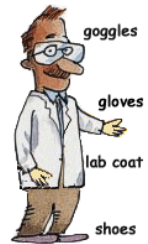
Name _____

Date _____

Lab safety: Full PPE required - Lab coat, long pants, closed-toe shoes and gloves; safety goggles (required)

Learning Objectives:

1. To investigate the abundance and diversity of microorganisms in our local environments.
2. To learn basic lab protocols including the use of solid media for microbial growth and labeling lab cultures
3. To distinguish between bacterial colony and fungal colony from their appearances
4. To understand the significance of hand washing to maintain better hygiene
5. To learn how to draw graphs using the data collected from an experiment



Pre-Lab Activity:

Read the article (Microbes) using this link – <https://www.ncbi.nlm.nih.gov/books/NBK279387/> and answer the following questions.

i) What are the four groups of microbes? Give one example of each.

ii) What are the differences between bacteria and virus?

iii) Are all bacteria harmful? _____

iv) Is flu caused by a bacteria or virus? _____

Introduction:

What are Microorganisms and How We Fight Against Them

The first evidence of the origin of life on Earth came from fossils underneath the sea which are called stromatolites. These fossils were formed by packed cyanobacteria (photosynthetic bacteria). From this evidence we can estimate that life on earth originated about 3.5 BYA (Billion years ago). The concept of these invisible creatures were there in ancient Egyptian and Indus Valley civilizations, but it was not until the 17th century that their existence was confirmed. Antonie van Leeuwenhoek first noted the existence of microorganisms in 1663 using the microscope invented by him. Once discovered, this opened up an entirely new field of studying microorganisms, called **microbiology**. Since Van Leeuwenhoek first identified microorganisms, he is now commonly referred as “the Father of Microbiology”.

Every possible surface of the earth is covered by microorganisms. The phrase, “**Ubiquity of Microorganisms**” refers to the concept that microorganisms are omnipresent, or present everywhere all around us. Microorganisms include prokaryotes (bacteria and archaea), unicellular eukaryotes (fungi, protozoa) and viruses. They can survive in extreme conditions; e.g. – some archaea are found in hot spring water and some bacteria survive in high acidic environments such as some pond water. There is a common misconception that bacteria are bad, however not all bacteria are harmful. Microorganisms are found naturally inside our body (in our guts in what is known as the microbiome) and control certain physiologies and are thereby called beneficial bacteria. There are certain places which are maintained in a carefully controlled or sterile environment, like our blood and cerebrospinal fluid in the brain. The body’s defense mechanism or immune system works efficiently to keep these fluids sterile or free from any contamination from bacteria or other living organisms. We get sick when our immunity system fails and is taken over by potentially harmful, microbes.

Microorganisms are most commonly studied in a microbiology lab where sterile conditions are maintained to avoid contamination. A growth medium is used to provide nutrients for the bacteria that are grown for experiments. Composition of the growth medium with a mixture of nutrients is an important part of the experiments as the medium determines the diversity of bacterial growth in it. Two types of growth mediums are very commonly used in the laboratories, a) a liquid nutrient media called broth and b) a solid nutrient media called agar. When agar (extracted from sea weed) is mixed in the nutrient broth it solidifies in a gel like consistency and is used for effective growth of different ranges of bacteria. One such solid growth medium often used in labs is **TSA** or **trypticase soy agar**. TSA has a broad mixture of nutrients which allows growth of a large diversity of microorganisms.

Microorganism growth can be inhibited by the addition of **antibiotics** (chemicals which can kill bacteria) which can limit or prevent the growth of specific types of bacteria. Although the era of antibiotic utilization truly began in 1928 with Alexander Fleming’s discovery of penicillin, plants had been used for thousands of years prior to this to treat infections and sickness. It is estimated that about 80,000 lives were saved in WWII by penicillin. Antibiotics help to treat a great deal of illnesses, but they do not work against viruses or other groups of microbes. Scientists have developed vaccines to cover the gap in treatment, specifically targeting viruses and some bacteria.

For humans, most of the infectious particles enter in our body through the nose (for airborne particles) and or mouth (for both airborne and food or waterborne particles. According to the National Institutes

of Health a **contagious disease** is any disease that can spread rapidly from person to person through direct contact, indirect contact, or droplet contact. Direct contact involves physical contact with the individual who is infected whereas indirect contact involves spread of the infection through a contaminated object. Spread that occurs via droplet contact involves inhalation of respiratory droplets released from a cough, sneeze, or even talking, which contain the illness.

Maintaining clean condition is key to sustain a healthy life style. A relatively easy, inexpensive and efficient way to keep us clean is to wash our hands thoroughly with soap and water. Maintaining absolute sterile condition is not possible outside of labs and hospitals, but if we follow some common sense and responsible behavior and basic knowledge of hygiene, we will be able to prevent infection and spread of germs.

Critical Thinking:

According to CDC (Center for Disease Control) – “COVID-19 is thought to spread mainly through close contact from person-to-person:

- Between people who are in close contact with one another (within about 6 feet).
- Through respiratory droplets produced when an infected person coughs, sneezes, or talks.
- These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs.
- COVID-19 may be spread by people who are not showing symptoms.”

What type of disease is Covid-19?

What type of protections are required to keep an individual safe and to prevent spread from person to person?

Activity 1A: What Microorganisms Are Around Us?

Materials Needed

For each team of 4, you will need

- 3 TSA plates
- 2 TSA + Antibiotic plates
- Cleansing reagents
- Cotton swabs
- Marker pen
- Incubator

Procedure: Day 1

1. Label 4 plates with your team’s names, date, the type of medium (TSA/TSA + antibiotics), the inoculum (“Air” in this case) and the location of the inoculum (ex. outside science hall). The plates should be labeled around the perimeter of the agar side, not on the lid.

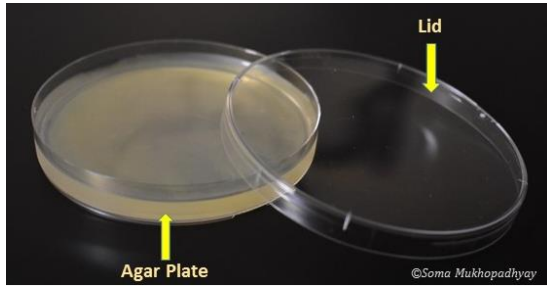


Figure 1A. Petri dish with agar.



Figure 1B. Example of label on side with agar.

Note:

We label the agar side for two reasons.

- First, the microbes will grow on the agar surface (not on the plastic Petri lid). So by labeling the agar-side, we will always know the identity of the plate, even if the lid and the agar-side become separated.
- Second, we incubate agar plates agar-side up. If condensation (water accumulation) occurs during incubation, it will accumulate on the lid, not on the agar surface.

We use **TSA, trypticase soy agar**, for this activity because it has a broad mixture of nutrients that allows the growth of a large diversity of microorganisms.

We are using a **generic antibiotic (ampicillin)** in the media to test for antibiotic resistant microorganisms.

Do you think there will be more or less colonies on the antibiotic plate compared to the other plate, why? _____

Most common microorganisms grow optimally at warm temperatures. We generally grow microbes that exist outside our bodies at 35°C. What incubation temperature should we use for organisms that grow inside our bodies? _____

2. Each group will place one set of plates inside the building (in the lab or other place) and one location outside. Record the location of your plates:

Inside: _____

Outside: _____

3. Leave a) one set the agar exposed to the air for 15 minutes inside the building and b) second set outside of the building).
4. After 15 minutes, close the plate and place those in the incubator.
5. You have to come back to the lab after ~48 hours to note the results.

Activity – 1B: What gets on your arms when you put your arms on the benchtop?

1. Take one TSA plate and label as described in above activity. Draw a line across it's diameter.

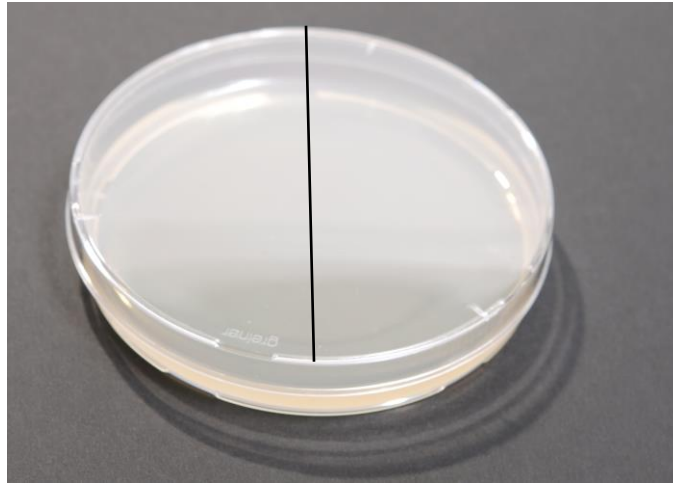


Figure 2. Labeling plate for benchtop experiment

2. The inocula will be “Benchtop Before” on one side of the line and “Benchtop After” for the other side.
3. Swab the benchtop with a cotton swab. Twirl the tip of the swab as you go over the entire benchtop.
4. Transfer cells to the “Benchtop Before” side by gently swabbing the agar surface. (Tip: hold the swab so that you are using the side of the cotton (not the very tip)).
5. Close the plate.
6. Using a tape mark a square area on your benchtop. Spray cleaner on this area. Each lab group will have a different cleaner, please label your plates with your cleaner: a) water only B) soap & water c) glass cleaner d) alcohol e) acetone f) multi-surface cleaner or other (as supplied). Use paper towels to spread the disinfectant uniformly. You are trying to wet down the bench, not dry it off. Allow the liquid to air-dry.
7. Inoculate the “Benchtop After” side by repeating steps 3 through 4. Incubate the plate at 35°C.

Note: Put a tape around your team’s 5 plates and put those in the incubator for ~48 hours.

Observation:

Every team needs to come back after 48 hours of this class/experiment to view your plates
Please be aware of other classes that meet in this lab.
DO NOT interrupt another class to check your plates.
Record the data in the tables on page 8 and answer the questions.

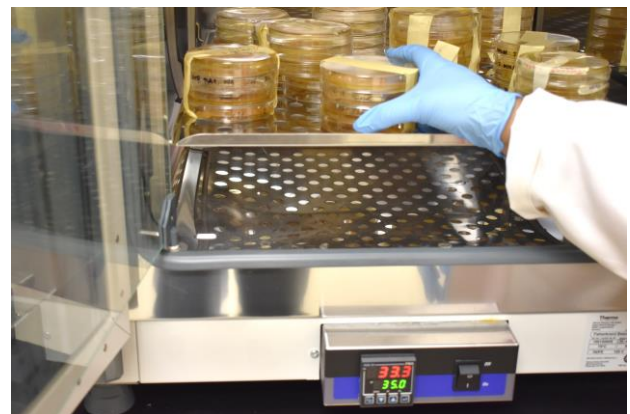


Figure 3. Placing the plates in the incubator at 35°C.

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Make sure you have your lab coat and gloves on when recording your data.

Disposal: After recording the data and taking pictures, put the plates in the refrigerator.

DO NOT throw the plates in the trash. Those will be discarded later following proper safety procedure.

Procedure: Day 2

You will make observations of the agar plates that you inoculated on Day 1. Each bump that has formed on the agar surface is called a colony. Fungal colonies tend to be furry; bacterial colonies tend to have rather smoothly-textured surfaces (there are many exceptions to these tendencies). Each bacterial colony is made of tens to hundreds of millions of cells—all descendants of one cell that was placed there during inoculation on Day 1. The millions of cells in a colony are all genetically-identical to one another having arisen through the process of bacterial reproduction called binary fission (essentially one cell splitting into two identical daughter cells).

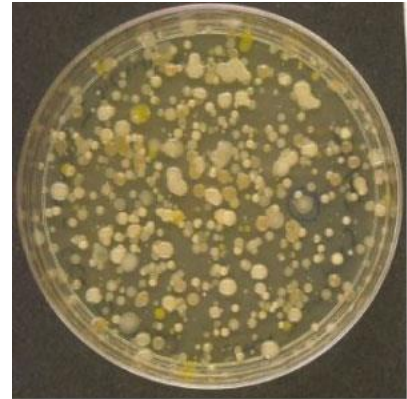


Figure 4. Example of a plate with bacterial colonies

Observations - Abundance and Diversity of Microbes

1. Estimate the number of colonies on each plate. Your estimation needs only to be very rough (e.g., “lots of colonies” or “a gabillion colonies”). Fill in the table below.
2. Estimate the number of different kinds of colonies (the diversity).
 - Two colonies that look different from one another are probably different species (although we can’t be sure without performing further identification tests).
 - Characteristics that are important indicators of differences are:
 - Colony color and size
 - Colony translucency vs opacity
 - Colony texture (rough vs smooth)
 - Margin (outer edge of the colony): entire (round), lobed, toothed, irregular.
 - Elevation (how tall off the agar the colony rises)

Table 1. Observing your microbes.

Plate	Abundance	Diversity – brief description
Air TSA (Inside)		
Air TSA+ antibiotic (Inside)		
Air TSA (Outside)		
Air TSA + antibiotic (Outside)		
Bench before		
Bench after		

3. Take pictures (make sure it is a clear picture) of all the plates and paste/insert those into the appropriated spaces on your post-lab.

Activity 2: Hand-Washing Activity

Do you wash your hands? You should—it's the best way to prevent the spread of microorganisms. These small organisms find nooks and crannies to hide in, so it takes good hand-washing technique to get rid of them. In this activity, you'll investigate which parts of the hand are the most difficult to wash microscopic organisms off from there.

According to health professionals, carefully washing your hands for 20 seconds (that's about as long as it takes to sing "Happy Birthday" twice) in warm water with soap will eliminate most germs and keep you healthier. When washing make sure to get not just the **palm** and **fingers**, but also the **back of hand**, **between fingers**, and around **fingerails**.

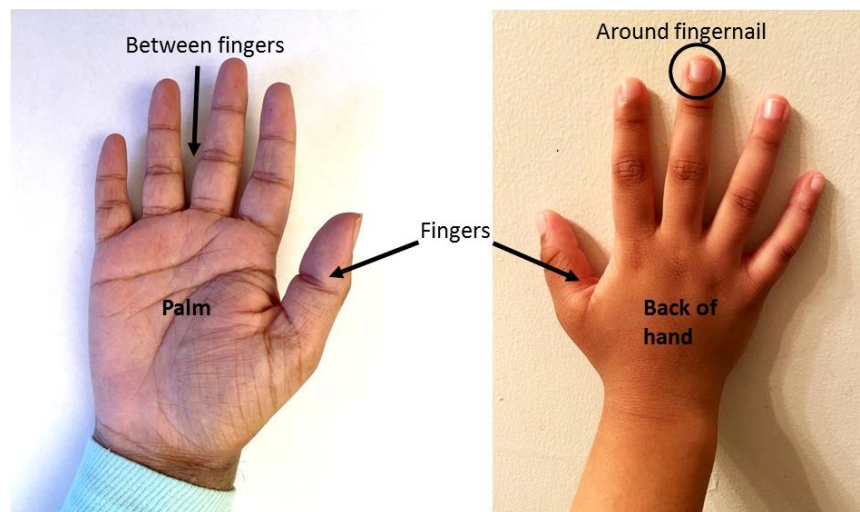


Figure 5: Parts of the hands.
Picture Courtesy: Volunteers

Even if you are one of those whom consistently wash your hands, you may be frequently missing several areas.



Figure 6: Areas most often missed during hand washing

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Materials Needed

For each team of 4, you will need

- soap
- Glow germ lotion
- UV lamp
- timer

Procedure

1. Select two volunteers from every team; one will be “water only” and the other will be “soap and water”.
2. Volunteers: Squeeze a nickel-sized amount of the Glo Germ gel on the palms of your hands. Rub your hands together as if you are applying hand lotion. Make sure you completely cover your hands, including around your fingernails and in between your fingers.
3. Have the “water only” individual wash their hands just with water for one second.
4. Then shine the ultraviolet light on his/her hands. Look carefully for any "glowing germs." Make sure everybody is wearing safety goggles when the UV lamp is on.

Are your hand's glowing? _____

Where is the dye concentrated—on the palms, fingers, back of hands, around the fingernails?

5. Record your data in the chart below. Use the following scale to indicate the amount of “germs:”

- 5 – Extremely dirty
- 4 – Very dirty
- 3 – Dirty
- 2 - Slightly dirty
- 1 – Clean

6. Now have the “water only” individual wash his/her hands for 4 more seconds with just water. Examine the glow and record the cleanliness in Table 2a.
7. Repeat with an additional 5 seconds (total of 10 seconds of hand washing) and an additional 10 secs (total of 20 seconds of hand washing).
8. After the first “water only” volunteer complete 20 seconds of washing, have the “soap and water” volunteer follow steps 3 – 7 using soap and water. Record the data in Table 2b.

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Table 2a: WATER ONLY

	1 sec	5 sec	10 sec	20 sec
Palm				
Back of Hand				
Fingers				
Around finger nails				
In between fingers				
Wrist				

Table 2b: SOAP & WATER

	1 sec	5 sec	10 sec	20 sec
Palm				
Back of Hand				
Fingers				
Around finger nails				
In between fingers				
Wrist				

References:

Lab Module 1: Ubiquity of Microorganisms Introduction - Clark College
Science Buddies Staff. (2020, June 23). *Spread the Soap, Not the Germs*. Retrieved from https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p018/microbiology/soap-wash-away-germs

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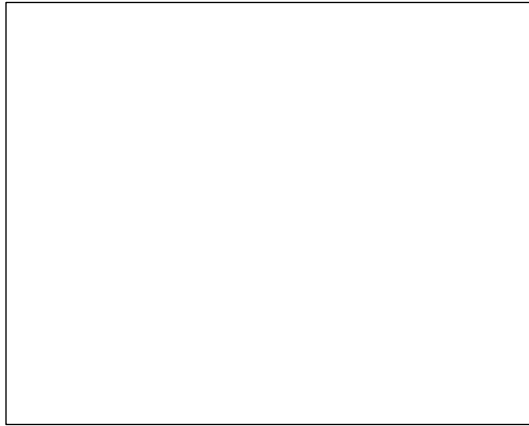
Post-Lab

Name: _____

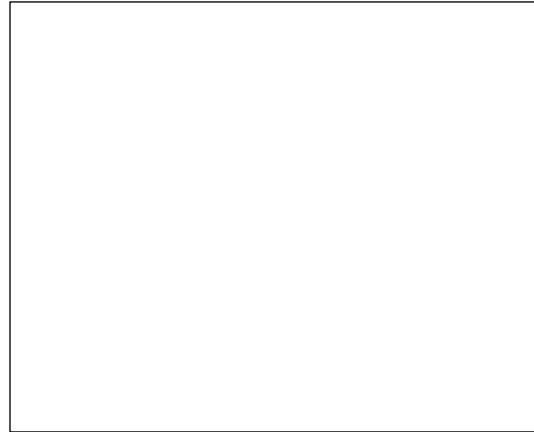
You will be creating a PowerPoint Presentation with images from Activity 1A and 1B, as well as the graphs created from Activity 2.

Part I. From activity A, take pictures of all the plates and insert electronically into the appropriate spaces below.

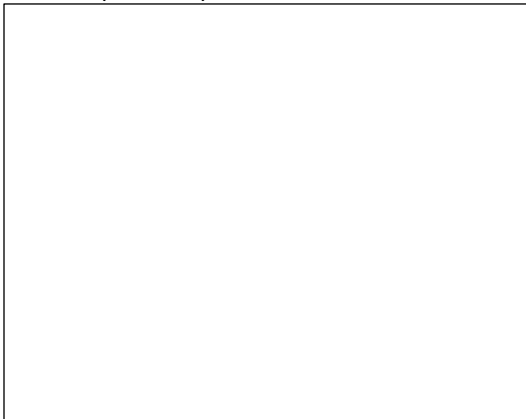
Air TSA (Inside)



Air TSA+ antibiotic (Inside)



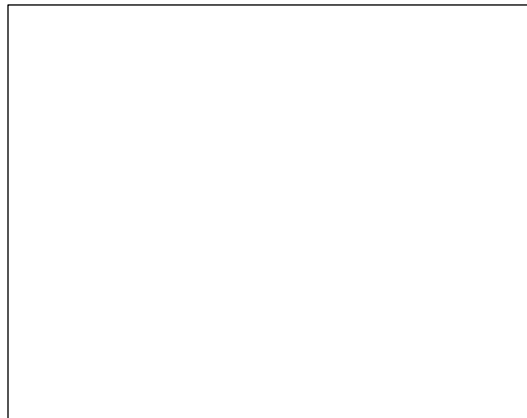
Air TSA (Outside)



Air TSA + antibiotic (Outside)



Bench before and after cleaner.



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Part II. Answer the following questions pertaining to activity A:

1. What is a benefit of solid agar medium?

2. What is a colony?

3. How do you tell the difference between a bacterial colony and a fungal colony?

4. How do you reduce airborne contamination?

5. Is there a difference in the observations when comparing the plates with and without antibiotics?

Explain.

6. Does table disinfection work? Compared to the data collected by other group members how do you rank the effectiveness of the different disinfectants?

7. If almost every surface we touch is inhabited by bacteria, why don't bacterial infections occur more often? _____

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8. Are all bacteria harmful? Explain your answer.

9. What effect, if any, did increased washing time have on the percentage of "germ" coverage on your hands? _____

10. What effect, if any, did using soap and water compared to water alone have on the percentage of "germ" coverage on your hands?

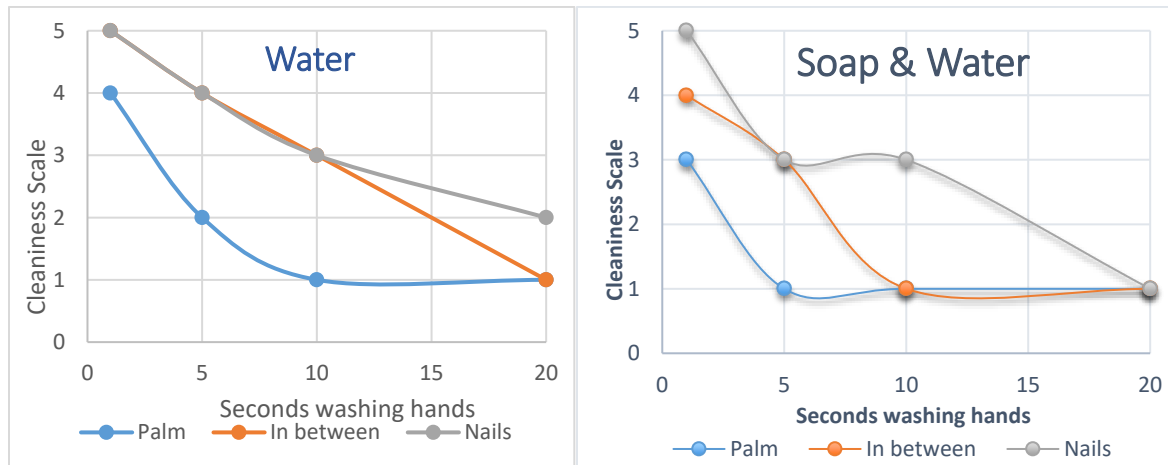
11. Name 3 areas of your home that are most susceptible to bacterial contamination. Explain steps you could take in each of these areas to prevent the spread of bacteria to other places in your home.

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Part III: Watch the video on how to make graphs using excel. Create two graphs using your data from the hand cleaning activities (Activity B).

Graph your results for - 1) Palm 2) In between fingers 3) Around fingernail. Plot two graphs showing the average cleanliness score at each time interval for the water only and the soap and water experiments. (Make sure you add proper title, label X-axis and Y-axis)

Example of the graphs are shown below:



Further instruction on creating your PowerPoint presentation will be provided by your instructor.