

IVC BIOL 15: MICROBIOLOGY LAB MANUAL



Emalee MacKenzie

IVC Microbiology Lab Manual

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This text was compiled on 10/14/2026

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Detailed Licensing

Detailed Licensing

Licensing

A detailed breakdown of this resource's licensing can be found in **Back Matter/Detailed Licensing**.

Lab Exercise Template

BACKGROUND

MATERIALS

METHODS/PROCEDURES

METHODS \(\PageIndex{1}\)

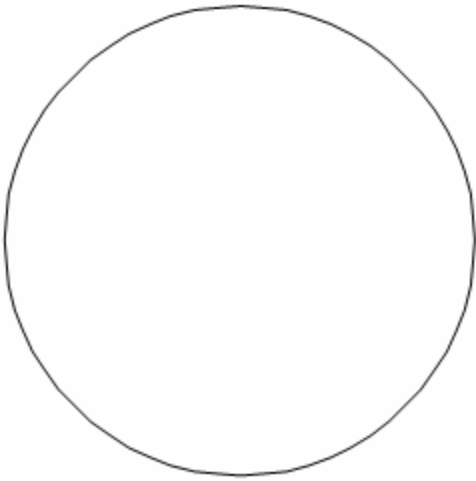
Print a hard copy to bring to lab ([PDF](#)).

👉 If you are filling this out on a digital iPad or tablet please note put your name here and take a screen shot.
You are also welcome to print the PDF and turn in a physical copy.

NAME _____

EXPECTATIONS

RESULTS



CONCLUSIONS

template

Learning Objectives

- Objective 1
- Objective 2

BACKGROUND

MATERIALS

METHODS/PROCEDURES

METHODS \(\PageIndex{1}\)

Note

The following is to be completed during lab then turned in on Canvas as a PDF

*If you are using an iPad or tablet you will need to take screen shots of your completed work, save the screenshots as one PDF then submit them on Canvas by the due date designated on Canvas.

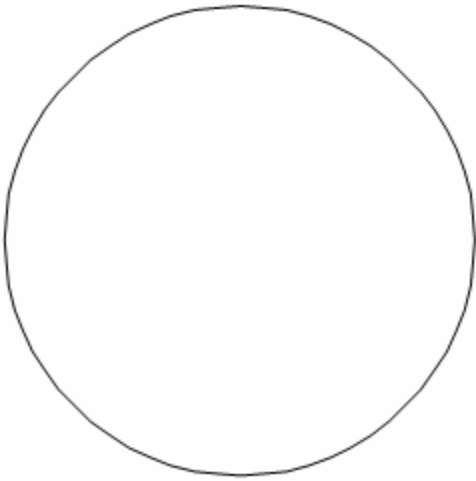
*You can also print out the entire exercise to bring to lab with you. If you choose to complete the lab on paper, take pictures of the completed results and conclusions sections only, save them as one PDF, then submit to Canvas by the due date designated on Canvas.

Exercise Name

NAME _____

EXPECTATIONS

RESULTS



CONCLUSIONS

1: INTRODUCTION. SAFETY AND AGAR PLATES

1.1: LABORATORY SAFETY RULES

1.2: REQUIRED LABORATORY SUPPLIES (Student Responsibility)

1.3: USING AGAR MEDIA

1.1: LABORATORY SAFETY RULES

Bio 15 RULES FOR LABORATORY SAFETY

Throughout this course, you will be working with living cultures of various microorganisms. While only some of these organisms are classified as **human pathogens** (organisms that can cause disease in humans), any microorganism has the potential to cause infection if present in large enough quantities.

THEREFORE, ALL CULTURES USED IN THIS LAB WILL BE HANDLED AS IF THEY ARE HUMAN PATHOGENS.

In addition, some laboratory procedures may involve the use of potentially hazardous chemicals and reagents. The safety guidelines and procedures outlined below must be followed at all times throughout the semester. These rules are designed to minimize microbial contamination to yourself and your environment, and to support safe and effective laboratory practices.

- **No food or drink is allowed in the lab at any time**—this includes water bottles.
- Lockers are located outside the lab room. Choose one to use for the session. You must bring your own padlock. Combination locks are recommended and can be purchased for \$1.25 at stores like Dollar Tree.
- **Closed-toed shoes are required at all times.** Students not wearing appropriate footwear will not be permitted to participate in lab and will not receive credit for that day's assignments. It is recommended to leave an extra pair of shoes in your locker just in case.
- Only bring materials needed for that day into the lab (lab exercises, pencil/pen, colored pencils, sharpie, lab coat etc.). Tablets/iPads are allowed but no computers.
- While in lab, keep your hands away from your mouth, eyes, and face to avoid accidental contamination or infection.
- You will need to purchase a white lab coat. The coat must be buttoned up at all times while in lab. The purpose of the lab coat is to protect your clothing and skin from contamination. An unbuttoned lab coat is pointless.
- The lab coat must:
 - Be non-disposable
 - Have long sleeves
 - Close with buttons or snaps
 - Extend beyond the height of the lab bench
 - Only used for this class
- **Always store your lab coat in your locker** between lab sessions. To prevent cross-contamination, do not take your coat home, leave it in your car, store it in your backpack or use it for another class.
- When you enter lab, place your essential lab materials on your chair then wash your hands. If you leave the lab during class, wash your hands before exiting then again when re-entering.
- After washing your hands decontaminate your workbench with disinfectant and paper towels. Repeat this process at the end of each lab session.
- Any cuts or scratches on your hands must be covered before participating in lab work. (Band-Aids are available upon request).
- Keep your workbench free of unnecessary items and clutter.
- Be aware of cords and other potential hazards—do not allow cords to come near open flames or hang over the edge of the table.
- Hair that falls below the shoulders must be tied back and secured at all times while in lab.
- Cultures, microscope slides, and lab equipment must remain in the lab. Do not store these items in your locker or remove them from the lab room for any reason.
- All inoculated media and microscope slides must be labeled properly using a **Sharpie** marker. Do not use tape. Each label must include:
 - Student name (first initial, full last name)
 - Date of inoculation
 - Name of the culture
 - Exercise number

*Any media found in the incubator that is not properly labeled will be discarded therefore you will lose points.

- Use caution when working with Bunsen burners and hot plates. Keep all flammable items (e.g., paper towels, notebooks, gas hoses) away from heat sources.
- After completing the lab exercises for the day decontaminate the bench top then wash your hands.
- **Never walk away from an open flame.** Open flames must be attended at all times. Failure to do so will result in a loss of points.
- If you smell gas, inspect the gas stopcock and hose for leaks. Turn off the gas immediately and notify your instructor. Sometimes the connections can become loose and potentially start a fire.
- Dispose of all glassware, plastics, and cultures in the designated waste bins.
- Dispose of chemical waste and reagents properly using the containers specified by your instructor. If you are unsure, always ask first.
- Know the locations of all emergency equipment, including the fire extinguisher, eyewash station, emergency shower, and all exits.
- Report all spills, cuts, burns, breakages, or accidents to your instructor immediately—**no matter how minor.**
- If you are pregnant, immunocompromised, or have any health condition that may require special precautions, please inform your instructor so accommodations can be made.
- A single student disregarding lab safety procedures puts everyone at risk. Failure to follow lab safety rules will result in point deductions and/or removal from the course.
- If you ever have questions about lab safety or procedures, **ask your instructor.**
- A clean and organized workspace is essential for microbiology. Each group is assigned clean-up responsibilities. All students are expected to participate in cleanup. Leaving before clean-up tasks are complete—without instructor permission—will result in loss of points.

I _____ (print name) have attended the safety orientation for the Bio 15 laboratory and agree to abide by the safety rules and regulations of the classroom.

Date: _____

Student Signature _____

After attending the safety orientation the first day of lab complete the above then submit on Canvas. Check Canvas for the due date.

1.2: REQUIRED LABORATORY SUPPLIES (Student Responsibility)

Each student is required to supply the following items to use in lab starting the first day:

1 White Lab coat - dedicated to microbio - cannot be shared with another class such as chemistry etc.

1 black Perennate marker medium tip

Combo lock

hair tie (if hair beyond shoulder length)

1 pair of Goggles

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1.3: USING AGAR MEDIA

LEARNING OBJECTIVES

- Describe the role of nutrient agar in microbiological experiments.
- Demonstrate proper technique when pouring nutrient agar plates.

BACKGROUND

Like all living organisms, microorganisms (microscopic organisms such as bacteria, fungi, and some algae) require nutrients and suitable environmental conditions to survive and reproduce. Microorganisms absorb nutrients from their surroundings, so early microbiologists commonly grew them in nutrient broth (a liquid containing the nutrients needed for microbial growth).

However, some experiments are difficult or impossible to perform in liquid media because cells move freely throughout the fluid. This movement makes isolating a single cell, tracing the growth of **daughter cells** (cells produced through the division of a parent cell), and determining the **purity** (the presence of only one type of organism) of a culture challenging.

Early attempts to create a solid growth surface included using slices of fruits or vegetables. However, variations in moisture and nutrient content made these materials unreliable. Microbiologists later began adding gelatin to nutrient solutions to create solid media. Although gelatin provided a smoother and more consistent surface than fruits or vegetables, gelatin often melted at the warm temperatures required for bacterial growth. Some microorganisms also produced enzymes that broke down gelatin, causing the solid medium to become liquid.

A major breakthrough occurred in 1881 through the work of Fanny Angelina Hesse. Fanny worked as an unpaid laboratory assistant and scientific illustrator alongside her husband, Walther Hesse, who conducted research in Robert Koch's laboratory. She helped prepare growth media, clean laboratory equipment, and create detailed illustrations of microorganisms and microbial colonies for scientific publications.

While Walther Hesse was studying microorganisms in the air, he experienced difficulty keeping gelatin-based media solid during warm weather. Fanny suggested replacing gelatin with **agar** (a gelatin-like substance derived from seaweed). She was familiar with agar because she had used the substance to prepare jams and jellies. Fanny had learned about agar from family acquaintances who had lived in Java, where cooks commonly used agar as a thickening and solidifying agent.

Agar offered several important advantages over gelatin. Agar remained solid at temperatures that caused gelatin to melt, and most microorganisms could not break agar down. Agar also formed a clear, stable surface that allowed microbial colonies to remain separated and visible. Walther Hesse and Robert Koch adopted Fanny's suggestion, and Koch reported using agar as a solidifying agent in 1882. Although Fanny received little recognition during her lifetime, her practical knowledge and scientific insight helped transform microbiology. Agar made reliable isolation and study of pure microbial cultures possible and remains one of the most widely used materials in microbiology laboratories today.

When a single bacterial cell lands on the surface of a nutrient agar plate under suitable conditions, the cell divides repeatedly and forms a **colony** (a visible cluster of genetically similar cells). Colonies may have distinctive sizes, shapes, colors, textures, and margins depending on the species. These characteristics can help with microbial identification.

**Agar provides no nutritional value for bacteria.
Agar serves only as a solidifying agent.
Without added nutrients, bacteria cannot grow.**

The ability to isolate genetically similar bacterial cells on solid media forms the foundation of many microbiological techniques. When individual cells land far apart on an agar plate, each cell can develop into an **isolated colony** (a single colony that does not touch other colonies). Isolated colonies allow microbiologists to count, examine, and transfer microorganisms accurately.

Contamination (the unintended presence of unwanted microorganisms) commonly occurs when working with microbial cultures. Preventing the introduction of unwanted organisms during the preparation and handling of agar plates helps maintain pure cultures and reduces the chance of false or misleading results.

Agar melts when heated to approximately 85°C and remains liquid at temperatures used for pouring plates. Before pouring, molten agar is usually cooled to approximately 45–50°C. This temperature keeps the agar liquid while reducing excessive condensation and preventing damage to heat-sensitive materials.

Throughout the semester, you will pour nutrient agar plates for several experiments. Before use, the plates must be incubated **uninoculated** (not intentionally exposed to microorganisms) for 48 hours to check for contamination. **Aseptic technique** (procedures used to transfer microorganisms without introducing unwanted contaminants) is essential for maintaining the quality and reliability of laboratory work.

MATERIALS (Per Group of Four)

- 1 flask containing 100 mL nutrient agar broth
- 4 sterile petri dishes

METHODS/PROCEDURES

Always Label Petri Dishes Prior to Pouring

Each Petri dish must be labeled before agar is dispensed. Label the bottom portion of the dish (the smaller, deeper half).

The day plates are poured write only your last name and first initial along the perimeter of the bottom portion of the plate. Additional information will be added during the subsequent lab session.

Do not remove the lid while labeling the plate to minimize the risk of contamination. Ensure that the dish is turned lid-side up before pouring to avoid accidentally pouring the agar into the incorrect section. Once agar is poured, the dishes must remain stationary until the medium has solidified completely.

Preparing the Nutrient Agar

1. Confirm the water bath is at 50°C.
2. Loosen, but do not remove the cap of the agar flask then heat the flask in the microwave for 30 seconds.
3. Using appropriate hand protection, carefully remove the flask from the microwave and gently swirl to mix the contents.
4. Repeat steps 2 and 3 as necessary until the agar is fully liquefied.
 - 👉 It is essential there are no clumps in the liquid agar when swirled.
 - 👉 Also essential to avoid overheating or allowing the medium to boil.
5. Once completely liquefied, place the flask with the loosened cap into the water bath.
6. Stabilize the flask by placing a weight around the neck.
7. Allow the melted agar flask to remain in the water bath for at least 10–15 minutes in order to cool to a temperature in which the agar can be poured into the plastic dish without cracking the dish.

Pouring Agar Plates

Any time after the 10–15-minute cooling period the melted agar can be poured into the plastic dishes without risk of cracking. Do not remove the flask until you are ready to immediately pour the melted agar into the plates. Melted agar flasks can remain in the 50°C water bath as long as needed while you work on other things.

1. Before removing the melted agar from the water bath arrange the Petri dishes along the edge of the workbench, away from the main workspace. You should not see your name on the plate, if you see writing confirm the bottom of the plate is on the bench not the lid.

2. Retrieve the agar flask from the water bath then remove any water from the flask with a paper towel.

3. Once back at the bench partially lift the lid of the dish as demonstrated by your instructor then pour approximately 25 mL of agar into the plate.

4. Immediately close the lid after pouring.

👉 Never remove the lid entirely or set the lid down on the bench.

5. Once poured do not move the plate until solid.

6. Once agar has completely solidified, invert the plates so that the agar side faces upward then put the plate in the incubator.

👉 You should be able to see your label when you place the plate in the incubator.



Figure 1-1a. Aseptically removing a bottle cap, freeing the rest of the hand to manipulate a plate lid, pipette, etc.

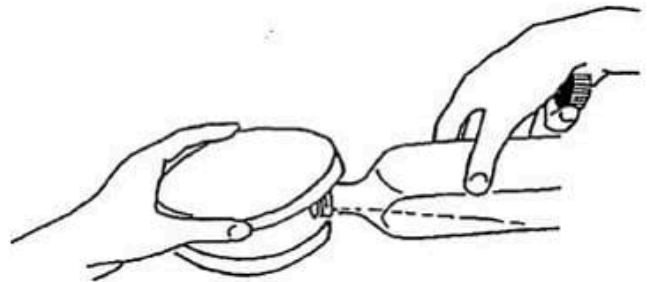


Figure 1-1b. Agar poured into a petri dish. The lid shields the lower plate and its contents from airborne contamination.

2.4: Lab Procedures- Prepare solid media, Aseptic Technique, T-streaking is shared under a CC BY-NC-SA license and was authored, remixed, and/or curated by Joan Petersen & Susan McLaughlin via source content that was edited to the style and standards of the LibreTexts platform.

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*You can also print out the entire exercise to bring to lab with you. If you choose to complete the lab on paper, take pictures of the completed results and conclusions sections only, save them as one PDF, then submit to Canvas by the due date designated on Canvas.

NAME _____

EX 1 AGAR MEDIA

EXPECTATIONS

Based on your experience of melting and pouring the agar plates today, do you think your plates will be free of contamination after incubating 48 hours, or do you expect some contamination? Explain why/why not.

CONCLUSIONS

1. After examining your agar plates, were you successful in preventing contamination? Yes or No
2. Based on your results, describe what went well and what could be improved in your technique.
3. What is the purpose of incubating nutrient agar plates for 48 hours before using them in an experiment?
4. A single bacterial cell can lead to the formation of a visible colony on a nutrient agar plate. If the colony is uniform in color and isolated (not touching any other colonies). What does this indicate about all the cells in the colony?

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2: EXPOSURE PLATES

LEARNING OBJECTIVES

- Observe and describe microbial growth on nutrient agar plates resulting from environmental exposure.
- Identify the appearance of colonies formed by different microorganisms.

BACKGROUND

Although they are invisible to the naked eye, millions of microorganisms surround us at all times. Becoming aware of their presence is an important first step in learning to work with them safely and effectively. While a microscope can help you observe microorganisms, it is not practical to scan every inch of your lab bench or hands to locate them.

Instead, we will use a method that allows individual microbial cells to multiply until they form visible colonies which can be seen without a microscope. This approach lets us observe microorganisms present in our environment by growing them on solid nutrient media.

In this exercise, you will test various environmental surfaces by exposing sterile agar plates to them. As each cell multiplies, visible colonies will appear where microorganisms landed and grew. However, it's important to note that we can't create conditions that support the growth of all types of microorganisms, and we intentionally avoid growing **fungi** (a group of eukaryotic microorganisms that includes molds and yeasts) in large quantities, as they can be difficult to control and may pose health.

Each student should choose a different surface to test. You may expose plates to surfaces such as doorknobs, countertops, elevator buttons, or personal items like coins or backpacks.

Important: Do not test your own skin, mouth, or any part of your body at this time. The purpose of this activity is to safely visualize environmental contamination, not to culture potentially infectious microbes from your own body. Later in the semester, once you have developed the skills necessary to safely handle human specimens, you will have an opportunity to examine your own skin and throat for microbial presence.

MATERIALS

- 4 sterile cotton swabs
- 1 tube of sterile water
- 2 marking pens to label plates
- 4 nutrient agar plates

METHODS/PROCEDURES

1. Carefully open the sterile cotton swab without touching the cotton tip.
2. If you are sampling a dry surface, moisten the swab slightly by dipping it into the tube of sterile water. This helps lift microorganisms more effectively.
3. Rub the swab firmly and thoroughly over a surface area approximately 4 inches by 4 inches.

4. Gently lift the lid of your Petri dish just enough to access the agar surface. Rub the swab lightly across the agar in a zigzag or sweeping motion. As you move the swab, gently roll it between your fingers to ensure maximum transfer of any collected microorganisms.
5. Label your agar plate according to the instructions provided in Exercise 1. Include your name, the date, the source of the sample, and "Ex. 2."
6. Place the plate in the incubator set to 30°C with the agar side facing up. (You should be able to see your label.)

EX 2 Exposure Plates

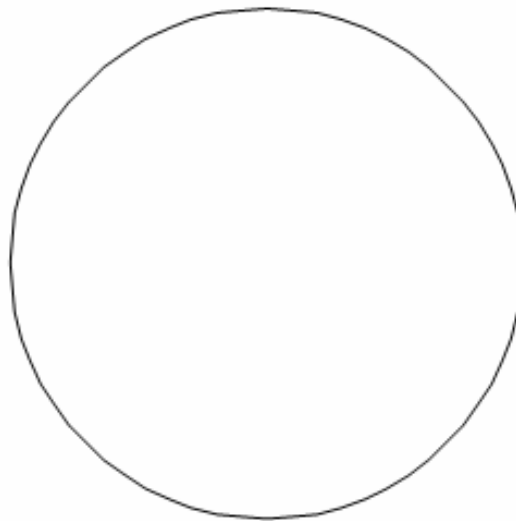
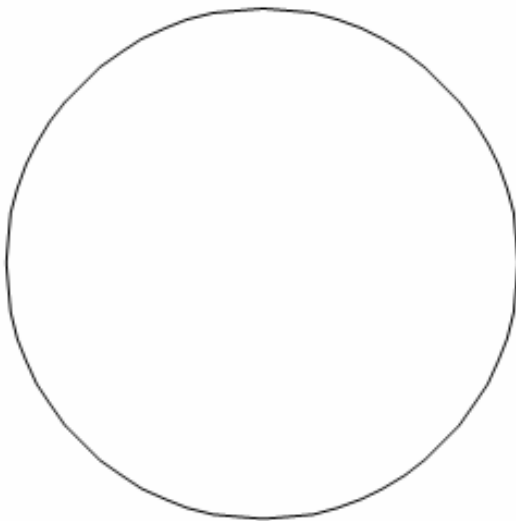
NAME _____

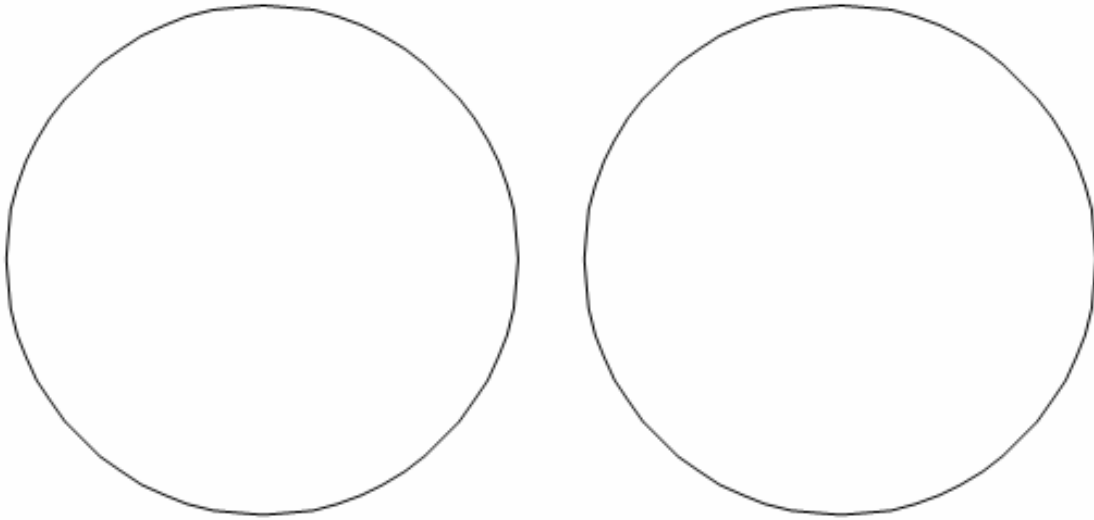
EXPECTATIONS

Talk to your three bench partners and record the sources (yours and theirs) tested below. Which of the four surfaces tested by your group do you predict will have the most microbial growth? Which do you expect will have the least? Explain your reasoning.

RESULTS

Use color pencils to draw and fully label your own plate and those of the three other students in your group. Place all labels **below** each drawing. Be sure to include the incubation time, temperature for each plate and sample source.





CONCLUSIONS

1. Which plate showed the most microbial growth? Describe the characteristics of the colonies you observed (e.g., size, shape, color, texture).
2. Were there any plates with very little or no visible growth? What are some possible reasons for this outcome?
3. After examining all four plates, yours and those of your three classmates—did the results match your expectations about where the most microbial growth would occur? If not, what might explain the differences?
4. A single swab may collect many different types of live microorganisms from a surface, but only some will grow into visible colonies on nutrient agar. This is because the medium or growth conditions may not support all types of organisms. For example, bacteria that require high temperatures won't grow if incubated at room temperature. What is one other reason a live microorganism might not grow into a colony on the plate?

3: DECONTAMINATION

LEARNING OBJECTIVES

- Observe and compare microbial growth on nutrient agar plates collected from different surfaces before and after handwashing and disinfection to evaluate the effectiveness of basic hygiene practices.
- Recognize the role of consistent handwashing and surface disinfection in minimizing microbial transmission.

BACKGROUND

Microorganisms are everywhere—including on our skin and the surfaces we touch daily. While many of these microorganisms are harmless or even beneficial, others have the potential to cause disease, especially when hygiene is neglected.

In addition, although many surfaces may appear visibly clean, they often harbor a diverse population of microorganisms. These organisms are typically undetectable without specialized tools or growth conditions, yet they persist on skin, objects, and in our surrounding environment.

This exercise is designed to visually demonstrate the presence of microorganisms on human hands and laboratory surfaces by comparing microbial growth under different conditions: prior to and following handwashing, and before and after surface disinfection. When these microorganisms are transferred to nutrient agar plates and incubated, many will multiply to form colonies allowing for direct observation of their growth.

While nutrient agar supports the growth of a wide range of bacterial species, it does not provide optimal conditions for all microorganisms. Thus, the colonies observed will represent only a subset of the total microbial population present on a given surface. Nevertheless, comparing growth between “clean” and “dirty” samples provides valuable insight into the effectiveness of basic hygiene and disinfection practices.

After completing this exercise, it is essential that all students practice proper hygiene protocols at the beginning and end of every class. Washing your hands and disinfecting your workspace not only helps maintain the integrity of your experiments but also reinforces habits that are critical in any health-related field.

MATERIALS (Per Group of Four)

4 nutrient agar plates
2 sterile swabs
1 tube of sterile water
1 lab table disinfectant
6 paper towel squares
Antibacterial hand soap

METHODS/PROCEDURES

Each student in the group chooses one of the four conditions below:

DIRTY HANDS - Student 1

1. Label the bottom of the plate (example label below)

2. Open the Petri dish just enough to gently rub the fingertips of one hand across the entire surface of the agar.
 3. Switch hands and repeat the process using the other hand, rubbing over the same plate.
 4. Close the lid and set the plate aside.
-

DIRTY BENCH - Student 2

1. Dip a sterile swab into a tube of sterile water.
 2. Rub the moistened swab over an area of the lab bench that has not been disinfected, preferably one that appears dusty or has not been recently cleaned.
 3. Partially open the Petri dish labeled *DIRTY BENCH*.
 4. Gently rub the swab over the entire surface of the agar, rotating the swab slightly to ensure full contact.
 5. Close the lid and set the plate aside.
 6. Throw the swab in the trash.
-

CLEAN HANDS - Student 3

1. Wash both hands thoroughly with antibacterial soap for at least 20 seconds (Yes, count). Pay special attention to fingertips and around the nails.
 2. Do **NOT** use a paper towel. Allow hands to air dry completely until no longer dripping.
 3. Once hands are air dried, ask a classmate to open the Petri dish labeled *CLEAN HANDS* (*this is the only time a classmate will help you with the lid*).
 4. Gently rub the fingertips of both hands one at a time over the surface of the agar.
 5. Close the lid and set the plate aside.
-

CLEAN BENCH - Student 4

1. The fourth student should apply a generous amount of disinfectant to the lab bench surface.
 2. Use a clean paper towel to spread the disinfectant across the entire bench area.
 3. Allow the disinfectant to sit for 3 minutes
 4. Dip a sterile swab into the sterile water tube then swab the cleaned bench surface thoroughly.
 5. Partially open the Petri dish labeled *CLEAN BENCH*.
 6. Gently rub the swab over the surface of the agar, rotating the swab to ensure even coverage.
 7. Close the lid and set the plate aside.
-

After all four plates are inoculated:

1. Place all four plates in the 30°C incubator, agar side up.
 2. Incubate until the next lab period.
- ◆ Reminder: From this point forward, all students must wash their hands with antiseptic soap and disinfect their lab benches at both the beginning and end of every lab session.

NAME _____

EX 3 Decontamination

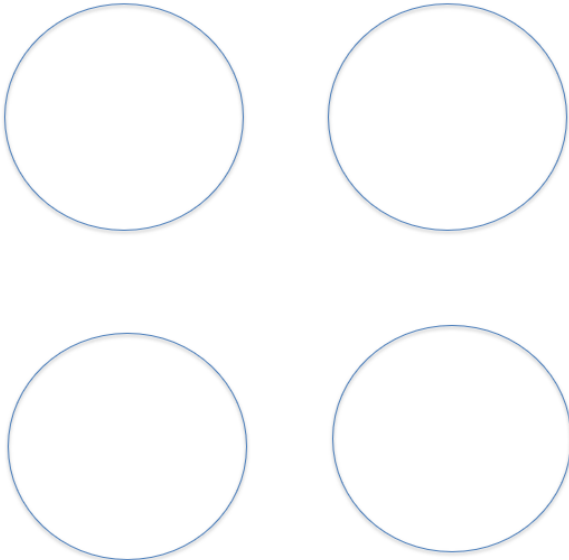
EXPECTATIONS

Do you expect to see microbial growth on any of the plates? Explain your reasoning.

RESULTS:

Using colored pencils draw what you observe on the plates after incubation.

Remember to properly label each drawing.



CONCLUSIONS:

1. Based on your observations, how effective were the disinfectants at eliminating microbes on the bench and hands?
2. If one or both plates, inoculated from the bench (or table) showed no visible microbial growth after incubation, does this mean the bench (or table) was sterile? Why or why not?
3. Describe two possible reasons why bacteria present on the surface of your hands might fail to grow into colonies when inoculated onto the agar plate.
4. Many commercials for disinfectants, toothpaste, and similar products claim to kill 99.9% of germs. How might companies use factors like temperature, growth conditions, or nutrient availability to design tests that make their products appear more effective

than they actually are? Explain your reasoning.

4: MICROSCOPY

LEARNING OBJECTIVES

- Students will be able to demonstrate proper care and handling of a compound light microscope, including cleaning, transport, and storage procedures.
- Students will accurately focus on a specimen at low and high magnification, including correct use of the oil immersion lens for observing bacterial cells.

BACKGROUND

One of the most essential tools in microbiology is the microscope (an instrument that uses lenses to magnify small objects). Before the invention of microscopes, the existence of microorganisms was completely unknown. Initially, it was difficult to persuade others—both scientists and the public—that such invisible life forms were real.

In this exercise, you will learn how to use a microscope effectively. Throughout this course, we will frequently examine bacteria which are extremely small. Proper care and precise use of your microscope will be necessary to observe them successfully.

A microscope functions similarly to a magnifying glass or eyeglasses by using glass lenses to enlarge objects. However, optimal performance depends on clean, undamaged lenses, sufficient lighting, proper alignment, and appropriate distance from the specimen. This exercise will teach you how to inspect and adjust these components to ensure accurate observations.

MICROSCOPE CARE

- Use only the microscope assigned to you.
- Always carry the microscope with two hands—one hand on the arm and the other supporting the base.
- Always lower the stage all the way down before removing a slide from the stage.
- Only use immersion oil with the 100x objective lens. Do not use oil with any other objective. If you accidentally get oil on any objective other than the 100x let the instructor know right away so it can be cleaned properly and prevent permanent damage.
- Always start with the scanning (4x) objective.

👉 Before returning the microscope to its cabinet:

- Wipe the oil immersion objective in one direction using only lens paper.
- Turn off the power and set the rheostat (brightness control) to the lowest setting.
- Rotate the 4x objective into place and lower the stage completely.
- Center the stage clip and holder so that they are balanced as much as possible over the stage.
- Coil the power cord neatly and secure it on the back of the scope.

MICROSCOPE FEATURES

OCULAR LENSES

The eyepieces, or ocular lenses, are located at the top of the microscope. Because your microscope has two, it is classified as binocular. Each ocular lens magnifies the image tenfold.

Adjust the distance between the oculars so that you can view a single, clear image with both eyes. If the image appears double or you can only see through one eye, the oculars need further adjustment.

You may remove your glasses while using the microscope. If your eyes require different prescriptions, one of the oculars can be adjusted to accommodate this difference.

👉 **Cleaning note:** Use only lens paper to clean the oculars. Dust, oils from skin, and cosmetic products like mascara can easily soil the lenses. If you are unsure whether the dirt is on the ocular lens, gently rotate it—any dirt present will move with the lens.

STAGE

The stage is the flat platform on which the microscope slide is placed. Metal clips secure the slide, which can be moved forward/backward and left/right using the adjustment knobs located underneath the stage.

LIGHT SOURCE

The light source is located in the base of the microscope. Turn it on using the “O/I” switch and adjust brightness using the rheostat on the side. Lower intensity light may appear yellow, while higher intensity becomes blue-white. Because resolution (the ability to distinguish fine detail) improves with shorter wavelengths of light, full brightness should be used with high-power lenses. Lower brightness is appropriate for viewing samples under low magnification.

OBJECTIVE LENSES & MAGNIFICATION

Above the stage is a rotating objective ring (nosepiece) that holds four objective lenses: 4x, 10x, 40x, and 100x. These provide the initial level of magnification. The image is then further magnified 10x by the ocular lenses.

For example:

- 4x objective × 10x ocular = 40x total magnification
- 100x objective × 10x ocular = 1000x total magnification

The 100x objective lens requires immersion oil to reduce light scatter and increase image clarity. **Use oil only with the 100x lens. Never allow oil to contact the other lenses. Oil will destroy the other objectives making it very difficult or impossible to focus.**

Always label your drawings with the final magnification.

The final magnification for bacteria is always 1000x.

Each objective lens must be at a specific distance from the slide in order for the landmark or sample to be in focus. Your microscope is parfocal, meaning once a slide is in focus at low power, it should remain close to focus at higher powers. Once the sample is in focus, you should not need to rotate the fine focus knob more than two full turns in either direction. If after moving to a higher power and slowly rotating the fine focus two rotations in both directions you cannot find the landmark or sample, go back to the 4x and start over. Unless there is a cover slip on the sample, once there is oil on the slide you cannot start over or back to the 40x. The lower objectives are not sealed. If oil touches any objective other than the 100x the objective oil will get into the lens and destroy the objective.

👉 **Technique tip:** Using a Sharpie pen, draw a circle (referred to as a landmark) on the slide around the sample. Be sure the circle and the sample are on the same side of the slide. Bacterial cells and the pen mark (circle) are on the same focus plane therefore when the circle is in focus the bacteria will also be in focus.

Remember: If at anytime (before adding oil to the slide) you experience difficulty focusing at higher magnifications, return to the 4x objective and refocus on the pen mark. Once there is oil on the slide DO NOT return to lower magnification. Ask your instructor for assistance.

MATERIALS (Per Group of 4)

• 4 Microscopes (One per student - only use your assigned microscope)

- 1 *Saccharomyces cerevisiae* slide
- 1 *Amoeba spp* slide
- 1 Mixed bacteria slide
- 1 Black Sharpie medium point pen

METHODS/PROCEDURES

STEPS TO FOCUS

1. Make sure the stage is in the lowest position by gently rotating the course focus knob (do not force it).
2. Rotate the scanning objective (4x) into position directly above the stage.
3. Place the slide on the stage and secure it in place with the stage clip.
4. Adjust the slide so that either the pen mark (landmark) is directly over the light source.
5. Use the course focus knob to raise the stage all the way up.
6. Use both eyes to look through the oculars while slowly rotating the coarse focus knob to slowly bringing the stage down until the landmark (pen mark/circle) comes into focus.
7. Use the fine focus to sharpen the landmark.
8. Increase the magnification by rotating to the 10x objective then use the fine focus to sharpen the landmark.
9. Center the landmark
10. Rotate to the 40x objective. **From now on use only the fine focus knob.**
11. Increase the light intensity.
12. Focus on the landmark - you may start to see bacterial cells under 40x objective.
13. Look through the oculars and slowly turn the coarse focus knob to lower the stage slightly, which will bring the pen mark into sharp focus. Once the image is clear at 4x, scan the slide to find a suitable area to observe. Make sure the region of interest is centered in the field of view. At higher magnifications, only a small portion of the slide is visible—any object not centered at low power will be lost from view at high power.

Using the Oil immersion Objective:

1. Once the landmark is centered and as sharp as possible (some of the 40x objectives have gotten oil on them by previous students and therefore the sharpness is limited) **DO NOT LOWER THE STAGE.** Rotate the objective ring past the oil objective lens until the space between the 100x and 4x is over the sample. Place a drop of immersion oil directly on the sample. Remember - do not lower the stage - rotate the 100x oil immersion lens into position. **Using only the fine focus knob**, adjust the image until it is sharp.

👉 Never use the coarse focus knob when the 100x lens is in place. The lens and/or slide may crack.

✎ Although it may appear that the 100x objective is too close and will hit the slide, as long as the sample is in focus at 40x the 100x lens will be at the right height to move into place without hitting the slide. The oil must touch the 100x objective in order for the light to be directed into the lens properly - otherwise you will not be able to see the bacteria.

You must focus the sample/landmark under each objective as you work your way up to the 100x objective, however, only draw the sample at the magnifications listed in the table below.

Pen Mark	X40	X100	X400	X1000
Amoeba	X40	X100	X400	-----
Saccharomyces	***	***	X400	X1000
Mixed bacteria	***	***	X400	X1000

*** view and focus but do not draw

--- do not view/focus or draw at that magnification

Drawings are to be done as you look through the microscope. You are welcome to take pictures if you want to, but drawings are to be completed in class while looking at the object through the microscope. All labels are to be placed directly under your drawing. Labels should include: genus, species (or name of the object) and the final magnification.

Remember, the genus can be abbreviated but not the species can not. Drawings should be done using colored pencils. Do not use ink pens as they will bleed through to the back of the page.

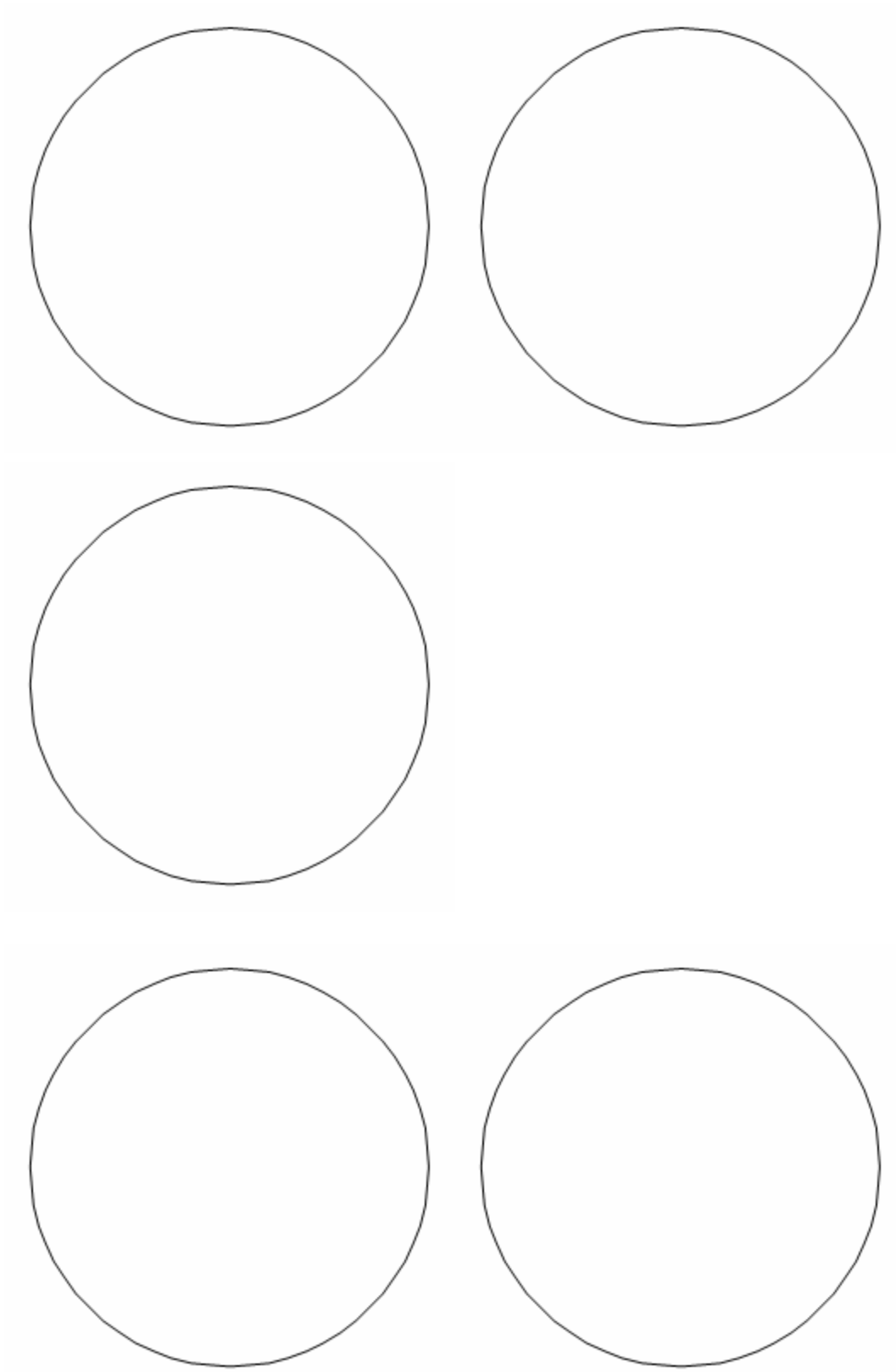
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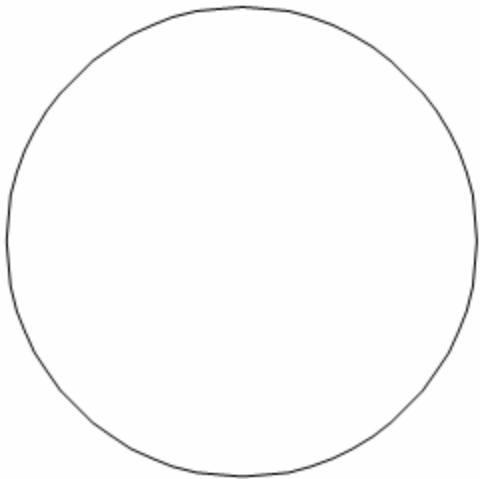
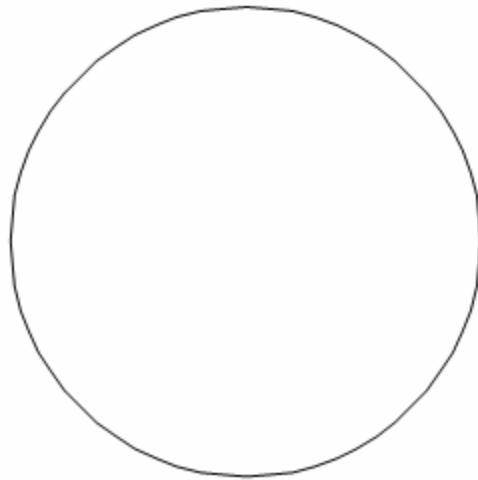
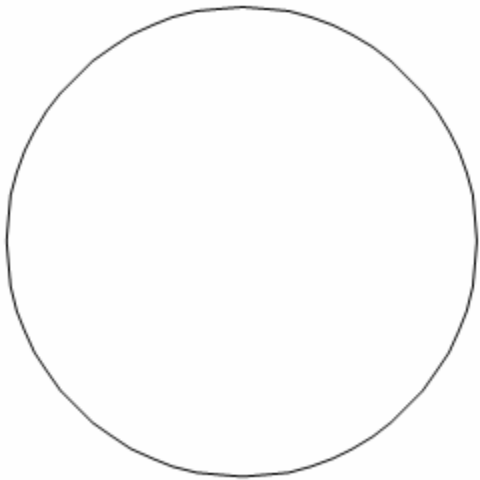
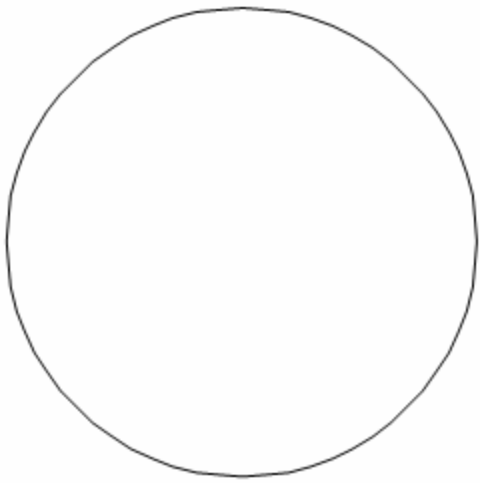
EX 4 Microscopy

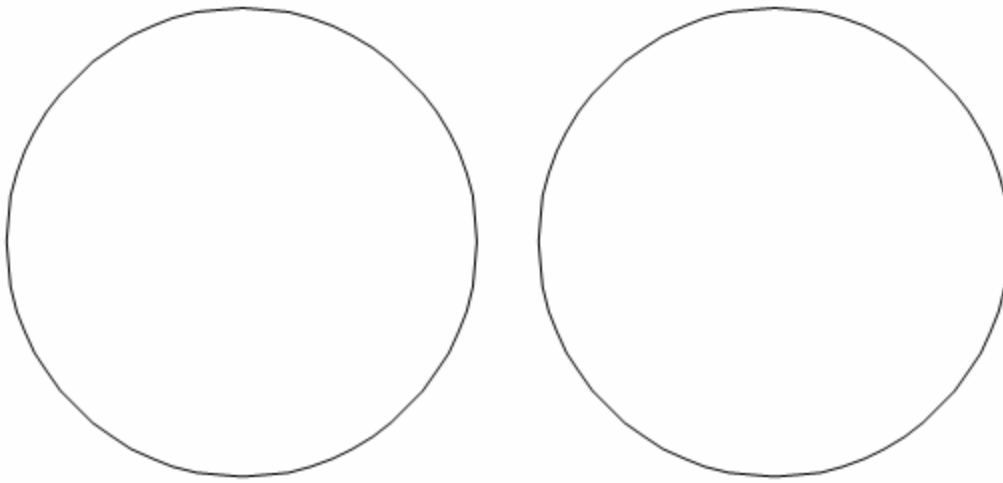
EXPECTATIONS

If you prepare a bacterial smear without staining it, what do you anticipate the bacteria will look like at 100x?

RESULTS







CONCLUSIONS:

1. When switching from 40x to 10x, would you expect to see more of the specimen or less of the specimen in your field of view? Why?
2. Which magnification provided the clearest view of the amoeba? Why is it difficult to focus clearly on thicker specimens when using high-power magnification? Explain your answer.
3. Which objectives can the course focus (the large dial) be used with? _____
4. Which objectives can the course focus (large dial) NOT be used with? _____
5. What objective(s) require the addition of immersion oil and what is the purpose of the oil?
6. What will happen if immersion oil is used with the wrong objective(s)?

5: MEASURING WITH A MICROSCOPE

LEARNING OBJECTIVES

- Describe the relationship between magnification and field of view in compound light microscopes.
- Identify how different objective lenses (4x, 10x, 40x, 100x) affect the visible area and level of detail.
- Estimate the actual size of specimens in micrometers by comparing them to calibrated measurements.

BACKGROUND

Microscopes make it possible to observe objects that are far too small to be seen with the naked eye, such as bacteria, plant cells, or grains of sand. When viewing these microscopic structures, it is important to determine their actual size. This is accomplished through a process called **calibration**, which allows accurate measurement through the microscope.

The area visible when looking through a microscope is called the **field of view**, and its size depends on the objective lens in use. Most microscopes include 4x, 10x, 40x and 100x objectives. The higher the magnification the more detail seen, however, the size of the field of view is decreased. For example, if a specimen fills the entire field at 4x magnification, it must be significantly larger than something that fills the field at 40x. This provides a general idea of an object's size, but more precise measurement requires an established reference.

To make accurate measurements, a **micrometer slide** is used. This special slide has a fine ruler etched into the glass with units marked in **micrometers (μm)**. One micrometer equals one-thousandth of a millimeter. For context, a human hair is roughly 70 micrometers wide.

Calibration is performed by placing the micrometer slide on the microscope stage and viewing it under each objective lens. By counting how many micrometer divisions span the field of view at each magnification, it is possible to calculate how much of the specimen is being viewed. This reference makes it possible to estimate the actual size of unknown samples. For instance, if the field of view is known to be 400 micrometers wide and a specimen fills half the field, then its approximate size is 200 micrometers.

Accurate measurement under the microscope plays an important role in many fields. Medical laboratory technicians measure blood cells to help diagnose disease. Environmental scientists study the size of microorganisms in water or soil to help in determining contamination. Forensic scientists compare the length and diameter of hairs or fibers found at crime scenes. In clinical settings, the size of a microorganism or cell is an essential clue for identification. Two species may appear visually similar but differ slightly in length or width. Even a few micrometers of difference can indicate a different organism or health condition.

In this lab, you will observe several microscopic specimens under various magnifications. By drawing each sample at different powers and comparing them to the calibrated micrometer slide, the actual size of each specimen can be estimated in micrometers.

MATERIALS (Per Student)

- 1 Micrometer slide
- 1 Prepared letter "e" slide
- 1 Clean microscope slide

- 1 Strand of hair
- 2 Pieces of Tape
- 1 Prepared WBC slide
- 1 Prepared RBC slide

METHODS/PROCEDURES

1. Place the micrometer slide on the microscope stage and center it so the etched scale is clearly visible in your field of view.
2. Focus carefully using the lowest objective lens. Once the image is sharp, draw what you see. Make sure your drawing includes the entire circular field of view and shows how much of the micrometer scale is visible. Label the magnification used.
3. Repeat step 2 for each of the remaining objective lenses. You should end up with four micrometer drawings, one for each magnification.
4. Tape a strand of your hair onto a clean microscope slide. Run the single strand of hair down the middle of the microscope slide horizontally then tape the hair to the edges of the slide. Be sure the tape is not in your field of view and lays flat on the slide. You do not need much tape. One 1 X 1cm piece on each edge is sufficient.
5. Observe and draw the prepared sample slides. For each sample, the magnifications to be drawn are listed in the table below. Always start with the 4x objective but only draw what you see the field of view at the magnifications listed in the table.

*It is important that you carefully observe and draw exactly what you see and your drawing is proportional to the full field of view—show how much of the circle the sample takes up. Inaccurate drawings will affect your results

6. Once all drawings are complete, use your micrometer drawings to estimate the actual size of each sample by comparing the sample drawing to the micrometer drawing made at the same magnification.
7. Record the estimated size of the samples in the results section.

*Remember that the full micrometer scale represents 1 millimeter (1 mm), which is equal to 1,000 micrometers (μm).

**Always start with the 4x objective.
Only draw the samples at the magnifications indicated:**

Slide micrometer	x40	x100	x400	x1000
Newsprint letter "e"	x40	x100		
Your own hair taped to a slide	x40	x100		
Blood smears (RBC and WBC)			x400	x1000

The following is to be completed during lab then turned in on Canvas as a PDF

*If you are using an iPad or tablet you will need to take screen shots of your competed work, save the screenshots as one PDF then submit them on Canvas by the due date designated on Canvas.

*You can also print out the entire exercise to bring to lab with you. If you choose to complete the lab on paper, take pictures of the completed results and conclusions sections only, save them as one PDF, then submit to Canvas by the due date designated on Canvas.

NAME _____

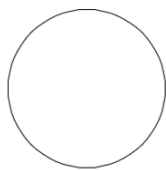
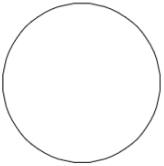
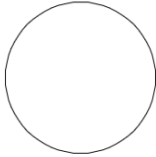
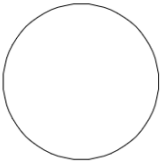
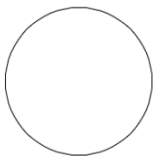
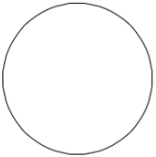
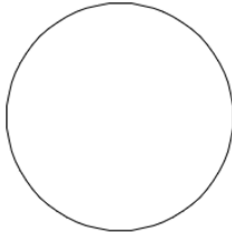
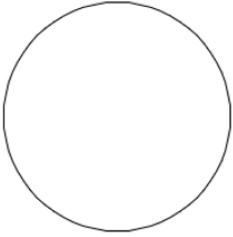
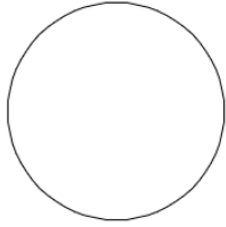
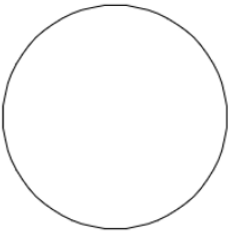
EX 5 Measuring with a Microscope

EXPECTATIONS

What do you think will happen to the size of the field of view as you switch from low power to high power on the microscope?

RESULTS

Draw your observations below. Remember to label underneath the circle with the name of the sample and final magnification.



Results Table			Estimated Size	
Specimen	Magnification	Estimated Specimen Size (um)	Newspaper Letter "e" _____	
Slide Micrometer			Hair Strand _____	
Slide Micrometer			White Blood Cell _____	
Slide Micrometer			Red Blood Cell _____	
Slide Micrometer				
Slide Micrometer				
Newspaper Letter "e"				
Newspaper Letter "e"				
Hair Strand				
Hair Strand				
White Blood Cell				
White Blood Cell				
Red Blood Cell				
Red Blood Cell				

CONCLUSIONS

1. Which specimen was the most difficult to measure? What challenges did you face, and how did you handle them?
2. Which specimen was the easiest to measure accurately? What made it easier to estimate its size?
3. If two different samples both fill the field of view at different magnifications, what might that tell you about their actual size?
4. How might an incorrect size estimate under the microscope lead to a mistake in diagnosing a patient's condition?

6: STERILE TECHNIQUE

LEARNING OBJECTIVES

- Differentiate between nutrient broth, nutrient agar slants, and deeps, and describe the purpose of each medium.
- Use an inoculating loop and needle correctly for transferring microorganisms to various media types.

BACKGROUND

A central challenge in microbiology is maintaining the purity of microbial cultures while accurately tracking where organisms have been introduced, despite the fact that individual microorganisms are not visible to the naked eye. It is essential to develop consistent habits that allow for reliable identification of inoculated versus uninoculated media throughout experimental procedures.

Maintaining a systematic approach is one of the most effective strategies for achieving this goal. For example, always flame sterilize the inoculating instrument before returning it to the test tube rack or work area unless it has already been sterilized. This practice minimizes the risk of unintentionally spreading viable organisms to laboratory surfaces, personal items, or other individuals. To further support accuracy, uninoculated media should be consistently positioned on one side of the workbench or test tube rack, and inoculated media should be placed on the opposite side.

In addition to tracking inoculations, it is essential to avoid contamination. Contamination occurs when unwanted microorganisms are inadvertently introduced into a culture. This can happen through exposure to airborne particles, contact with the laboratory bench or hands, use of improperly sterilized instruments, or even from exhaled breath. Avoiding contamination is especially critical in clinical and diagnostic applications. For instance, if a patient sample becomes contaminated during processing, it may lead to an incorrect identification of the pathogen and inappropriate treatment. Such errors can result in prolonged illness, treatment failure, or serious complications for the patient.

The prevention of contamination relies on the use of aseptic technique, a set of practices designed to minimize the introduction of extraneous microorganisms into cultures and the surrounding environment. Even for individuals not pursuing careers in microbiology, aseptic technique has practical benefits. These include limiting the transmission of illness in everyday settings, reducing the risk of foodborne disease, and extending the shelf life of perishable items.

This exercise introduces fundamental aseptic techniques used to transfer microorganisms between different types of culture media. In this lab, you will work with nutrient broth (a liquid medium) and nutrient agar (a solid medium). Nutrient agar is commonly prepared in three configurations: slants, deeps, and Petri plates.

When nutrient agar is dispensed into a test tube and allowed to solidify at an angle, it forms a slant. The slanted surface provides a larger area for microbial growth than if the medium had solidified in a vertical position. Slants are commonly used for storing cultures over extended periods. Once growth is established, the slant can typically be stored under refrigeration for several weeks or months, depending on the organism. Although Petri plates can also be used for storage, they are more susceptible to contamination and tend to dry out more quickly than slants.

When nutrient agar solidifies in an upright position within a test tube, it forms a deep. Deepes are used to support the growth of anaerobic organisms—microorganisms that do not require oxygen—and to assess whether an organism can grow in the presence or absence of oxygen. The top of the deep provides an oxygen-rich environment, while the bottom is largely anaerobic. Inoculation of deeps is performed using an inoculating needle, which is similar to a loop but lacks the circular wire end. The needle is used to insert cells deep into the agar while minimizing the introduction of air.

Proficiency in these foundational techniques is essential for future exercises, particularly those involving pathogenic organisms. The types of media used in this activity represent standard tools in microbiological practice: slants for maintaining cultures, broths for generating large cell populations, and deeps for investigating anaerobic growth.

MATERIALS

1 nutrient agar slant

1 nutrient agar deep

1 nutrient agar broth

Inoculating loop

Bunsen burner

Sterile nutrient broth tube (liquid medium)

Source culture (agar plate or broth tube)

Test tube rack

Permanent marker

One of the following: (Instructor will hand out)

Escherichia coli

Saccharomyces cerevisiae

Penicillium roqueforti

Micrococcus luteus

METHODS/PROCEDURES

SAFETY REMINDERS

Never place the cap of the test tube on the bench.

Always carry test tubes in a rack.

Be aware of the open flame – never leave a flame unattended.

Always rotate the tube not the cap

Use a permanent marker (Sharpie) to label your tubes of media

First initial

Full last name

Genus, species and strain (if designated)

Exercise #

Date

👉 Only write on the glass – do not write on the white portion of the tube.

👉 You can abbreviate the genus but not the species

Arrange your workspace

Place your materials within easy reach. Position the sterile broth tube on one side of your test tube rack and the source culture on the other side to help avoid cross-contamination.

1. Light your Bunsen burner

2. **Inoculate the broth:**

a. Sterilize the inoculating loop

Hold the loop in the flame until it becomes red-hot. Be sure the entire wire is sterilized. Then, allow the loop to cool for 10–15 seconds. Do not wave the loop in the air or blow on it to cool it.

b. Obtain the source culture

c. If the culture is on a plate: slightly lift one side the lid and gently touch the loop to a colony on the surface of the agar. Close the lid immediately.

d. If the culture is in a broth tube: remove the cap with the little finger of your loop hand. Flame the lip of the tube. Insert the cooled loop and collect a small amount of culture. Flame the lip again and recap the tube.

e. Remove the cap of the sterile broth tube using the same hand that holds the loop.

f. Briefly flame the lip of the broth tube.

g. Insert the loop into the broth and gently swirl or stir to release the cells.

h. Remove the loop, flame the lip of the tube again, and replace the cap loosely by rotating the tube not the cap.

i. Re-sterilize the loop by holding it in the flame until it is red-hot. Return it to the instrument block when cool.

j. Place the inoculated broth tube back in the test tube rack on the same side as the source culture.

3. **Inoculate the slant**

a. Sterilize the inoculating loop

Hold the loop in the flame until it becomes red-hot. Be sure the entire wire is sterilized. Then, allow the loop to cool for 10–15 seconds. Do not wave the loop in the air or blow on it to cool it.

b. Obtain the source culture.

c. If culture is on a plate: slightly lift the lid on one side and gently touch the loop to a colony on the surface of the agar. Close the lid immediately.

d. If culture is in a broth tube: remove the cap with the little finger of your loop hand. Flame the lip of the tube. Insert the cooled loop and collect a small amount of culture. Flame the lip again and recap the tube.

e. Hold the slant in your non-dominant hand and remove the cap with the little finger of your dominant hand (the one holding the loop). Do not set the cap down. Flame the lip of the slant tube.

f. Quickly pass the open mouth of the tube through the flame.
Inoculate the slant

g. Insert the loop and gently touch the surface of the agar at the bottom of the slant. Slowly draw the loop upward in a zigzag or wavy line along the surface of the slant to the top. Avoid gouging or cutting the agar.

Finish the transfer

- h. Carefully remove the loop. Flame the mouth of the tube again and replace the cap loosely. Do not tighten the cap to allow for gas exchange during incubation.
Flame the loop.
- i. Re-sterilize the loop by holding it in the flame until it is red-hot. Return it to the instrument block when cool.
- j. Place the inoculated slant tube back in the test tube rack on the same side as the source culture.

4. Inoculate a nutrient agar deep

Sterilize the inoculating loop

- a. Hold the loop in the flame until it becomes red-hot. Be sure the entire wire is sterilized. Then, allow the loop to cool for 10–15 seconds. Do not wave the loop in the air or blow on it to cool it.

Obtain the source culture.

- b. If culture is on a plate: slightly lift the lid on one side and gently touch the loop to a colony on the surface of the agar. Close the lid immediately.

If culture is in a broth tube: remove the cap with the little finger of your loop hand. Flame the lip of the tube. Insert the cooled loop and collect a small amount of culture. Flame the lip again and recap the tube.

- c. Open the sterile deep tube.

Hold the deep tube in your non-dominant hand. Remove the cap with the little finger of your dominant hand, which is also holding the inoculated needle. Do not set the cap down.

- d. Flame the lip of the deep tube.

Quickly pass the open mouth of the tube through the flame to maintain sterility.

- e. Inoculate the deep.

Insert the needle straight down into the center of the agar. Push the needle almost to the bottom of the tube in a single, steady motion. Do not let the handle touch the agar. Then withdraw the needle along the same path without wiggling or disturbing the agar. This technique minimizes oxygen introduction into the stab line.

- f. Finish the transfer.

Flame the mouth of the tube again and loosely replace the cap to allow for gas exchange during incubation.

- g. Flame the needle.

Re-sterilize the needle by heating it until red-hot. Return it to the rack or holder once cooled.

5. Incubate the inoculated media

Transfer the 3 inoculated test tubes in to the appropriate colored test tube racks for your section then place the rack in the class incubator.

NAME _____

Ex 6 Sterile Technique

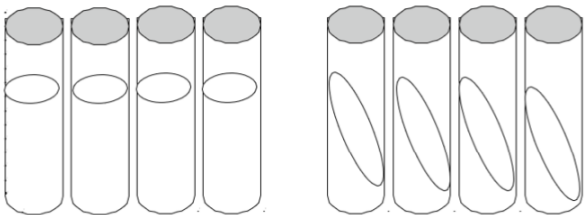
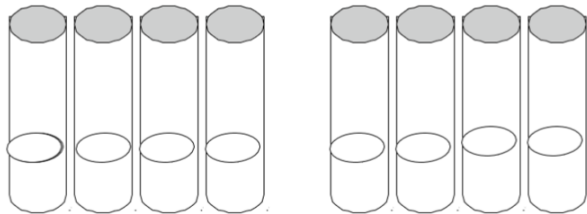
EXPECTATIONS

Do you think you were able to transfer cells from the original culture to the new medium without introducing contamination? Yes or No _____

How will you be able to tell if you were successful?

RESULTS

Draw your observations of the growth in the tubes after incubation. Label each tube separately. Draw the broths as they appear when you remove them from the incubator then again after shaking them. Make sure everyone in your group has had the opportunity to draw the broths before shaking.



CONCLUSIONS

Were you successful in transferring the organisms without introducing contamination? _____

Explain how you know.

2. Why is it important to keep culture tube caps slightly loosened during incubation?

3. Which of the three culture conditions (broth, deep, slant) is the only one in which you can make a judgment about oxygen use? Explain your answer.

4. If you accidentally introduced air bubbles while inoculating the deep how might that interfere with interpreting the organism's oxygen requirements?

5. Which, if any, of the four organisms was able to grow anaerobically?

6. Which, if any, of the four cultures is an absolute aerobe?

7. Which, if any, the cultures are(is) facultative anaerobes?

7: SMEAR AND SIMPLE STAIN

LEARNING OBJECTIVES

- Prepare bacterial smears from both liquid and solid cultures and apply a simple staining technique to enhance visualization of bacterial cell morphology under the microscope.
- Evaluate the quality of a smear and staining result based on the clarity, distribution, and visibility of bacterial cells when viewed using oil immersion at 1000x magnification.

BACKGROUND

Even under a high-quality light microscope, bacteria appear as extremely small, nearly transparent dots. To distinguish differences in shape and arrangement, bacterial cells must first be stained. In some cases, specific types of bacteria can also be identified based on how they react to particular chemical stains.

The initial step in observing and identifying bacteria microscopically is to prepare a **smear** (a thin, even layer of bacteria spread onto a glass slide) and apply a staining technique. Accurate interpretation is not possible if the smear is too thick, clumped, or sparsely populated. A well-prepared smear distributes an appropriate number of cells evenly across the slide, allowing for clear observation under the microscope.

Several factors should be considered when preparing smears:

- **Smear Size:** Avoid making smears that are unnecessarily large. Larger smears may take longer to dry and may spread cells too thinly, making them difficult to locate. Aim to use only the area needed for effective viewing.
- **Cell Concentration:** The density of the original culture will affect how the smear should be prepared. For dilute liquid cultures, it may be necessary to layer several drops to increase the cell concentration. For samples from solid media, such as agar plates, a small portion should be mixed with water on the slide to prevent overcrowding. It is essential to maintain an appropriate cell density to observe individual bacterial shapes clearly.
- **Slide Cleanliness:** A clean, smooth glass surface is essential. Oil, dust, or scratches can interfere with smear quality, causing poor adherence or visual artifacts. Slides should be cleaned with **95% ethanol** and dried thoroughly prior to use.
- **Sample Location:** Clearly marking the slide is recommended to help locate the smear during microscopy. It is also helpful to scan the entire smear at low power before switching to higher magnification to avoid clumped or unevenly stained areas.

Because bacteria are transparent, they must be stained to become visible under the microscope. A **simple stain** involves the use of a single dye that uniformly colors all cells. In today's exercise, we will use **Loeffler's Methylene Blue**, a commonly employed stain that enhances visibility of bacterial morphology. While many different dyes may be used, as long as only one is applied and it stains all cells similarly, the procedure qualifies as a simple stain.

MATERIALS

- 1 Nutrient broth culture of bacteria
- 1 Culture of bacteria on a nutrient agar slant
- 1 Culture of bacteria in nutrient broth

1 Staining tray and rack

1 Bottle Loeffler's methylene blue syain

METHODS

◇ The glass slides are *not* sterile. Once your loop touches a slide it is contaminated.

You must re-sterilize the loop in the flame every time before inserting it into a culture.

1. Label two glass microscope slides with your name, name of the organism you are working with, type of stain, (simple stain) and the date.
2. Place the two labeled slides onto a paper towel or white piece of paper. Use a pencil to trace the outline of the slides then remove the slides and place them on the staining rack. Add a line on the diagram that designates the label space. Then draw three circles that represent where you will place the cells. There is an example diagram at the end of the methods section.

◇ Be sure to leave at least a centimeter between the edge of the slide and the third circle on the diagram or your slide will fall into the opening of the stage when you try to focus.

3. Place the slides on top of the diagram you drew.

Preparing a Smear From a Solid Medium (agar plate or slant)

◇ Cell from a solid culture (agar plate or slant) are highly concentrated and must be diluted in water on the slide in order to see the individual cells.

Always add the water to the slide *before* the bacteria are added.

4. Use the DI water bottle to place 1-2 drops of water on a blank slide.
5. Sterilize your inoculating loop by holding it in the blue flame of the Bunsen burner until it glows red-hot. Allow it to cool for 5-10 seconds before touching any materials.
6. Using your sterilized loop, transfer two-three loopful of water to each of the circles outlined on the diagram under the slide.
7. Using a sterilized loop collect enough of the cells from the solid culture to easily see on the loop and place them in the first circle on the slide. Mix the cells in the water to dilute them. Be sure to stay within the boundaries of the first circle on the diagram.
8. Using a sterilized loop collect a tiny pinpoint of cells from the same solid culture then place the cells in the middle circle on the slide. Mix the cells in the water to spread them out but remain within the boundaries of the middle circle on the diagram.
9. Using a sterilized loop barely touch the surface of the culture (you should not be able to see any cells on the loop) then place the cells in the third circle on the slide. Mix the cells in the water to spread them out but remain within the boundaries of the third circle on the diagram.
10. Allow the smear to air dry on the slide warmer until completely before moving on to heat-fixing and staining.

Preparing a Smear From a Liquid (broth)

1. Wipe any remaining water off the slide from above Place the slide on the diagram you drew.

- Using a sterilized loop add one loopful of the culture in the nutrient broth (liquid medium) to the first circle on the diagram. use care when spreading the cells in the circle – stay within the boundaries.
- Using a sterilized loop add 3 loopful of the culture in the nutrient broth (liquid medium) to the middle circle on the diagram. Remember to use care when spreading the cells in the circle – stay within the boundaries.
- Using a sterilized loop add 5 loopful of the culture in the nutrient broth (liquid medium) to the first circle on the diagram. Remember to use care when spreading the cells in the circle – stay within the boundaries.
- Allow the smear to air dry on the slide warmer until completely before moving on to heat-fixing and staining.

Example

Name Culture name Type of stain Date	
---	--

Heat Fixing Smears:

Heat fixing ensures the cells adhere to the slide and are no longer viable (alive).

- Using a **clothes pin**, hold the slide at an angle and pass it slowly through the **blue cone of the Bunsen burner flame** for 2-3 seconds.

⚠ If you detect a burning odor, the slide is too hot.

- Allow the slide to cool before staining to prevent altering the chemical properties of the stain.

Staining Smears:

- Once the slide is cool, cover each smear completely with **Loeffler's Methylene Blue** stain.
- Let the stain sit for **30-60 seconds**.
- Pick up the slide by the frosted end using your clothes pin and tilt it slightly.
- Gently rinse off the stain using the distilled water wash bottle until no excess dye remains.
- Dispose of used stain and rinse water from your staining tray in the sink.
- Rinse and clean both your tray and the drying rack thoroughly. Dried stain on the rack can transfer to future slides and affect staining results.

Viewing the Cells:

- Place the dried, stained slide back on the diagram you drew.
- Using a medium sharpie pen, trace the landmark circles onto the top of the glass. The circles should surround the inoculate on the slide. These reference marks will help you locate samples under the microscope.
- Place the slide in the stage clip on the microscope stage. Focus on the sharpie circle under the 4x objective. Progress through the objectives (10x then 40x) centering the sharpie edge in the center of the field of view as you go up in magnification.

4. Once the pen mark is focused under the 40x objective, rotate the objectives to the open space between the 100x and 4x objectives. Place a drop or two of oil directly on the slide in the middle of your field of view where the light source is shining through. Use the the black ring to rotate the 100x (oil immersion lens) in place.
5. Once you have the cells on the slide in focus at 100x (oil immersion lens) do not lower the stage to view other areas on the slide. You can place oil anywhere on the slide then scan over to view it without lowering the stage and starting over.
6. Draw what you see for all three smears on each slide under oil in the results section.
 - ◇ Bacteria can only clearly be viewed under 1000x with immersion oil, however, always start with the 4x objective then progress through each objective focusing as you go. Since bacteria cannot be seen at lower magnification always focus on the inner edge of the landmark (sharpie circle) then scan the area inside the circle to find the cells. If the sharpie is in focus the bacteria will be too.

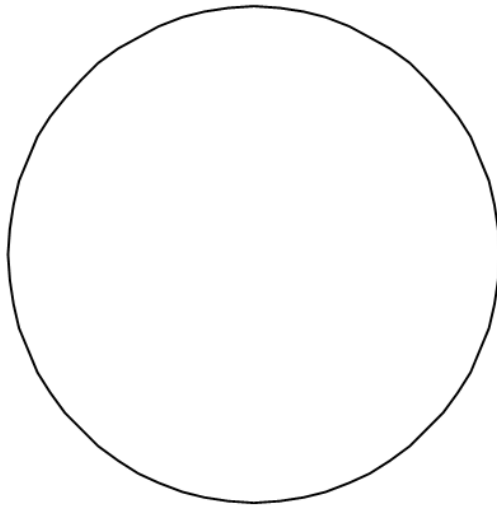
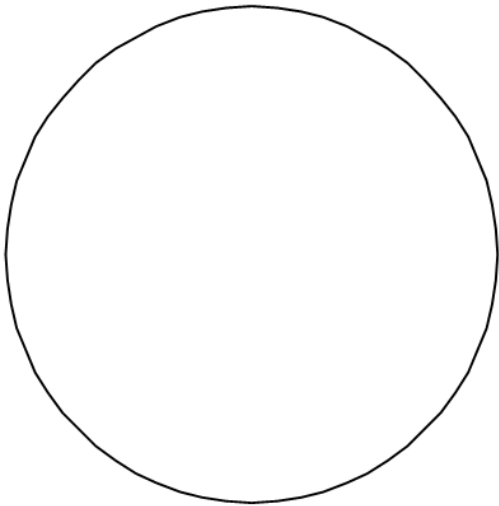
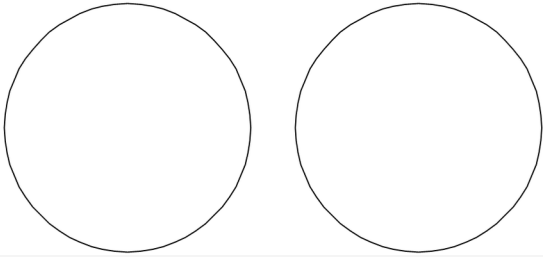
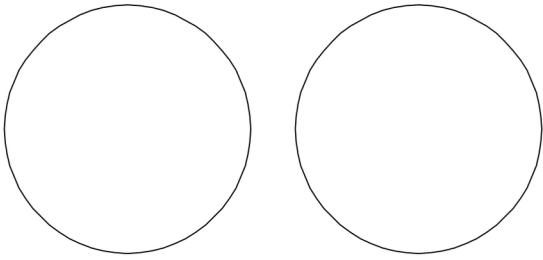
NAME _____

EX 7 Smear and Simple Stain

EXPECTATIONS

How many loopful of the broth do you think are necessary in order to see any cells on the slide?

RESULTS



CONCLUSIONS

1. Compare your results from the liquid (broth) culture and the solid (agar slant) culture.

Explain which technique gave you the clearest, most useful smear and why.

2. Why do bacteria have to be stained before viewing under a microscope?

3. Are simple stains always blue?

4. List two important reasons why heat fixation is necessary before staining.

5. Explain two reasons a sharpie is used to draw a circle on the slide?

6. Why should you not use a sharpie to label the slide?

8: GRAM STAIN

LEARNING OBJECTIVES

- Interpret Gram stain results to determine bacterial classification.
- Apply the Gram staining technique in a laboratory setting with attention to timing and accuracy.

BACKGROUND

The Gram stain is considered the most essential staining technique in microbiology. Developed by Hans Christian Gram, it originated from his experiments with various dyes and their interactions with bacterial cells. Through this work, he discovered a sequence of staining and rinsing steps that caused some bacteria to retain color while others did not. Today, the method includes a second stain to color the previously unstained cells, allowing both types to be seen under a microscope. As long as the two stains are different colors, the two groups of bacteria can be distinguished. This differential staining became the foundation for classifying bacteria, with most falling into one of two categories: Gram-positive, which retain the primary stain, and Gram-negative, which do not and instead take up the counterstain.

Although the Gram stain was developed before scientists understood bacterial cell wall structure, we now know that the staining outcome is determined by the composition of the cell wall. Gram-positive bacteria have thick layers of a substance called peptidoglycan, which traps the stain. In contrast, Gram-negative bacteria have much thinner peptidoglycan layers, allowing the stain to be washed away more easily. Some bacteria are Gram-variable, meaning their staining results are inconsistent, while others have unusual or even absent cell walls. Because the type of cell wall is so critical to identifying bacteria, the Gram stain is often the first test performed in microbiological analysis and remains essential for accurate classification.

The effectiveness of the Gram stain depends heavily on precise timing and technique. The duration of each staining and rinsing step can significantly affect the final appearance of the cells. For example, if the primary stain and mordant are left on too long, Gram-positive cells may appear nearly black, and Gram-negative cells may look dark blue. On the other hand, excessive rinsing with alcohol can cause Gram-positive cells to lose their color, making them appear pale lavender, while Gram-negative cells may become faintly pink. Because of these variables, it is crucial to include control samples with known Gram-positive and Gram-negative bacteria on the same slide. Without these controls, it is impossible to interpret the results with confidence.

Several factors can interfere with the accuracy of a Gram stain. If bacterial cells are unhealthy or dead, their cell walls may become compromised, causing Gram-positive cells to appear Gram-negative. Clumps of cells can also lead to misleading results, as uneven exposure to stains and rinses may cause Gram-negative cells in the center of a clump to retain the primary stain. This can create the false impression of a mixed population. Similarly, if too few cells are present on the slide, it becomes difficult to distinguish their color or shape, and artifacts such as scratches or stain crystals may be mistaken for bacteria. In many cases, a sparse sample indicates poor bacterial growth, which often correlates with unreliable staining results.

MATERIALS (Supplies Per Group of 4: Each student makes their own slides)

Gram positive control

Gram negative control

Alcohol (95% ethanol)

Microscope slides

Grams iodine

Safranin

Crystal violet

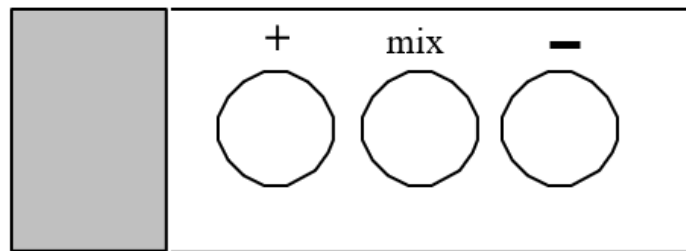
Staining tray

Water wash bottle

METHODS

Make 2-3 slides as follows:

1. Place a slide on top of the diagram below. Smear the Gram positive control in the left circle (+). Smear the Gram negative control in the circle to the right (-). In the center circle (mix) place one loopful of the gram positive control and one loopful of the Gram negative control then use the loop to mix the two controls together. Take care to keep the smears within their designated circles.



2. Place the slides on the slide warmer and allow all smears to dry completely.

3. Heat-fix all slides. Place **one** slide on the staining tray rack and set the others aside for later use.

DO NOT STAIN ALL SLIDES AT ONCE - ONLY STAIN **ONE** THEN LOOK AT IT UNDER THE MICROSCOPE

4. Cover the three smears on the slide with crystal violet and let it sit for 60 seconds.

5. Holding the slide with a clothes pin, tilt the slide and gently rinse with water for 2 to 3 seconds.

6. Flood the slide with Gram's iodine then let it sit for 60 seconds.

7. Holding the slide with a clothes pin, tilt the slide again and rinse gently with water for 2 to 3 seconds. Drain thoroughly.

8. Flood the slide with alcohol for 2-3 seconds then immediately gently rinse the slide with water to stop the decolorization.

9. Flood the slide with safranin and allow it to sit for approximately 60 seconds.

10. Holding the slide with a clothes pin, tilt the slide and gently rinse with water until the rinse is clear.

11. Dry the bottom of the slide then place the slide on the slide warmer until completely dry (about 5 minutes)

12. Put the slide on top of the diagram then use a sharpie pen to trace the landmarks on to the side.

12. Examine the smears under a microscope. If the color difference between Gram-negative and Gram-positive control cells is unclear, try staining another dried smear using slightly adjusted timing. Consult your instructor if you are unable to achieve a distinct color contrast between the two cell types.

NAME _____

EX 8 Gram Stain

EXPECTATIONS

Take a moment to examine your smears without using the microscope. Do they appear too thin or overly clumped? Are cells visible across all smear areas? Can you clearly distinguish between Gram-negative and Gram-positive cells based on the color differences in your staining

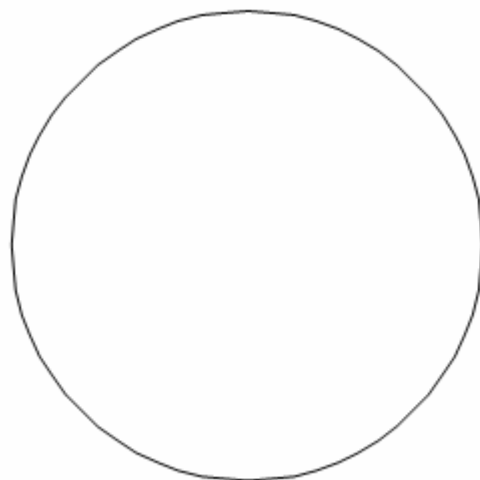
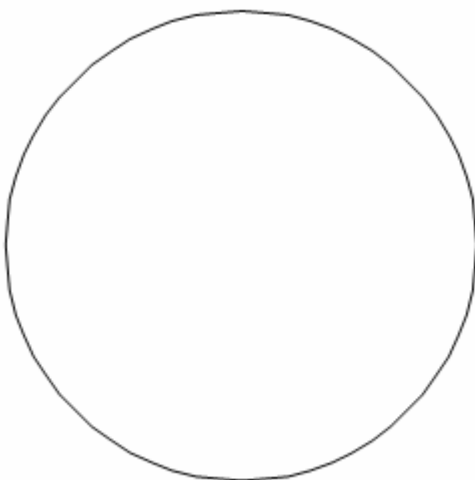
RESULTS

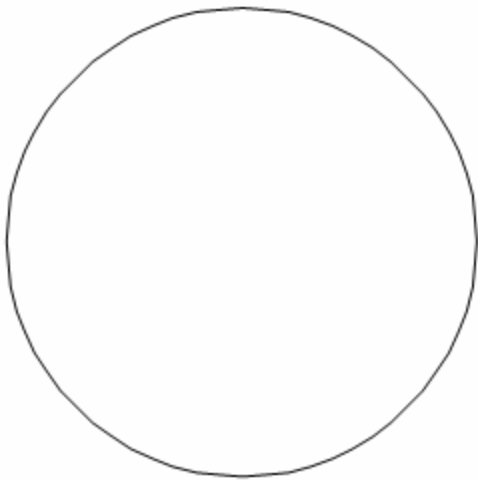
After staining one slide, examine the cells under the microscope.

Remember to always begin with the lowest magnification (4x). If the smear is difficult to locate, use the landmark (the circle you drew with the Sharpie) as a guide. When the Sharpe is in focus the cells will be as well. Make sure the area you center in the field of view accurately represents the overall smear, not just an isolated or atypical section.

Once the cells are in focus at 40x slide the objective to the side and apply immersion oil directly to the slide. slide the oil immersion objective (100x) in place and carefully scan across the smear. Take your time and observe thoroughly. Avoid drawing conclusions based on cells at the edges of the smear, as they may be poorly stained. Likewise, be cautious with clumped cells, which can retain excess stain and give misleading results. Scan the slide until you see a region where the cells are spread out and you can see the individual cells clearly. Check the controls first. If the control are the correct colors G+ purple and G- pink then look at the center smear. If the controls are not the correct colors or they are both pink and purple. Stain one of the other slides you have. Think about what could have gone wrong are adjust your timing accordingly.

Sketch what the cells look like to you for both control cultures and the mixed culture smear. Be sure to include the magnification used and the observed colors for each sample.





CONCLUSIONS

- Describe the colors of Gram negative and positive control cultures on your slide.
- Are all the Gram positive cells on your slide a single color? Are the Gram negatives all one color? Where on your smear are the colors uneven or inconsistent?
- If you did **all the other steps** of a Gram stain perfectly but **accidentally left out just one step**, **WHAT COLOR** would you see?

	Gram positive	Gram negative
A. Safranin stain left out		
B. Iodine step left out		
C. Slide <u>not heat</u> fixed		
D. <u>Alcohol rinse</u> left out		
E. Crystal violet stain left out		

4. Explain why you would see the colors you choose in the table above.

A.

B.

C.

D.

E.

9: ISOLATION BY STREAK PLATE

LEARNING OBJECTIVES

- Demonstrate correct use of the streak plate technique to isolate individual bacterial colonies
- Evaluate culture purity based on colony separation and appearance.

BACKGROUND

When working with an unknown microorganism, the first step is to obtain a **pure culture** (a population of cells growing in the absence of any other species). On solid media, such as a nutrient agar plate, a pure culture often produces **isolated colonies** (visible clusters of cells that originate from a single cell). Because all the cells in an isolated colony come from one parent cell, they are considered genetic clones.

Our environment is full of microorganisms. Even a single stray cell accidentally introduced into a pure culture can grow and multiply until it outnumbers the intended organism. This is why microbiologists must carefully check that a culture is not mixed, but truly pure.

In medical settings, obtaining a pure culture is especially important. When samples are collected from patients, it is nearly impossible to pick up only the infectious agent without also collecting normal flora (the body's natural microorganisms) or environmental organisms. To correctly identify the infectious agent, mixtures of organisms must be separated into pure cultures.

The classic way to demonstrate culture purity is to spread a sample on nutrient agar so that individual cells are deposited far enough apart to form isolated colonies. Each isolated cell divides repeatedly to form a visible colony. If colonies are separated from one another and all look alike, the culture is considered pure. To ensure purity, microbiologists select a single colony and transfer it to a fresh plate. Colonies that appear on this second plate are all derived from the original colony, meaning the cells are genetically identical.

To achieve this, microbiologists use a method called the **streak plate technique**. A sterile loop is dipped into a sample and drawn across the surface of a nutrient agar plate. Where the loop first touches the agar, many cells are deposited, and the resulting colonies will grow too close together to be isolated. As the loop is moved into new sections of the plate, fewer and fewer cells are deposited. Eventually, the cells are spread out enough that individual colonies can form without touching each other.

Your instructor will demonstrate the streak plate technique before you attempt it. Practicing the hand motion with an empty loop can be very helpful before working with live organisms.

Key point: It does not matter in which quadrant an isolated colony forms—what matters is that it is clearly separated from other colonies, with the full perimeter visible.

MATERIALS

(Per Group of 1)

1 Tube of mixed bacteria per bench

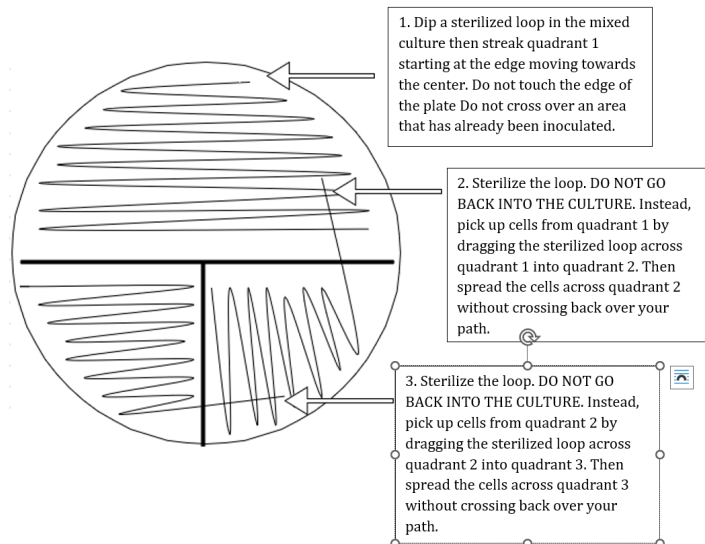
4 Nutrient agar plates (1 poured last class period, 3 poured today to use for next class period)

METHODS/PROCEDURES

METHODS

1. Divide the bottom of your Petri dish into three sections (in the shape of a “T”), as demonstrated by your instructor.
2. Label your plate clearly,
3. Using a sterile loop, pick up cells from the mixed culture and spread them carefully across the large top section of the plate. Avoid crossing back over areas you have already streaked.
4. Flame-sterilize your loop. **Do not return to the culture tube.** Instead, lightly drag the sterile loop once through the edge of section one to pick up a very small number of cells.
5. Spread these cells evenly across section two. Avoid crossing back over areas you have already streaked
6. Flame-sterilize your loop again. Drag it lightly once across section two to pick up a few cells.
7. Spread these cells across section three. Avoid crossing back over areas you have already streaked This section should contain very few cells, allowing isolated colonies to form.
8. Incubate the plate agar up until the next class meeting.

Next Class: You will select isolated colonies of each of the three colony types you observe and prepare a new T-streak plate with each one.



NAME _____

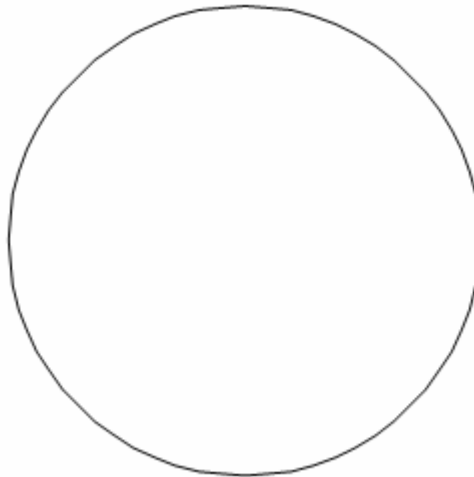
EX 9 Isolation by T-Streak Plate Method

EXPECTATIONS

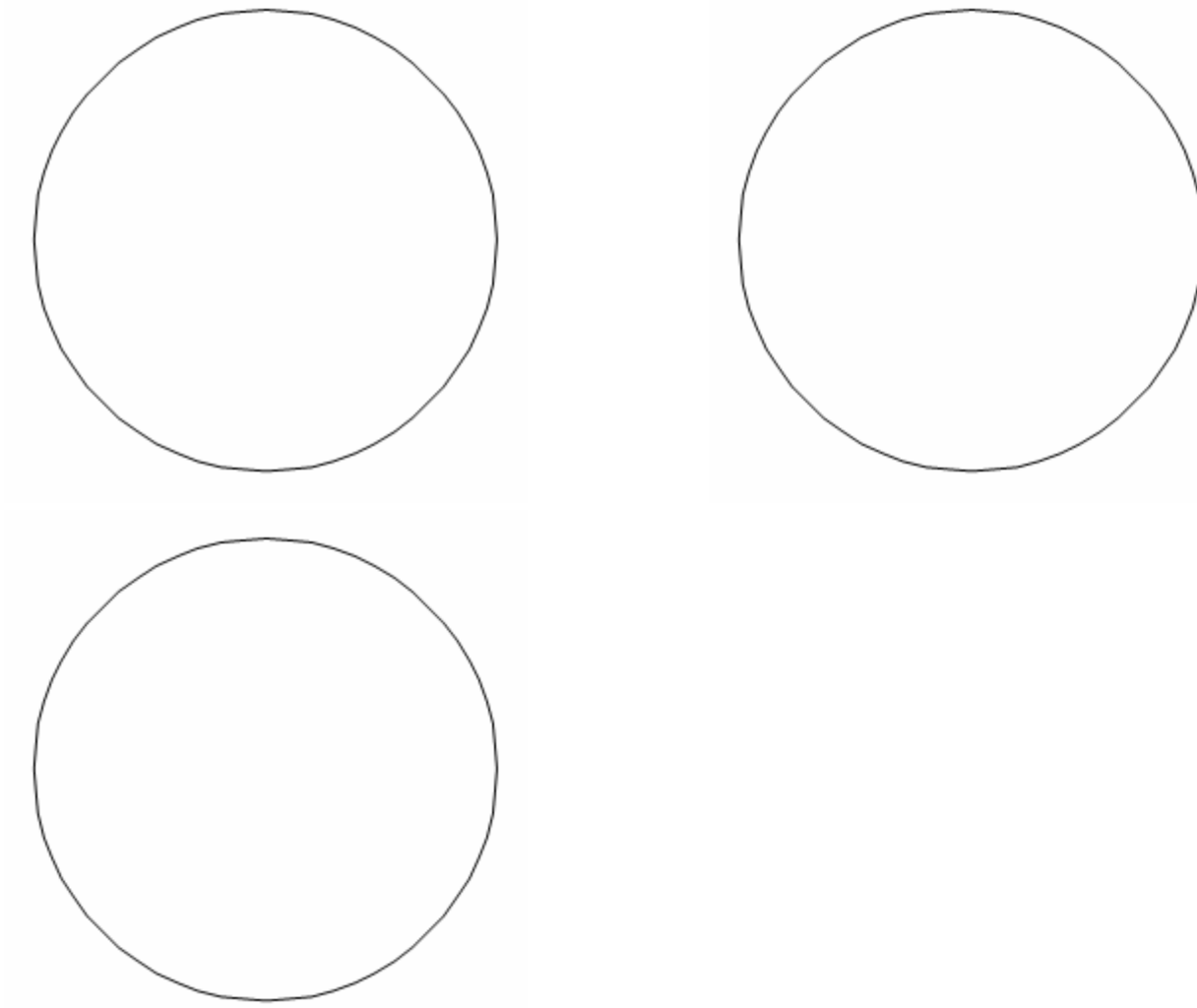
Do you think you'll have too many colonies or too few? In which quadrant of the plate do you think you will get the best isolated colonies, one, two or three?

RESULTS

Draw your **T** streak of the mixed culture after incubation.



At the following lab draw the three streak plates you made from the isolated colonies below. (Be careful to correctly label all your drawings.)



CONCLUSIONS

1. Were you successful at isolating all three cultures? If not, what could you do differently in the future to get better isolation?
2. Describe everything you can see that is different about the three types of colonies. Think about things like color, size, texture, shape, edges, and transparency.

3. If you had a broth mixture of three kinds of cells and one kind had capsules (or other sticky substances) on its surface, how might the capsules alter the effectiveness of a T-streak.

4. Imagine you are working with a broth culture into which you have just put exactly the same number of three different kinds of bacteria. In this growth medium one of the three bacteria grows much faster than the others. You make a T-streak from the broth right after you added the three cell types. The broth is left in an incubator and then four hours later you take another sample of the broth and make another T-streak. How would you expect the two T-streak plates to differ?

10: BACTERIAL GROWTH CURVE

LEARNING OBJECTIVES

- Monitor and interpret the bacterial growth curve by measuring optical density over time and identifying the four distinct phases of bacterial population growth: lag, log, stationary, and death.
- Calculate the generation time of a bacterial culture using spectrophotometer data and explain the importance of timing in microbial growth for experimental accuracy and clinical decision-making.

BACKGROUND

When bacteria are introduced into a nutrient-rich environment, they undergo population growth that follows a characteristic pattern known as the **bacterial growth curve**. This curve consists of four distinct phases, each representing a different stage in the life cycle of the population: the **lag phase**, **log phase**, **stationary phase**, and **death phase**.

During the **lag phase**, cells are metabolically active but not dividing. They are adjusting to their new environment, synthesizing enzymes, and preparing for population growth. There may be little to no increase in cell number during this time, but critical physiological changes are taking place.

Once cells have adjusted, they enter the **log phase** (also called the **exponential phase**), where they begin to divide at a constant and rapid rate. This phase is characterized by exponential growth—each cell divides into two, then four, then eight, and so on. Because of this multiplication pattern, the number of cells increases dramatically in a short period. This is also the phase where cells are most uniform in terms of size, shape, and metabolic activity, making it ideal for many microbiological experiments.

Eventually, nutrients become depleted and waste products begin to accumulate, leading to the **stationary phase**. In this phase, the rate of cell division slows and is approximately equal to the rate of cell death. The total number of viable cells remains relatively constant, but cellular stress and survival mechanisms become more prominent.

Finally, if environmental conditions continue to deteriorate, the culture enters the **death phase** (also known as the decline phase), during which the number of dying cells exceeds the number of new cells being produced. This decline continues until nutrients are exhausted or toxic conditions prevent further growth.

To observe and record this growth pattern in real time, we will use a **spectrophotometer** (an instrument that measures the amount of light absorbed by a sample). The spectrophotometer works by passing a beam of light through a liquid culture and measuring how much light is absorbed by the bacterial cells. The more cells present in the medium, the cloudier (or more **turbid**) it becomes, and the more light is absorbed.

The instrument emits light at a specific **wavelength** (the distance between peaks in a light wave), and the **optical density (OD)** (a measure of how much light is absorbed by a liquid sample) of the sample is determined based on how much light is blocked. Bacteria absorb red light efficiently, so in this exercise, we will use red light to assess culture density.

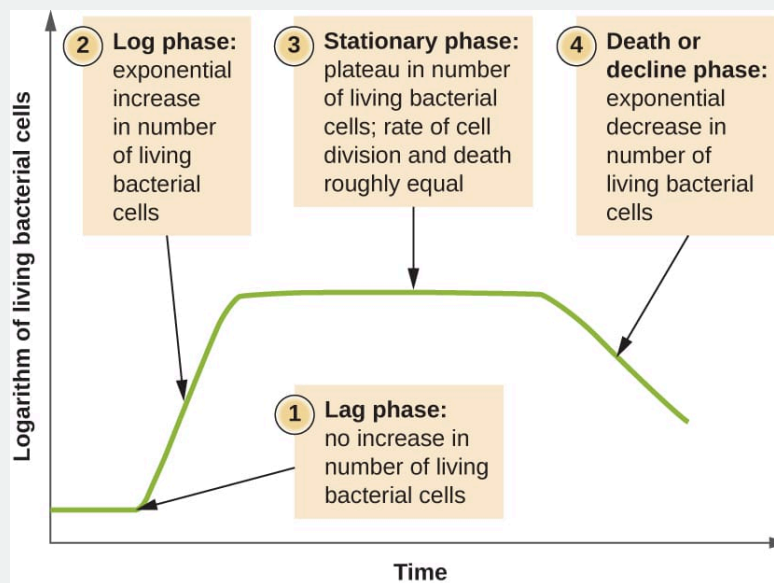
Two types of readings are possible: **transmittance** (the amount of light that passes through the sample) and **absorbance** (the amount that is blocked). We will be using the absorbance scale, which is more commonly used in scientific applications. Be sure to read only from the absorbance (OD) scale for consistency. Switching between transmittance and absorbance mid-experiment will produce invalid results.

Absorbance is measured on a **logarithmic scale**, which means the values increase in multiples rather than in equal steps. This is appropriate because bacterial populations also increase logarithmically during the log phase of growth. Therefore, optical density is directly proportional to the number of cells per milliliter (cells/mL) in the culture. By taking OD measurements at regular time intervals, you will be able to graph the bacterial growth curve and visually identify each phase based on changes in population density.

Knowing the generation time of a bacterial species is critical in microbiology and allied health fields. It helps predict the speed at which infections can spread, informs decisions in antibiotic treatment timing, and plays a central role in industrial and clinical applications involving microbial growth. Understanding these growth dynamics also aids in the design of experiments and in the development of protocols to control or encourage microbial proliferation.

Knowing the generation time is essential when conducting metabolic testing, which refers to laboratory procedures used to identify microorganisms based on the chemical reactions they perform to acquire energy and nutrients. These tests detect specific metabolic activities—such as sugar fermentation, enzyme production, or utilization of particular substrates—that help distinguish one bacterial species from another. To obtain accurate results, it is important that cultures are in the log phase of growth, when cells are most metabolically active. If testing is performed during the lag, stationary, or death phases, the cells may be too inactive or stressed to express key metabolic traits, increasing the risk of inaccurate or false-negative results.

Accurately monitoring bacterial growth and identifying when a culture is in the log phase are critical for ensuring reliable results in both clinical diagnostics and microbiological research.



9.1: How Microbes Grow is shared under a [CC BY 4.0](#) license and was authored, remixed, and/or curated by [OpenStax](#) via [source content](#) that was edited to the style and standards of the LibreTexts platform.

1.

MATERIALS

- 1 Nutrient Broth flask
- 2 Cuvettes
- Spectrophotometer
- 5 mL *E. coli*

METHODS/PROCEDURES

The spectrophotometers will be turned on and warmed up prior to the start of lab. Use the same spectrophotometer for all readings.

Step 1: Zero the Spectrophotometer

1. Set the spectrophotometer to absorbance
2. Set the wavelength to 620 nanometers
3. Use a sterile transfer pipette to transfer 1mL of nutrient broth from the flask into a clean cuvette.
4. Follow the directions next to the spectrophotometer to zero out the machine.
5. Set the cuvette with the nutrient broth aside incase you need to re-zero the spectrophotometer.

Step 2: Record Your Time Zero Reading

1. Add 5 mL of *E. coli* to the flask (obtain the culture from your instructor)
2. Gently swirl the flask to evenly distribute the bacteria in the medium
3. Use a sterile transfer pipette to transfer 1mL of the culture from the flask into a clean cuvette.
4. Place the cuvette in the spectrophotometer and record the OD in the results section
5. Transfer the culture from the cuvette back into the culture flask.
6. Place the culture flask in the incubator shaker. Remember to leave the cap slightly loose to enable gas exchange.
7. Repeat steps 3-6 every 15 minutes until the end of the lab period

NAME _____

EX 10 Bacterial Growth Curve

EXPECTATIONS

Which phases of growth do you anticipate you will be able to see during the lab period today?

RESULTS

Record the OD readings in the table below.

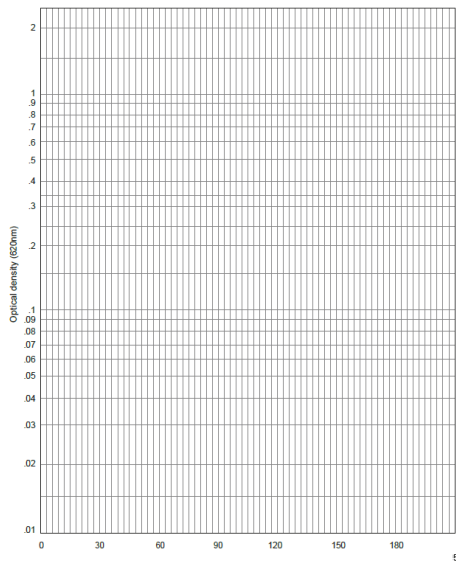
Volume of cells added to start culture

Minutes of incubation	Time of Reading	Optical Density (620nm)
0 min.		
15 min.		
30 min.		
45 min.		
60 min.		
75 min.		
90 min.		
105 min.		
120 min.		
135 min.		
150 min.		

Logarithmic growth (the phase during which bacterial cells divide at a constant and rapid rate) will appear as a straight line when plotted on semi-logarithmic graph paper. When you connect your recorded data points, the resulting line may show minor fluctuations due to slight inconsistencies in measurement. Despite this, the underlying pattern of growth remains linear during the log phase. To accurately represent this trend, draw a “best-fit” straight line through the points that correspond to this phase.

Use your data to estimate the most accurate straight line possible through the log phase. It is expected that some early or late data points may not align perfectly with this line, as they may represent the lag or stationary phases. The **slope** of the line indicates the rate of growth: a steeper slope corresponds to a more rapidly growing culture, while a less steep slope indicates slower growth.

Plot your data on the semi log graph paper



Calculate the Generation Time

1. Using a red pen or pencil, place a red dot on your graph at any point along the best-fit straight line representing the **logarithmic growth** phase. Record the **optical density (OD)** and the corresponding **time** for this point in the spaces provided below.
2. Next, place a second red dot on the same best-fit line at the point where the OD is exactly **double** that of the first dot. Record the OD and the corresponding time for this second point. Since the OD has doubled, this indicates that the number of cells has also doubled.

The time it took to go from the first OD value to the second represents the **generation time**—the time required for the bacterial population to double.

OD at 1st red dot _____ Time at 1st red dot _____

OD at 2nd red dot _____ Time 2nd at red dot _____

Generation time _____

CONCLUSIONS

1. Were you able to record all four phases of bacterial growth? If any phases were missing or unclear, identify which ones and explain why you think they were not observed.
2. If a culture has an **optical density (OD)** reading of 0.03 when there are one million cells per milliliter, how many cells per milliliter would be present if the OD reads 0.015?
3. Based on the culture described in question 3, what OD reading would you expect for a sample containing six million cells per milliliter?
4. What does it indicate if the OD of a culture remains unchanged over several consecutive sample times?

5. If a sample of *Escherichia coli* containing one million cells per milliliter has an OD reading of 0.23, would you expect the same OD, a higher OD, or a lower OD for a sample containing one million fungal cells per milliliter? Explain your reasoning.

11: EPIDEMIC

LEARNING OBJECTIVES

- Record and organize experimental data related to simulated disease transmission using observation and peer interaction tracking.
- Describe how chain-of-contact tracing contributes to disease tracking.

BACKGROUND

An epidemic occurs when the number of individuals affected by a specific infectious disease rises significantly above what is normally expected within a particular population or region. The study of epidemics, known as epidemiology, focuses on identifying the causes of disease outbreaks, understanding how they spread, and developing strategies to control them. Much of this work involves careful investigation, pattern recognition, and tracing the origin of the pathogen—similar to solving a mystery.

When the source of an outbreak can be identified, public health officials are better equipped to contain the spread and prevent future infections. However, when the origin remains unknown, the risk of repeated outbreaks increases.

In today's lab, you will participate in a simulated epidemic that models how a disease can spread within a population. To conduct this simulation, the bacterium *Serratia marcescens* will be used. *S. marcescens* is a gram-negative organism commonly used in microbiology labs. It is generally harmless to healthy individuals and easily recognized due to its production of a natural red pigment called prodigiosin. This pigment allows you to visually track the transmission of the "infection" during the exercise.

In real outbreaks, infections often spread in a stepwise fashion through chains of contact. This lab will replicate that process. By recording your interactions and analyzing patterns of spread, the class will work together to identify the original source of the infection.

It is important that you follow all instructions carefully and label correctly, as the success of this simulation depends on the accuracy of your data. During the next lab session, the information on the inoculated plates will be used to determine which student was the initial source of the epidemic.

MATERIALS (Per Student)

- 1 plastic glove
- 1 candy in numbered dish (one is contaminated with *Serratia marcescens*)
- 1 tube of sterile water
- 1 sterile cotton swab
- 1 petri dish of nutrient agar

METHODS/PROCEDURES

1. Label the bottom of your petri dish with five sections labeled 1 through 5. Numbers should be large and easy to read from the top of the dish. In the center, write your last name, today's date, and the candy ID number.
2. When directed by your instructor, put a glove on your non dominant hand (the hand you do not write with). With your ungloved right hand, open the plastic bag containing the candy. Use your gloved hand to pick up the candy and smear it all over the palm of the glove. Do not touch anything else with the candy.
3. When told to do so, shake hands (glove to glove only) with one person in the lab. After shaking hands, exchange ID numbers. This person is your first contact.
4. After the handshake, swab the surface of your gloved palm using a sterile swab. Rub the swab across section 1 of your petri dish. Return the swab to the sterile water tube. Record the ID number of your first contact in section 1.
5. When instructed, shake hands with a new person in the room (do not shake hands with the same person more than once). After the handshake, swab your gloved palm again with the same swab and streak section 2 of your plate. Place the swab back into the water tube. Record the new contact's ID number in section 2.
6. Repeat the handshaking and swabbing process until you have completed five rounds. Remember to always shake hands with someone new. Each time, use the same swab and streak the appropriate numbered section of your plate.

NAME _____

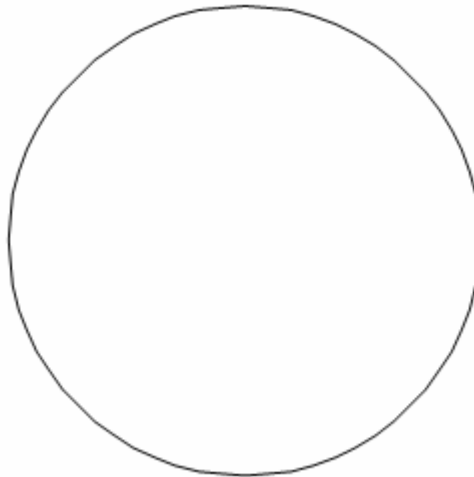
EX 11 Epidemic

EXPECTATIONS

How many students do you think will be infected by the fifth round of contact? Explain your reasoning.

RESULTS

Draw the growth on your test plate after incubation. Be sure to note the color of growth.



CONCLUSIONS

1. Why was red colony growth the only type recorded during this experiment?
2. How would using white candy instead of red have compromised the results of this simulation?

12: OUTBREAK

LEARNING OBJECTIVES

- Interpret laboratory data to determine causative agent of food borne illness based on DNA analysis.
- Explain how DNA analysis is used to identify a causative agent.

BACKGROUND

According to the Centers for Disease Control and Prevention (CDC), about 48 million Americans become ill from foodborne diseases each year. About 128,000 are hospitalized, and 3,000 die. In 2000, the CDC received reports of 406 cases of the same foodborne illness across ten states, primarily on the West Coast. Epidemiologists investigating the outbreak determined that the pathogen spread through the fecal-oral route (the spread of a pathogen when fecal material enters a person's mouth, often through contaminated food, water, hands, or surfaces). The same type of bacterium was isolated from stool samples collected from 98% of people with similar signs and symptoms.

Several members of the Enterobacteriaceae family (a group of Gram-negative, non-endospore-forming, facultative anaerobic bacteria) could have caused the outbreak. Foodborne pathogens in this family include *Shigella*, *Salmonella*, and *Escherichia coli*. These bacteria can cause similar signs and symptoms, but the safest and most effective treatment may differ depending on the species or strain causing the infection.

Illnesses caused by these pathogens can range from mild diarrhea to severe dysentery. The severity of the illness depends partly on the toxins produced by the bacteria. Some strains of *Shigella* produce Shiga toxin (a powerful toxin that damages human cells). This toxin can cause severe illness and may lead to death. Shigellosis is usually self-limiting (an illness that generally resolves without medication), but the affected person should be monitored and kept hydrated.

Enteroinvasive *E. coli* (EIEC) (a type of *E. coli* that enters and damages cells lining the intestine) is closely related to *Shigella* and can cause similar signs and symptoms. Infections caused by *E. coli* O157:H7 may also begin with symptoms that resemble shigellosis. However, these infections can progress to serious complications if they are not identified and managed correctly.

Salmonella bacteria cause an illness called salmonellosis. Treatment usually focuses on managing diarrhea and replacing fluids and electrolytes to prevent dehydration. Antibiotics are generally reserved for severe infections or for people who have a greater risk of complications.

The toxins and other disease-causing traits produced by a bacterium depend on its genome (all the genetic material within a cell, including its chromosome and plasmids). For diagnosis and treatment, it is often critical to quickly identify the species and strain causing an infection.

Identifying the exact organism is important because different pathogens can cause similar signs and symptoms but may require different treatments. Some infections require only fluids, rest, and monitoring, while others may require antibiotics. In some cases, the wrong antibiotic could make the illness worse. Antibiotics are generally avoided in infections caused by Shiga toxin-producing *E. coli*, such as *E. coli* O157:H7, because they may increase the risk of serious kidney damage. Rapid identification helps healthcare providers choose the safest treatment, monitor for possible complications, and limit the spread of the pathogen.

In the past, laboratories relied mainly on signs and symptoms, bacterial cultures, and metabolic tests to identify pathogens. Most metabolic tests require 48–72 hours of incubation before results are available, which can delay diagnosis and treatment. In addition, some metabolic tests cannot distinguish between closely related bacterial strains. Hospitals and clinical laboratories now commonly use polymerase chain reaction and gel electrophoresis to analyze pathogen DNA and identify infectious organisms more quickly.

Polymerase chain reaction (PCR) (a laboratory method used to make millions of copies of a selected DNA region) uses repeated cycles of heating and cooling. Heating separates the two DNA strands. Cooling allows primers to attach to matching sequences on the separated strands. DNA polymerase then builds new DNA strands, copying the selected region.

Primers (short, single-stranded pieces of DNA that mark the beginning and end of the region to be copied) give PCR its specificity. PCR uses a set of two primers because DNA polymerase can build DNA in only one direction. Each set contains a forward primer and a reverse primer. The primers attach to opposite DNA strands on either side of the target region. Together, they mark the exact DNA section that will be copied and allow both strands to be copied during each PCR cycle.

Each primer set is designed to attach to DNA sequences from a particular pathogen. If both matching sequences are present, the primers attach to the DNA, and DNA polymerase copies the region between them. If either matching sequence is absent, the primers do not attach correctly, and the expected DNA fragment is not produced. Different pathogen-specific primer sets can therefore be used to distinguish among pathogens that cause similar signs and symptoms.

During PCR, the reaction mixture passes through repeated cycles of heating and cooling. Heating separates the two strands of bacterial DNA. As the mixture cools, the forward and reverse primers attach to matching sequences on opposite strands. DNA polymerase then extends from each primer and makes new copies of the target region. Repeating these cycles produces millions of copies of the target DNA.

If the unknown sample contains the sequences recognized by a primer set, PCR will produce a DNA fragment with a predictable number of base pairs. If the target sequences are absent, the expected fragment will not be produced.

The PCR products will be examined using gel electrophoresis (a laboratory method that separates DNA fragments by size). DNA has a negative charge. When an electric current is applied, the DNA fragments move through the gel toward the positive electrode. The agarose gel contains tiny pores that act like a filter. Smaller DNA fragments pass through the pores more easily and travel farther than larger fragments.

The bands produced by the unknown sample will be compared with a DNA ladder (a mixture of DNA fragments of known sizes) and known positive and negative controls. The DNA ladder will help determine the size of each PCR product. The positive controls will show the expected results when the target DNA is present. The negative control will help detect contamination or other errors.

A band at the expected location will show that the unknown sample contains the DNA sequence targeted by that primer set. The pathogen will be identified by determining which pathogen-specific primer set produces a band of the expected size. This information can help healthcare providers choose the safest treatment and respond quickly to a foodborne outbreak.

During this lab, you will use pathogen-specific primer sets and PCR to copy selected DNA regions from an unknown bacterial sample. You will then use gel electrophoresis to separate the PCR products by size. By comparing the bands from the unknown sample with a DNA ladder and known positive and negative controls, you will determine whether the sample contains DNA from *Shigella*, *Salmonella*, or a disease-causing strain of *E. coli*.

MATERIALS (Per Group of 4)

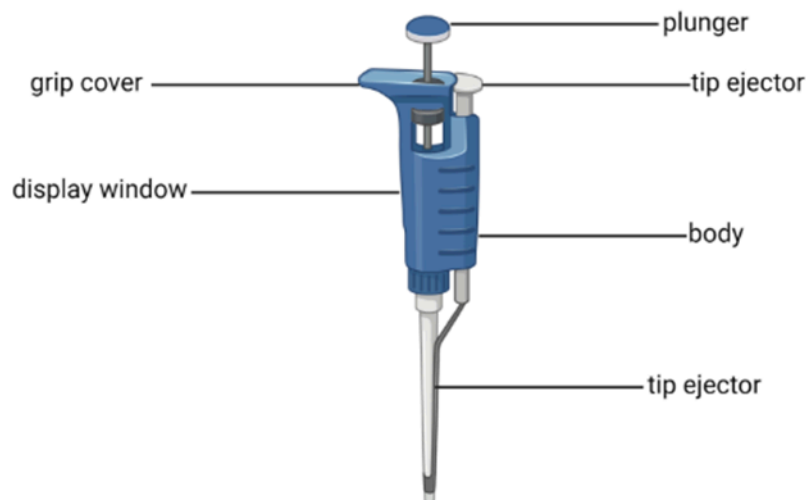
- 1 P-20 Micropipettes
- 1 Micropipettes tips
- 1 Beaker for waste
- 1 1% Agarose TAE gel
- 1 Gel electrophoresis system
- 1 Microfuge tube rack
- 5 1X TAE DNA samples
- 1 1X TAE Size marker

METHODS/PROCEDURES

HOW TO USE MICROPIPETTES

Adjusting Volumes

The instrument pictured at the top right is a typical P20 micropipette (a precision tool designed

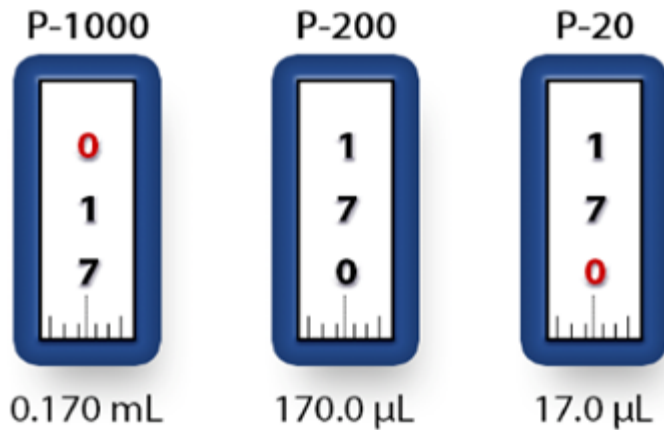


to measure and transfer very small liquid volumes). The “P20” name means it can hold up to 20.0 microliters (μL) of liquid. This micropipette is accurate for volumes between 2.0 μL and 20.0 μL . It should not be used for amounts smaller or larger than this range.

Micropipette volumes are adjusted using a three-digit dial located on the handle. Many micropipettes use a color-coded digit to indicate the decimal point. For example, in a P20 pipette, a setting of black 0, black 2, red 0 indicates 2.0 μL .

Loading the Micropipette

1. **Attach a disposable tip**
Always use a clean, disposable plastic tip. Push the micropipette's shaft straight down into a tip (located in the tip box) until it clicks firmly into place.
2. **Set your volume**
Dial in the volume needed using the adjustment knob. Make sure the setting is within the P20's valid range.
3. **Grip the pipette correctly**
Hold the micropipette like a pencil, wrapping your fingers beneath the curved finger rest, with your thumb on the plunger button.



4. **Understand the two “stops”**
- **First stop:** Pressing the plunger gently until you feel the first stop is used to draw up liquid.
 - **Second stop:** Pressing beyond the first stop to the second stop is used to eject all the liquid from the tip.

Transferring Liquids

- Aspirate (draw up liquid)**
 - Press the plunger down to the **first stop** and hold.
 - Submerge the tip in the liquid sample.
 - Slowly release the plunger to draw liquid into the tip.
 - Check for smooth intake with **no air bubbles**.
- Dispense (release liquid)**
 - Move the pipette to the receiving tube or surface.
 - Press the plunger to the **first stop** to begin dispensing the liquid.
 - Continue pressing to the **second stop** to ensure all the liquid is released.
 -
- Eject the used tip**
 - Always **change the tip** between samples to avoid contamination.
 - Press the **tip ejector** button to discard the used tip into a designated waste container.

METHODS

- Set the P20 micropipet to 10.0 µL. The dial should read 100, top to bottom
- Place the 1% agarose gel into the electrophoresis chamber. Pour enough 1X TAE buffer to just cover the gel (entire gel needs to be completely submerged).
- Load 10.0 µL of each DNA sample into individual wells of the gel in the order indicated in the chart below changing tips for each sample.
- Once all samples have been loaded, place the cover on the gel tank, matching colored electrodes.
- Plug the gel box into the power supply and set the voltage to 120V.
- Run the gel for 15-30 minutes or until the samples are separated into distinct bands.
- Remove the gel from the electrophoresis box and view it on the light box

1.3 Micropipetting is shared under a [CC BY 4.0](#) license and was authored, remixed, and/or curated by [Orange County Biotechnology Education Collaborative \(ASCCC Open Educational Resources Initiative\)](#).

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EX 12

Outbreak

EXPECTATIONS

The *E. coli* O157:H7 strain has a plasmid that *E. coli* does not have. Would you expect the two organisms to have the same or different DNA band patterns on the gel? Explain.

RESULTS

Draw the results of the gel electrophoresis below

Outbreak Strain	(A) <i>S. Typhi</i>	(B) <i>V. cholera</i>	(C) <i>E. coli</i> (EPEC)	(D) <i>E. Coli</i> O157:H7	(E) <i>E. coli</i> EIEC

CONCLUSIONS

1. Based on the results above, what pathogen caused the outbreak? _____
2. Explain how you made your determination.
3. Explain how you used the DNA banding patterns to identify the pathogen.

4. Describe one advantage and one disadvantage of using DNA-based testing rather than metabolic testing to identify bacteria.

5.

13: SURVEY OF FUNGI

LEARNING OBJECTIVES

- Identify and describe the microscopic structures of medically important fungi, including yeast cells, hyphae, conidia, zygospores, and asci, using prepared slides and appropriate magnification.
- Explain the relationship between fungal morphology and pathogenic potential, with emphasis on opportunistic infections caused by species such as *Candida albicans* and *Aspergillus* spp.

BACKGROUND

Fungi (a group of eukaryotic organisms that includes yeasts, molds, and mushrooms) are important to both the environment and human health. While fungi are known for their roles in nutrient recycling and food production, they also play a major role in medicine—sometimes as life-saving allies, and sometimes as harmful pathogens. For future healthcare workers, understanding how fungi function in the body and in clinical environments is essential.

Many fungi are opportunistic pathogens, meaning they typically don't cause disease unless the host's immune system is weakened. Fungal infections, or mycoses, can affect the skin, mucous membranes, lungs, bloodstream, and internal organs. These infections are often more difficult to treat than bacterial infections because fungal cells are structurally similar to human cells, which limits the number of safe and effective drug options. In addition, drug resistance and toxicity are ongoing concerns in antifungal therapy.

One of the most common fungal pathogens is *Candida albicans*, a yeast that normally lives in the human mouth, digestive tract, and vaginal area. When conditions change—such as during antibiotic use, pregnancy, diabetes, or immune suppression—*Candida* can multiply out of control. This overgrowth can lead to infections such as oral thrush, vaginal yeast infections, or more serious bloodstream infections called invasive candidiasis, especially in hospitalized patients.

Another important fungal group is *Aspergillus*, a mold commonly found in soil, air, and decaying vegetation. *Aspergillus fumigatus* is known to cause allergic reactions in some individuals and can form fungal balls in the lungs of those with pre-existing conditions. In more severe cases, it can invade the lungs and spread through the bloodstream, particularly in people receiving chemotherapy or organ transplants. *Aspergillus flavus* is a related species that produces aflatoxins—dangerous compounds that can contaminate stored food and contribute to liver disease when ingested. *Schizosaccharomyces*, a fission yeast used in fermentation processes, is not commonly pathogenic but may occasionally be associated with infections when introduced into a disrupted or immunocompromised system. Similarly, *Penicillium notatum*, while not typically harmful, is famous for its contribution to medicine. This mold was the original source of penicillin, the first antibiotic, which revolutionized the treatment of bacterial infections. Today, other fungi are used to create immunosuppressive drugs, cholesterol-lowering agents, and powerful enzymes for laboratory testing and biotechnology.

As fungal infections become more frequent in hospitals—due to the use of catheters, ventilators, and immunosuppressive treatments—it is increasingly important for healthcare professionals to recognize their signs and understand their sources. Fast and accurate diagnosis, combined with knowledge of fungal biology, is essential to protecting patient health.

In this lab, you will examine key fungal structures and learn how to identify medically relevant species under the microscope. This experience will help you connect the biology of fungi with the diseases they can cause, strengthening your diagnostic skills and preparing you for clinical practice.

Pay attention to the characteristics and appearance of the organisms, structures and features you observe and draw, you may be asked to identify them on the lab exam.

MATERIALS (per student)

Prepared slides of various fungi

METHODS/PROCEDURES

1. Draw what you observe as you look through the microscope from each of the six slides in the results section. The final magnification for each organism is indicated in the table below.
2. Label the structures and or features indicated in the table for each organism.
3. Keep in mind that fungal cells are much larger than bacterial cells. Some of the structures you will observe—like hyphae or spore-bearing bodies—are composed of many cells and are best viewed at lower magnifications. Not all slides are perfect, so don't be discouraged if some features are harder to see.
4. Ask your instructor for help if you're having trouble locating key structures. Use caution when focusing. Fungal slides are often thick, and it's easy to damage them by accidentally striking the glass with the microscope objective. Move slowly and carefully to avoid breaking the slide or scratching the lens.

**Rhizopus and Aspergillus have very thick cover slips.
DO NOT USE ANY MAGNIFICATION HIGHER THAN LISTED BELOW**

Rhizopus	100x	Draw the zygosporangia (not conidia)
Aspergillus	100x	Draw conidia covered with spores
Penicillium	400x	Draw the branched conidia
Candida albicans	1000x	Draw both hyphae & round yeast type cells
Saccharomyces cerevisiae	1000x	Draw budding (dividing) cells
Schizo-saccharomyces	1000x	Draw ascus with spores inside

NAME _____

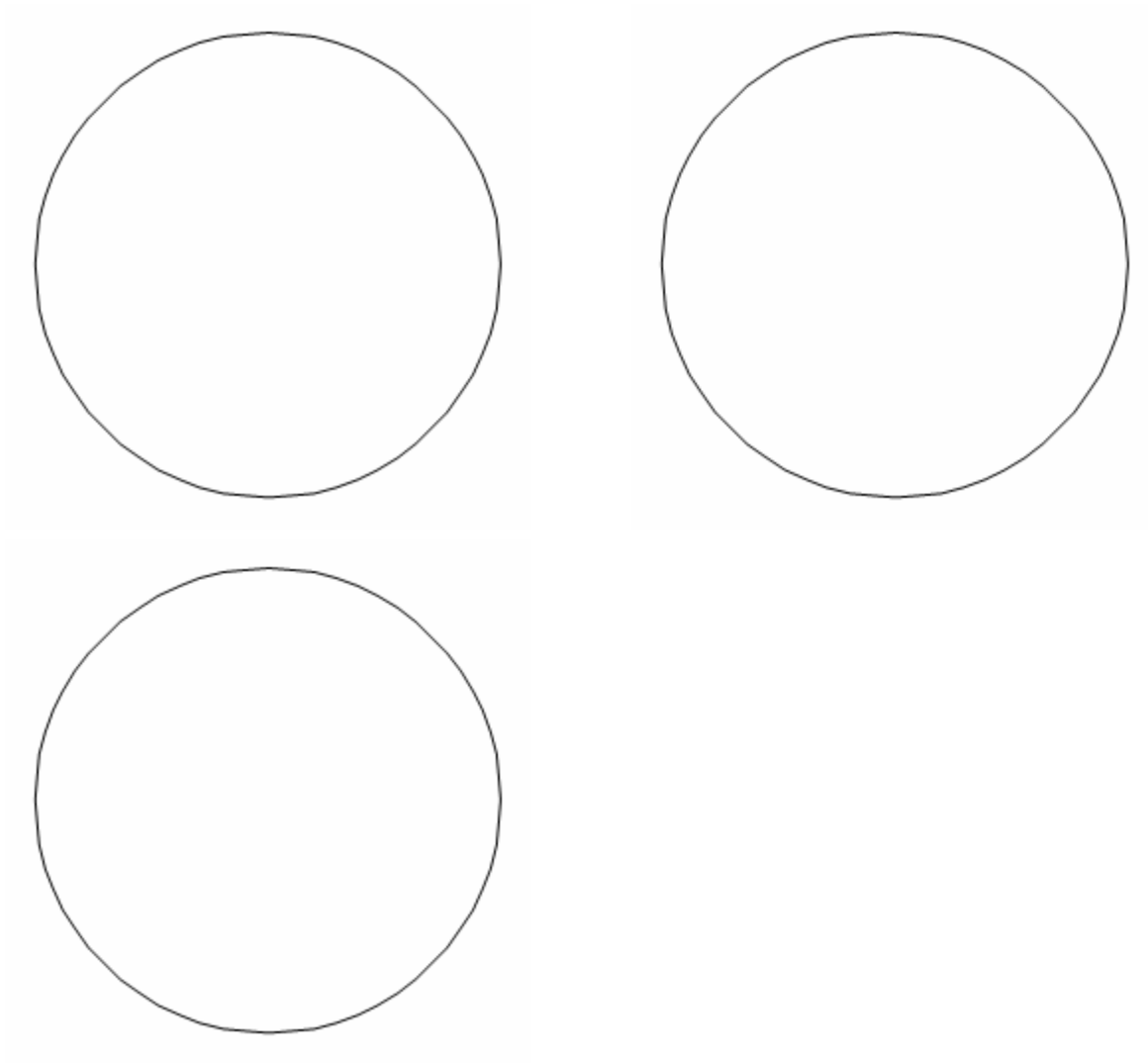
EX 13 Fungi

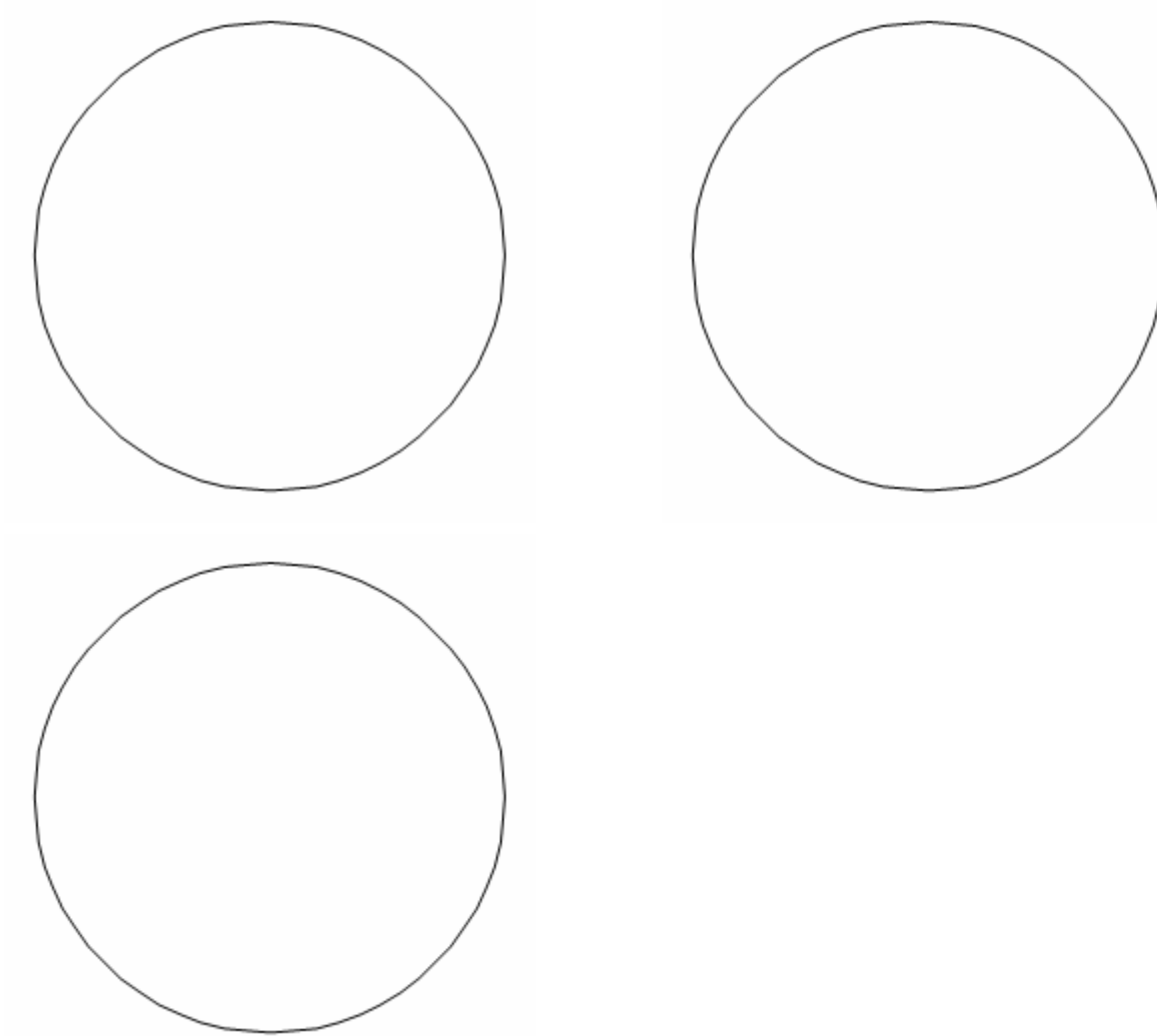
EXPECTATIONS

Based on your background reading, what differences do you expect to notice between fungal cells and bacterial cells under the microscope?

Why might some fungal slides be more easily observed at low magnification rather than high magnification?

RESULTS





CONCLUSIONS

1. How did the appearance of *Candida albicans* differ from that of a mold like *Aspergillus* or *Penicillium*?
2. Describe two features you observed that differentiate *Saccharomyces cerevisiae* from *Schizosaccharomyces*

3. If you were given an unknown fungal sample from a patient, what observations from today's lab would help you begin to identify it?

4. Explain one similarity and one difference between endospores and fungal spores

5. Which fungal species in today's lab are considered opportunistic pathogens? What conditions might allow these fungi to cause infection?

14: ALGAE

Learning Objectives

- Identify and compare the major structural forms of algae, unicellular, filamentous, and colonial, using prepared microscope slides of representative species such as *Ceratium*, *Diatoms*, *Spirogyra*, and *Volvox*.
- Explain the ecological and medical relevance of algae, including their roles in toxin production, nutrient cycling, and contributions to biotechnology and public health.

BACKGROUND

The kingdom Protista includes a diverse range of aquatic organisms, many of which are microscopic. Within this kingdom are two major groups of interest in microbiology: protozoa (animal-like protists) and algae (photosynthetic, plant-like protists). This section focuses on algae—their structure, classification, and how they differ from both plants and bacteria.

Algae are single-celled or simple multicellular, eukaryotic organisms that perform photosynthesis. They inhabit both freshwater and marine environments and use chloroplasts containing the pigment chlorophyll (a molecule that captures light energy to produce organic compounds). Although algae share several characteristics with plants, such as photosynthesis and the presence of a cell wall, they lack the specialized tissues found in higher plants.

Historically, organisms were often classified by outward characteristics like color and habitat. As a result, some photosynthetic bacteria were mistakenly labeled as algae. For example, cyanobacteria were once called “blue-green algae” due to their color and photosynthetic ability. However, we now know that cyanobacteria are prokaryotes—cells without a nucleus or membrane-bound organelles—while true algae are eukaryotic. Cyanobacteria perform photosynthesis using bacteriochlorophyll, which is chemically distinct from plant chlorophyll, and they lack the internal structures that define eukaryotic cells.

While algae and plants are both eukaryotic and photosynthetic, algae do not have the tissue specialization seen in plants. Algae may be unicellular, form simple filaments, or grow as colonies, but all the cells are generally similar in structure and function. In contrast, plants have differentiated tissues such as roots, stems, and leaves.

Unicellular Algae

Unicellular algae exist as independent, free-floating cells. One of the most ecologically important types is the diatom. Diatoms are enclosed within a hard silica shell, or test, composed of two interlocking halves. These microscopic organisms glide using a thin extension that protrudes through a slit in the test. Diatoms play a crucial role in aquatic ecosystems by forming the base of the food web and producing significant amounts of oxygen. Their fossilized remains accumulate as diatomaceous earth, which is mined and used in water filtration systems, abrasives, and insecticides.

Filamentous Algae

Filamentous algae consist of long, unbranched chains of cells. These cells remain connected after division, forming visible strands in aquatic environments. All cells within the filament are structurally and functionally similar.

An excellent example of filamentous algae is *Spirogyra*, recognized by its internal spiral chloroplasts. Since the cells are not specialized, any fragment of the filament can detach and grow independently, giving these organisms a high capacity for dispersion and colonization.

Colonial Algae

Colonial algae form groups of similar cells arranged in sheets, clusters, or spheres. While each cell maintains individual function,

they may coordinate activities such as movement and reproduction. Colonies can reproduce by forming smaller internal daughter colonies. Volvox is a well-known colonial algae that forms spherical colonies composed of thousands of cells, each with flagella that allow the colony to rotate and move through water. When stained, Volvox colonies may appear in vibrant colors, aiding in microscopic visualization.

Medical Significance of Algae

While algae are not typically pathogenic to humans, they do have medical and public health relevance. Certain species of marine algae can produce toxins that accumulate in shellfish, leading to serious human illnesses when consumed. For example, Gonyaulax and related dinoflagellates can produce neurotoxins responsible for paralytic shellfish poisoning (PSP).

Additionally, cyanobacteria (though not algae) can form harmful algal blooms (HABs) in freshwater systems. These blooms release toxins that can affect the liver, nervous system, and skin, posing health risks to humans and animals that come into contact with contaminated water.

On a more positive note, algae also contribute to biotechnology and medical research. Species such as Chlorella and Spirulina are cultivated as nutritional supplements due to their high protein, vitamin, and mineral content. Algal polysaccharides like agar and carrageenan, derived from red algae, are widely used in microbiological media, pharmaceuticals, and cosmetics as stabilizers and gelling agents. Understanding the role of algae in both environmental systems and medical contexts is essential for allied health professionals, especially those working in public health, clinical microbiology, or environmental safety.

MATERIALS

Prepared slides of the following:

- Ceratium
- Diatoms
- Volvox
- Spirogyra filamentous algae

METHODS/PROCEDURES

1. Observe each of the above organisms listed at 10x only.

Bacterium	Objective Resolution
<i>Ceratium</i> single cell algae	x100
<i>Volvox</i> colonial	x100
diatoms single cell algae	x100
<i>Spirogyra</i> filamentous algae	x100

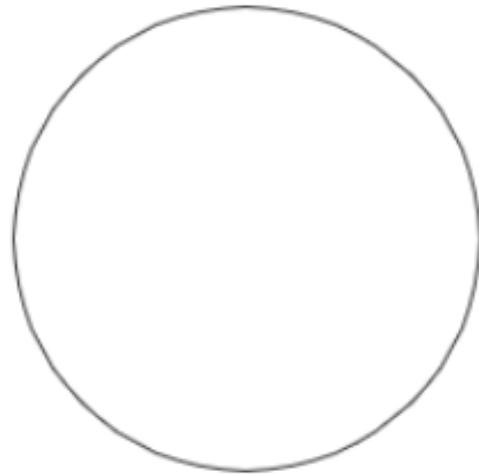
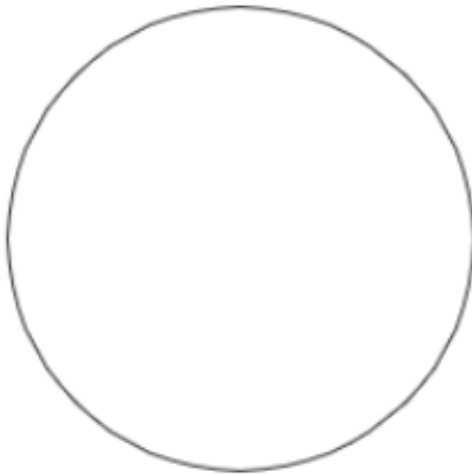
NAME _____

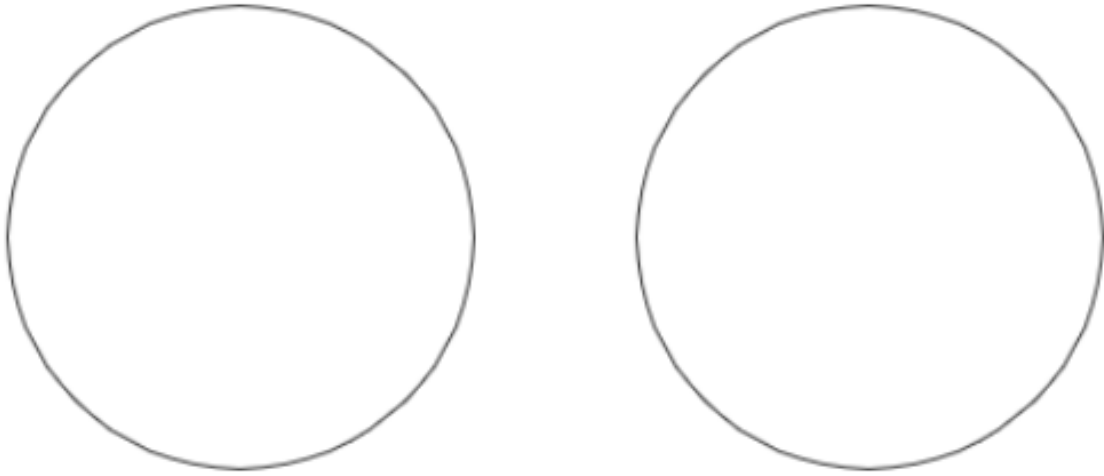
EX 14 Algae

EXPECTATIONS

Algae are eukaryotes. Based on that, what differences do you expect to see between algae cells and bacterial cells when viewed under the microscope?

RESULTS





CONCLUSIONS

1. Identify one example of each type of algal growth form that you observed today:

Unicellular: _____

Filamentous: _____

Colonial: _____

2. What visible features helped you distinguish between unicellular, filamentous, and colonial algae? Provide at least two distinguishing characteristics.

3. Some algae and cyanobacteria produce toxins. Describe one way these organisms can impact human or environmental health.

4. Based on your observations, which organism(s) appeared to have the most complex structure? What features led you to that conclusion?

15: PROTOZOA

LEARNING OBJECTIVES

- Identify medically important protozoa, such as *Entamoeba histolytica*, *Giardia lamblia*, *Trichomonas vaginalis*, *Trypanosoma* spp., *Plasmodium* spp., and *Balantidium coli*.
- Associate each organism with the disease it causes and/or tissues, organ systems affected.

BACKGROUND

Protozoa are one of the two major groups within the Kingdom Protista, along with algae. Like all protists, protozoa are made up of eukaryotic cells (cells that contain a true nucleus and membrane-bound organelles). While algae are often described as “plant-like” due to their ability to photosynthesize, protozoa are considered more “animal-like” because they must consume other organisms for food.

The name “protozoa” literally means “first animals”, and these organisms often resemble tiny animals in behavior and lifestyle. Many protozoa are pathogens in humans or animals and understanding them is essential in clinical microbiology.

Unlike algae, protozoa cannot photosynthesize, so they must find and absorb nutrients from their environment. Since they are single-celled, they do not have mouths or intestines. Instead, they absorb nutrients through their cell membrane or ingest food particles using a process called **endocytosis** (a method by which a cell surrounds and takes in material from its environment).

Historically, protozoa were grouped based on how they move. Today, modern classification also uses DNA sequencing and rRNA typing (methods that analyze genetic material), but for the purposes of this lab, we will focus on their method of movement. The four major groups are:

- **Flagellates** – move using long whip-like flagella
- **Amoeboids** – move by extending parts of their body called pseudopods (“false feet”)
- **Ciliates** – are covered in short, hair-like cilia that beat in unison to allow movement
- **Apicomplexans** – are obligate intracellular parasites (they must live inside a host cell and cannot move on their own); they use a specialized structure called an apical complex to invade host cells

Some protozoa can form cysts (dormant, protective forms that survive harsh environments). These cysts help them survive outside a host and allow transmission from one host to another.

Examples of Medically Important Protozoa

- **Entamoeba histolytica** is an amoeboid organism that causes amoebic dysentery, a serious intestinal disease. The amoeba invades the large intestine and consumes red blood cells. It is spread by ingestion of food or water contaminated with feces and is a major public health concern in many parts of the world, though less common in the United States.
- **Giardia lamblia** is a flagellated protozoan that causes giardiasis, an intestinal infection. It is often contracted by drinking water contaminated by animal feces or through direct person-to-person contact in childcare settings.
- **Trichomonas vaginalis** is a flagellated protozoan responsible for the sexually transmitted disease **trichomoniasis** (commonly called “tric”).
- **Trypanosoma** species cause diseases such as African sleeping sickness. These protozoa have a single flagellum attached to an undulating membrane (a wave-like membrane that helps in movement). They are typically spread through the bites of infected insects like the tsetse fly.
- **Plasmodium**, a member of the Apicomplexa group, causes malaria—the most common protozoan infection worldwide. These parasites live inside red blood cells and are spread by the bite of an infected mosquito, which also serves as the site for the organism’s sexual reproduction. At least four *Plasmodium* species infect humans.

- **Balantidium coli**, a ciliate, is the only ciliated protozoan known to infect humans. Though rare, it can cause intestinal illness in immuno-compromised individuals. It is named after its location—the colon—just like *Escherichia coli* (*E. coli*), although the two are very different organisms.

MATERIALS (Per Group of 1)

Prepared slides of the following organisms:

Organism	Feature/Structure	Final magnification
<i>Paramecium sp.</i>	Cilia	x100
<i>Balantidium coli</i>	Cilia	x400
<i>Amoeba proteus</i> or <i>chaos</i>	Pseudopods	x100
<i>Entamoeba coli</i> or <i>histolytica</i>	Pseudopods	x1000
<i>Trichomonas muris</i> or <i>vaginalis</i>	Flagella	x1000
<i>Trypanosoma cruzi</i>	Flagella	x1000
<i>Giardia lamblia</i>	Flagella	x1000
<i>Plasmodium berghei</i>	Intracellular	x1000

METHODS/PROCEDURES

1. Make accurate drawings off all slides listed at the magnifications listed in the table above.
2. Make note of any features such as flagella, cilia, nucleoli, intracellular, etc. that will help you identify the organisms on the lab exam.

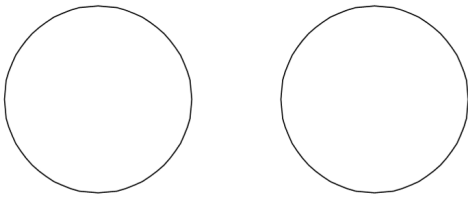
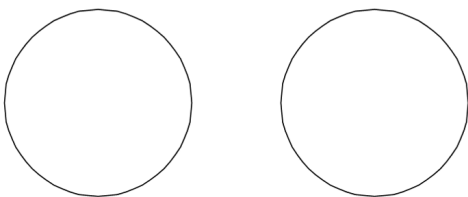
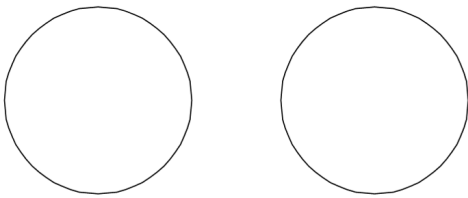
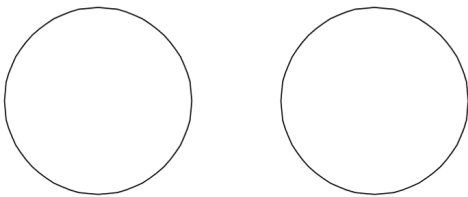
NAME _____

EX 15 Protozoa

EXPECTATIONS

Which protozoan do you think will be an intracellular pathogen?

RESULTS



CONCLUSIONS

1. What visual evidence on the microscope slides suggests that *Plasmodium* and *Trypanosoma* are parasitic organisms? Describe how their appearance and location relative to host cells support this conclusion.
2. How does the ability to form cysts help protozoa survive outside the host? How might this affect sanitation practices?
3. If you were testing a water sample for protozoan contamination, which species from today's lab might you expect to find and why?
4. What would you expect to see if a sample contains both protozoa and bacteria? How would you tell the difference between them under the microscope?

16: TEMPERATURE EFFECTS

LEARNING OBJECTIVES

- Describe how temperature influences the rate of biochemical reactions in bacterial cells.
- Describe the effect of small temperature shifts on bacteria with conditional lethal or non-lethal mutations.

BACKGROUND

Temperature plays a major role in the growth and survival of bacteria because it affects the speed of biochemical reactions—the chemical processes that occur inside living organisms. As temperature increases, these reactions speed up, allowing bacteria to grow and divide more quickly. However, this only continues up to a point.

Biological reactions are controlled by enzymes, which are specialized proteins that speed up chemical reactions. Enzymes have a specific three-dimensional shape that allows them to function properly. If the temperature gets too high, enzymes begin to denature (lose their shape). When this happens, the enzyme can no longer carry out its function. If the enzyme is essential for life, the bacterial cell will die.

Sometimes, the enzyme is not essential. In that case, the bacterial cell may still survive, but it may grow differently or at a slower rate because a specific function is no longer working.

A clear example of enzyme temperature sensitivity in animals can be seen in Siamese cats. These cats are pale cream in color except on their cooler body parts—such as the nose, ears, paws, and tail—which appear dark brown. This difference occurs because one of the enzymes needed to make dark pigment is temperature-sensitive. In the warmer parts of the cat's body, the enzyme denatures and cannot function. In cooler areas, the enzyme remains active and pigment is produced, resulting in darker fur.

Bacteria also vary in the temperatures they can tolerate. Some grow on snow, while others thrive in hot springs. Each bacterial species has an optimal temperature range (the range in which it grows best). This range can help microbiologists identify and classify bacteria.

In this lab, you will investigate how temperature affects the growth of four different bacteria. Each one has a unique response to temperature:

- One strain has a conditional lethal mutation. A small temperature increase—from 34°C to 37°C—is enough to damage an essential enzyme and cause cell death.
- One is a thermophile, or heat-loving bacterium. It grows well at high temperatures like those found in hot springs. At cooler temperatures, it does not die right away, but it cannot grow.
- A third strain has a conditional non-lethal mutation. When the temperature increases slightly, one of its enzymes becomes nonfunctional. However, because this enzyme is not essential, the cells survive and grow, although they may grow differently.
- The fourth strain is a mesophile. It grows best at moderate temperatures between 25°C and 40°C, which includes room temperature and body temperature.

Your task is to observe the growth patterns of these four organisms at different temperatures to help determine which is which.

MATERIALS (Per Group of 4)

3 different bacterial cultures

4 sterile petri dishes (1 dish per student)

METHODS/PROCEDURES

1. Divide the agar plate into three quadrants as shown on the board.
2. Inoculate organism 1 in quadrants 1, organism 2 in quadrant 2 and organism 3 in quadrant 3.
3. Place your inoculated plate (agar up) into the tub that corresponds to your assigned incubation temperature.
4. At the next lab session observe the class results then answer the questions below.

NAME _____

EX 16 Temperature Effects

EXPECTATIONS

If your culture is a thermophile. What would you expect to see after incubation at 30°C, 38°C, and 55°C? Explain why.

RESULTS

Record your observations in the table below. Record growth as either strong, weak or none. Include notes regarding any physical differences you observe such as color, texture etc.

T E M P E R A T U R E	Name of Culture			
	20°C			
	30°C			
	38°C			
	55°C			

CONCLUSIONS

1. Which culture showed the best growth at the highest temperature (55°C)? What does this suggest about the enzymes in that organism?
2. Which culture failed to grow at 38°C but grew well at 30°C? What type of mutation might this strain have and what is the likelihood of it being a human pathogen?

3. Which culture grew differently at 38°C compared to 30°C but was still viable. What kind of mutation does this represent? How can you tell?
4. Which culture grew well at 38°C and 30°C but did not grow at 55°C? What does this indicate about its temperature tolerance and the likelihood of it being a human pathogen?

17: ANTIBIOTICS

LEARNING OBJECTIVES

- Explain the purpose of antibiotic susceptibility testing and describe why it is essential for selecting effective antibiotic treatments in clinical settings
- Describe how the Kirby-Bauer disk diffusion method works, including the role of antibiotic diffusion, bacterial sensitivity, and standardized reference tables in interpreting results.

BACKGROUND

Antibiotics have revolutionized the treatment of bacterial infections and remain one of the most powerful tools in modern medicine. However, to use them responsibly and effectively, it is essential to determine which antibiotics are likely to be successful against a specific infection. Choosing the wrong antibiotic—or using the right one incorrectly—can have serious consequences. Not only does the patient receive no benefit, but side effects may occur unnecessarily, and effective treatment may be delayed. Worse still, inappropriate antibiotic use increases the likelihood of developing **antibiotic resistance** (the ability of bacteria to survive and grow in the presence of drugs designed to kill them).

Antibiotic resistance can arise from several factors, including:

- Using antibiotics when they are not needed
- Selecting the wrong drug for the type of infection
- Prescribing incorrect dosages
- Ending treatment too early

Each exposure to an antibiotic increases the selective pressure on bacteria, encouraging the survival of resistant strains. Over time, this can lead to the loss of effectiveness for antibiotics that were once reliable.

In life-threatening situations, healthcare providers may start treatment with a "best guess" antibiotic before laboratory test results are available. Even in these cases, it is critical to collect a sample and perform an **antibiotic susceptibility test** (a laboratory test that determines how effective different antibiotics are against a particular bacterial strain). This allows physicians to adjust therapy if the initial treatment proves ineffective.

One of the most widely used tests to determine antibiotic effectiveness is the **Kirby-Bauer disk diffusion test**, also known as a **culture and sensitivity test**. In this method, the bacteria isolated from the patient are spread evenly across the surface of a special nutrient-rich agar medium called **Mueller-Hinton agar**. This medium supports the growth of most bacteria and allows for consistent and reliable diffusion of antibiotics.

Sterile paper discs containing known concentrations of different antibiotics are placed on the surface of the agar. As the plate is incubated, the antibiotics diffuse outward from each disc, creating a gradient of concentration in the surrounding agar. If the bacteria are sensitive to a particular antibiotic, a **zone of inhibition** (a clear, circular area where bacteria do not grow) will appear around the disc.

The size of the zone of inhibition provides important information:

- A **large zone** suggests the bacteria are very sensitive to the antibiotic.
- A **small zone** may indicate that the bacteria are only slightly sensitive.
- If there is **no clear zone**, the bacteria are resistant.

However, zone size alone is not enough to determine effectiveness. Different antibiotics diffuse through agar at different rates depending on their molecular size and chemical properties. For example, smaller antibiotic molecules spread farther than larger ones, so a small zone for a large molecule antibiotic might still indicate effectiveness at low concentrations.

To account for these differences, standardized reference tables have been developed. These tables list the **minimum zone sizes** that correspond to effective concentrations in the human body. By measuring the diameter of the clear zone around each antibiotic disc and comparing it to the table, microbiologists can classify each bacterial response as:

- **Sensitive (S):** The antibiotic is likely to work at standard doses.
- **Intermediate (I):** The antibiotic may be effective at higher doses or in combination with others.
- **Resistant (R):** The antibiotic is unlikely to be effective.

If the organism is resistant to all tested antibiotics, the lab may recommend combining two or more drugs to which the bacteria showed intermediate responses. However, an antibiotic with a clear resistance profile should never be used.

Accurate antibiotic testing is essential not only for effective patient care but also for preserving the long-term usefulness of antibiotic medications. Tests like the Kirby-Bauer method help ensure that treatment decisions are guided by scientific evidence rather than guesswork.

MATERIALS (Per Student Group of 4)

- 4 Large plates Mueller-Hinton agar
- 2 Cell spreaders
- 1 Jars of alcohol
- 2 Turn tables
- 8 Antibiotic Discs
- 1 Forcep
- 4 Sterile 1.0 ml pipets
- 2 Gram negative cultures
- 2 Gram positive cultures

. METHODS

1. Each student will work with one of the four assigned bacterial cultures. Record the name of the bacterial species you are using in the results table.
2. Record the name of the antibiotics you are going to test and the associated code in the results table.
3. Using aseptic technique, transfer approximately 0.5–0.6 mL of your assigned bacterial culture onto the surface of the agar plate.
4. Immediately spread the culture evenly across the surface of the plate using a sterile cell spreader to create a bacterial lawn (a continuous, even layer of bacterial growth).
5. Allow the culture to absorb into the agar by letting the plate sit undisturbed for a few minutes with the lid closed and the agar side facing down.
6. Using flamed, alcohol-sterilized forceps, gently press each antibiotic disc to ensure full contact with the surface of the agar. This step is essential for consistent diffusion of the antibiotic into the medium. Make sure the discs are evenly spaced on the agar surface to prevent overlapping zones of inhibition. Try not to touch the forceps to the surface of the agar as this would compromise sterility and could disturb the lawn of bacteria. Once a disc touches the agar, do not move the disc. If a disc accidentally lands in the wrong location. If a disc falls off the plate completely, do not reuse it—replace it with a new sterile disc if needed.

7. Incubate the plates **agar side down** at 30°C until the next class period.

NAME _____

EX 17 Antibiotic Resistance

EXPECTATIONS

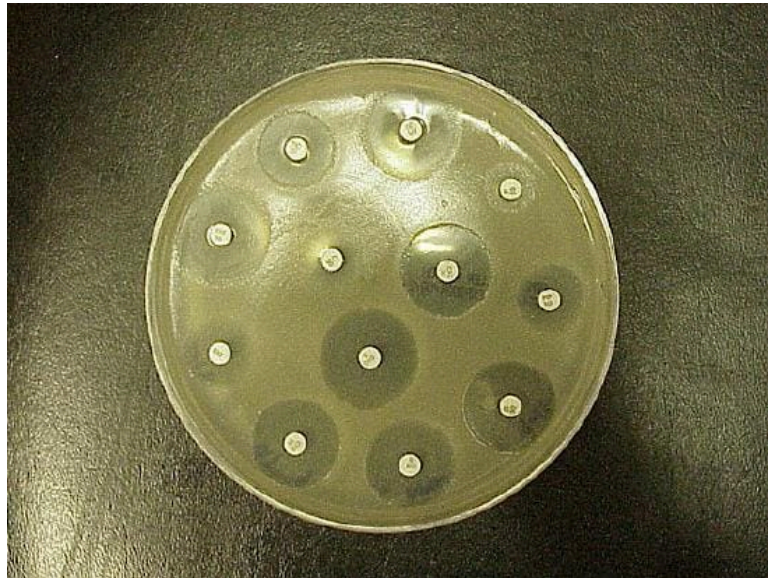
Do you think all antibiotics will produce the same size zone of inhibition for your assigned bacterium? Why or why not?

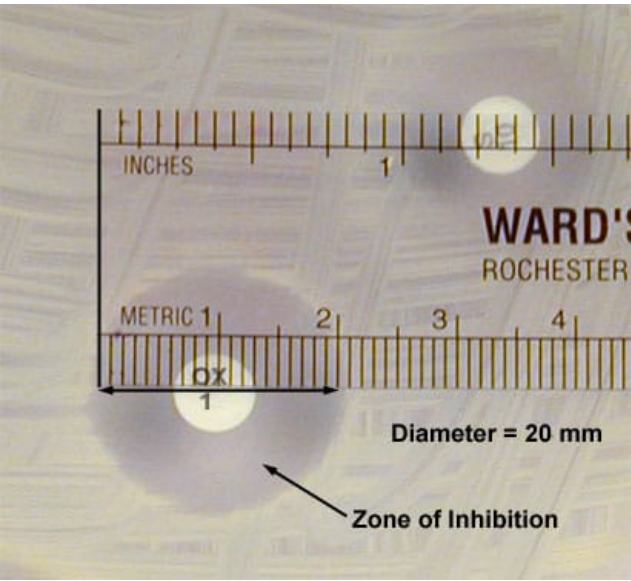
Do you expect all the zones of inhibition for the bacteria that are susceptible to a particular antibiotic to be the same or different? Explain your reasoning.

RESULTS

Assigned Organism _____

1. If a zone of inhibition is present (without any colonies growing in the zone), measure the zone diameter in millimeters then record the value in the table below.





Antibiotic Tested	Zone of Inhibition in mm	R, S, or I

2. Compare the diameter you measure for each antibiotic to the value in the Kirby-Bauer Zone Diameter table to determine if the bacteria is resistant, intermediate or sensitive to the antibiotic.

Kirby-Bauer Zone Diameter

Antibiotic	Disk Potency	Susceptible (S) $\geq$ mm	Intermediate (I) mm	Resistant (R) $\leq$ mm
Ampicillin	10 μ g	17	15–16	14
Penicillin G	10 units	29	22–28	21
Chloramphenicol	30 μ g	18	13–17	12
Erythromycin	15 μ g	23	14–22	13
Streptomycin	10 μ g	15	12–14	11
Tetracycline	30 μ g	19	15–18	14
Gentamicin	10 μ g	15	13–14	12
Ciprofloxacin	5 μ g	21	16–20	15
Clindamycin	2 μ g	21	15–20	14
Cefoxitin	30 μ g	18	15–17	14
Oxacillin	1 μ g	13	11–12	10
Sulfamethoxazole/Trimethoprim	25 μ g	16	11–15	10
Amoxicillin/Clavulanic acid	30 μ g	18	14–17	13
Tobramycin	10 μ g	15	13–14	12
Vancomycin	30 μ g	12	10–11	9
Cephalothin	30 μ g	18	15–17	14
Azithromycin	15 μ g	18	14–17	13
Levofloxacin	5 μ g	17	14–16	13
Nitrofurantoin	300 μ g	17	15–16	14
Doxycycline	30 μ g	16	13–15	12
Imipenem	10 μ g	23	14–22	13
Ceftriaxone	30 μ g	23	20–22	19
Linezolid	30 μ g	23	21–22	20
Piperacillin/Tazobactam	100/10 μ g	21	18–20	17
Meropenem	10 μ g	23	20–22	19
Nalidixic Acid	30 μ g	19	14–18	13

CONCLUSIONS

1. Explain what it means for bacteria to be susceptible to an antibiotic.

2. Define zone of inhibition. What does the presence or absence of a zone indicate about the bacterial response to an antibiotic?

3. Why is the diameter of the zone of inhibition alone insufficient for determining whether an antibiotic is effective?

4. How did performing the Kirby-Bauer test help you better understand the relationship between microbiology laboratory data and real-world clinical decisions?

5. It is not unusual for a healthcare provider to begin antibiotic treatment before susceptibility results are available. Based on this lab, explain why it is still important to perform antibiotic susceptibility testing even after treatment has already started.

18: ANTISEPTICS AND DISINFECTANTS

LEARNING OBJECTIVES

- Define disinfectants, antiseptics, and antibiotics, and explain their proper applications.
- Analyze your experimental results to determine which agents were most successful at controlling microbial growth.

BACKGROUND

Unlike antibiotics, which are used internally to treat infections, **disinfectants** (chemicals used to kill microorganisms on external surfaces) are applied to objects and surfaces outside the body. A special type of disinfectant is called an **antiseptic** (a disinfectant that is safe to use on human skin). Disinfectants and antiseptics are among the most important tools that make modern surgery possible—alongside effective **anesthesia** (a method for preventing pain during procedures).

Before these tools were developed, surgery was considered a last resort. It was generally used only for emergencies such as repairing traumatic injuries, sewing up wounds, or amputating limbs that could not be saved. Infections after surgery were often fatal, making most surgical attempts extremely risky. During wartime, many soldiers died not from their wounds but from the infections that followed. Even minor cuts from knives or bullets could turn deadly once an infection set in. In fact, it was not unusual for people to die from the infection of a simple scratch or abrasion.

Before germ theory was well understood, people relied on a variety of techniques to try to prevent infection—some more effective than others. Surprisingly, a few traditional remedies were actually useful. Certain herbs and spices, alcohol, and powdered sulfur all have antimicrobial properties. When used consistently, these substances could reduce the risk of infection.

A major turning point in infection control came when **Florence Nightingale** introduced the routine use of hot water and soap in the care of wounded soldiers. This simple practice led to a dramatic drop in infection and death rates and marked the beginning of modern antiseptic technique.

Some of the same compounds used to prevent wound infections were also used to preserve food. Before refrigeration, food would quickly spoil as **microbial cultures** multiplied. To slow this growth and prevent **food poisoning** (illness caused by ingesting food contaminated with harmful microorganisms), people used salt, sugar, acids, alcohol, and spices. These substances helped suppress or kill bacteria and fungi, acting as simple antiseptics or disinfectants both on the body and in food.

MATERIALS (Per Group of 4)

- 4 Nutrient agar plate
- 4 Pure cultures
- 4 Related disinfectants (spices, cosmetics, toothpastes, household cleaners etc.)
- 2 Cell spreaders
- 2 Jars of alcohol
- 2 Turn tables
- 4 Sterile 1.0 ml pipets

16 Sterile filter paper disks

4 Small beakers

METHODS/PROCEDURES

Choose and Plan Your Test Compound

1. As a group, decide on four compounds to test that are related in purpose.
 - ✓ Examples: Four different herbs, four brands of mouthwash, or four types of household cleaners.
 - ✗ Avoid unrelated items (e.g., comparing mouthwash to a surface disinfectant).
2. Choose something your group finds interesting—you'll be more engaged in the results.

Inoculate Your Agar Plate

3. Obtain your assigned bacterial culture (a liquid suspension of bacteria).
4. Use a sterile pipette or dropper to place about 0.3–0.5 mL of the culture onto an agar plate.
5. Spread the bacteria evenly across the surface using a metal rod that has been flamed and cooled.
6. Each student in your group should use a different bacterium but test the same four compounds.
7. Share results to compare how each compound affects different bacteria.

Apply the Test Compounds

8. Label the bottom of your agar plate to identify the position of each disc.
9. If your compound is **solid**:
 - a. Use flamed forceps to pick up a sterile paper disc.
 - b. Gently touch the disc to the solid compound.
 - c. Tap lightly to remove any excess material.
 - d. Place the disc onto the surface of the inoculated plate.
10. If your compound is **liquid**:
 - a. Pour a small amount of the liquid into the container lid or a clean watch glass.
 - b. Use flamed forceps to pick up a sterile disc.
 - c. Dip the disc into the liquid.
 - d. Place the disc onto the surface of the agar.
11. Apply all four compounds using the same technique so your results are consistent and comparable.
12. Double-check that your plate is labeled correctly to match each disc's location.
13. Carefully carry your plate to the incubator.

14. Place the plate in the incubator agar side down to keep the discs in place and prevent condensation from dripping on the agar.

NAME _____

EX 18 Antiseptics and Disinfectants

EXPECTATIONS

What four compounds did your team choose to test? List each compound and include any specific brand names or sources if applicable.

Why did your group think these compounds might have disinfectant or antimicrobial properties?
Explain your reasoning.

Which compound do you predict will be the most effective at inhibiting bacterial growth?
Explain why you made this prediction.

RESULTS

Organism tested _____

Tested Compound	Diameter Cleared	Susceptible or Resistant

Organism tested _____

Tested Compound	Diameter Cleared	Susceptible or Resistant

Organism tested _____

Tested Compound	Diameter Cleared	Susceptible or Resistant

Organism tested _____

Tested Compound	Diameter Cleared	Susceptible or Resistant

CONCLUSIONS

1. After examining the plates for all four bacteria, did any of the compounds appear effective at suppressing bacterial growth?
2. Were the most effective compounds the ones you predicted would work best? Explain your answer and include any surprises.
3. Did any of the compounds work against some bacteria but not others? If so, which compounds were selective, and which bacteria were affected?
4. Was one of the four bacteria generally more resistant to all the compounds tested? If yes, identify the bacterium and describe what you observed.

19: ALCOHOL FERMENTATION

LEARNING OBJECTIVES

- Define fermentation and explain how yeast converts sugars into alcohol and carbon dioxide.
- Monitor and record the fermentation process by observing visible signs such as bubbling or pH changes.

BACKGROUND

Wine making is an ancient form of fermentation, a metabolic process in which microorganisms convert sugars into alcohol and other by-products. This technique has been practiced by human civilizations for thousands of years. The process likely began by accident, when overripe or crushed fruit was left at room temperature and naturally occurring microbes began to ferment the sugars, producing alcohol and changing the fruit's flavor and aroma. This accidental discovery introduced early humans to the intoxicating and preservative effects of fermentation.

Wine can be made from virtually any sweet fruit, including grapes, plums, cherries, currants, and strawberries. It can even be made from sweetened water. Mead, a traditional beverage made by fermenting honey and water, is one of the oldest examples of wine-like drinks and was especially popular in early Celtic and Nordic cultures.

In modern wine production, the yeast species *Saccharomyces cerevisiae* is commonly used because of its reliability and efficiency in converting sugars into ethanol (alcohol) and carbon dioxide. The amount of sugar in the starting fruit juice determines the potential alcohol content of the final wine: more sugar results in more alcohol. During fermentation, yeast continues to convert sugars until the supply runs out or the alcohol level becomes too high for the yeast to survive.

Since alcohol inhibits the growth of many other microbes, it acts as a natural preservative. This quality made wine especially useful in ancient times as a way to preserve the calories and nutrients of fruit. In fact, in some regions where clean drinking water was scarce or unreliable, wine and other fermented beverages were safer to consume than untreated water.

Although wild yeasts and bacteria are naturally present on the skins of grapes, not all of these organisms are beneficial for wine making. Some can spoil the wine by producing acetic acid or other unwanted by-products. In modern wineries, grapes are often treated with sulfur dioxide or gently pasteurized to eliminate wild microbes. After this step, a known culture of yeast is added to begin a controlled fermentation.

The visual haze seen on the surface of fresh grapes is often a layer of wild yeast. Historically, this natural yeast helped start the fermentation process. Grapes became a favored fruit for wine production in part because of this yeast-rich surface. However, controlling fermentation by using selected strains has led to more consistent and higher-quality wines.

The difference between red and white wine lies in how the grapes are handled before fermentation. Red wine is produced by fermenting the crushed grapes along with their skins and seeds. The skins contribute color, flavor, and tannins—compounds that influence the texture and taste of the wine. In contrast, white wine is made by removing the grape skins before fermentation. This results in a lighter color and different flavor profile, as the juice ferments without extracting compounds from the skin.

In some fermentation processes, very little carbon dioxide is retained. This produces still or non-sparkling wines. If the carbon dioxide is allowed to escape during fermentation, the wine remains still. However, when fermentation occurs in a sealed container, the carbon dioxide stays dissolved in the liquid. This process results in sparkling wine, such as champagne.

A significant milestone in understanding fermentation came in the mid-1800s, when Louis Pasteur demonstrated that *Saccharomyces cerevisiae* was responsible for the desirable fermentation in wine. He also introduced the process of pasteurization, which involves gently heating liquids to reduce or eliminate unwanted microorganisms. By reducing contamination and allowing the controlled addition of a known yeast culture, pasteurization greatly improved the quality and consistency of wine production.

In this laboratory exercise, you will create your own small batch of wine using pasteurized fruit juice and a commercial yeast culture. You'll apply techniques developed over centuries to begin and monitor the fermentation process. Be sure to follow each step carefully—while fermentation is a natural process, small errors in handling can lead to contamination or spoilage.

MATERIALS (Per Group of 4)

3 large screw-cap glass tubes

Fresh grapes or other soft fruit

1 Scalpel or sterile cutting tool

1 Glass stirring rod

1 DI water bottle

4 Sterile transfer pipets

2 pH test strips

1 *Saccharomyces cerevisiae* culture

1 500 mL Beaker

1 Hot plate

1 Thermometer

1 bottle of methylene blue

Microscope slides

METHODS/PROCEDURES

1. Label your tubes as follows:
 - a. Tube #1: Crushed fruit; unpasteurized; uninoculated
 - b. Tube #2: Crushed fruit; pasteurized; uninoculated
 - c. Tube #3: Crushed fruit; pasteurized; inoculated with *Saccharomyces cerevisiae*.
2. Wash your hands thoroughly with soap and water. Rinse your fruit under running water to remove any surface debris.
3. Using a scalpel, carefully cut the grapes (or other selected fruit) into quarters. Place 2–3 cut pieces into each of three large, screw-cap glass tubes.

4. Gently mash the fruit in each tube using a clean glass stirring rod. Be careful not to crack or break the glass. If your fruit does not release much juice (ask your instructor to confirm), add 1 mL of sterile water to each tube using a sterile pipet.
5. Measure and record the pH of each sample in Figure 3. Use a transfer pipet to remove a small amount of liquid—do not insert the pH strip directly into the tube.
6. Prepare a boiling water bath using a beaker with approximately 300 mL of water. The water level should be higher than the fruit level in the tubes, but not so high that water can enter the tubes. Do not allow water from the bath to spill into the samples.
7. Once the water reaches a boil, carefully place Tubes #2 and #3 into the water bath. Turn off the hot plate immediately after adding the tubes.
Pasteurize these samples by leaving them in the hot water bath for 5 minutes. Then, using test tube holders, remove the tubes and allow them to cool to room temperature in a test tube rack.
8. Inoculate Tube #3 with *Saccharomyces cerevisiae* using a transfer pipet (approximately 4–5 drops).
9. Once the tubes have cooled, recheck and record the pH of all three tubes. Also observe and note the color, smell, and any other distinguishing characteristics of each sample at this time.
10. Tightly screw the caps onto all three tubes. Incubate them at room temperature or at 30°C for one week.

After one week of incubation:

11. Before opening the tubes, examine each for signs of gas production (bubbles may indicate fermentation).
12. Perform a simple stain on the contents of all three tubes. Record your observations.
13. Measure and record the final pH of each tube.
14. Record any signs of fermentation, such as a change in pH, gas bubbles, or the characteristic aroma of alcohol.

NAME _____

EX 19 Alcohol Fermentation

EXPECTATIONS

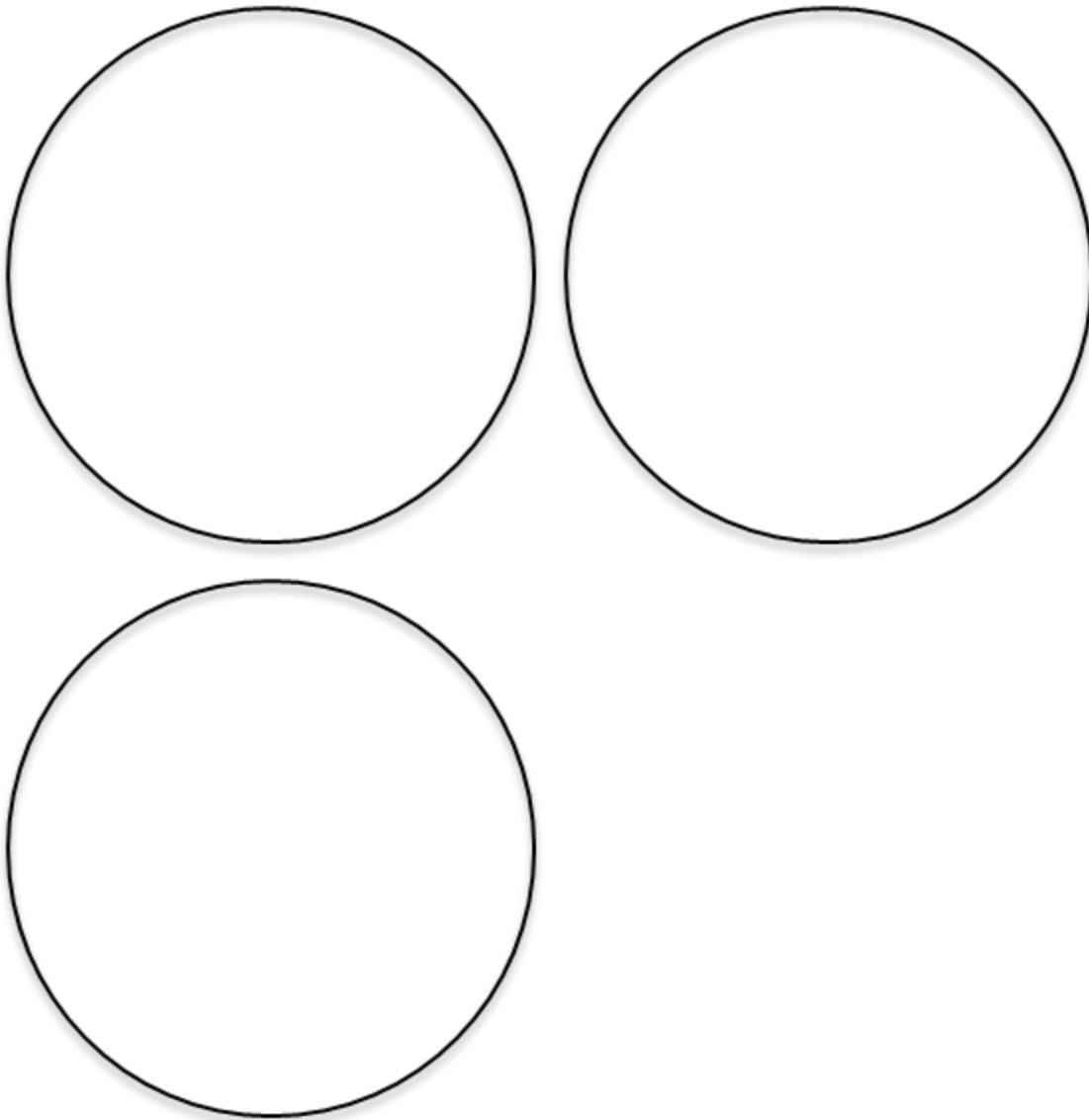
What visible or measurable changes do you expect to observe in a tube where fermentation has successfully occurred? List at least two signs.

RESULTS

Fermentation Observations:

Tube	Treatment Description	pH Before Incubation	pH After Incubation	Bubbles Present? (Y/N)	Alcohol Smell or aroma? (Y/N)	Color / Appearance after incubation
A	Crushed fruit, uninoculated & unpasteurized					
B	Crushed fruit, uninoculated & pasteurized					
C	Crushed fruit, inoculated & pasteurized					

Simple Stain Observations:



CONCLUSIONS

1. Which tube showed the clearest signs of fermentation and alcohol production? Describe the evidence that supports your conclusion
2. Examine your simple stain results. What types of microbial cells did you observe? Were there differences in shape or abundance between the tubes? What can the differences tell you about the tubes?

3. Alcohol is known to inhibit the growth of many microorganisms. How do you think this property benefits wine production? What effect might it have on contamination?

4. Alcohol is known to inhibit the growth of many microorganisms. How do you think this property benefits wine production? What effect might it have on contamination?

5. Given that wine can be made from many types of fruit, how might the sugar content of the fruit you choose affect the fermentation process and final alcohol content?

20: SKIN AND UPPER RESPIRATORY BACTERIA

LEARNING OBJECTIVES

- Differentiate between alpha, beta, and gamma hemolysis on blood agar plates.
- Use hemolytic patterns to identify potential pathogenic organisms, such as *Streptococcus pyogenes*.

BACKGROUND

The skin and upper respiratory tract are ideal environments for microbial growth. Under normal conditions, the microorganisms that naturally reside in these areas—known as the normal flora—do not cause harm. In fact, many of these microbes are beneficial and contribute to the overall health of the tissues they inhabit.

The throat is a particularly accessible part of the upper respiratory tract for sampling and analysis. It also serves as a common entry point for infectious agents. The normal flora of the throat includes species from several genera, such as *Streptococcus*, *Staphylococcus*, *Branhamella*, *Corynebacterium*, *Haemophilus*, *Actinomyces*, and *Bacteroides*. Many of these organisms can be preliminarily identified using selective media and a Gram stain.

Interestingly, pathogenic organisms can belong to the same genera as the normal flora. Examples include *Streptococcus pyogenes*, a major cause of strep throat, and *Corynebacterium diphtheriae*, which causes diphtheria. Other pathogens in the throat may include yeast and viruses.

Of particular importance is *Streptococcus pyogenes*, a frequent upper respiratory tract pathogen. This organism causes strep throat and produces enzymes capable of lysing red blood cells and degrading hemoglobin, contributing to tissue damage during infection. A classic diagnostic method involves growing throat swabs on blood agar, a medium that contains intact red blood cells. Colonies of *S. pyogenes* often produce a clear zone around them, where the red blood cells have been completely lysed. This phenomenon is called beta hemolysis and serves as a strong indicator of a pathogenic streptococcal infection.

In contrast, many nonpathogenic streptococci produce enzymes that only partially lyse red blood cells. This results in greenish discoloration and partial clearing around colonies, known as alpha hemolysis. Some organisms do not lyse red blood cells at all; these are referred to as gamma hemolytic or non-hemolytic. Blood agar is a useful tool for identifying the hemolytic characteristics of streptococci, and a follow-up Gram stain can help differentiate other organisms that may appear on the plate.

The skin is another body site rich in normal flora. One of their primary benefits is their ability to outcompete potential pathogens for space and resources. Many skin bacteria also produce enzymes or metabolic byproducts that inhibit the growth of invading microbes, including harmful bacteria, fungi, and viruses.

However, when the protective barrier of the skin is compromised—through irritation, cuts, or invasive medical procedures, the same organisms can become opportunistic pathogens. This risk is a major concern in surgical settings, where aseptic technique is essential to prevent the introduction of skin flora into sterile body sites. Even common skin organisms can become dangerous if they gain access to the bloodstream or internal tissues.

Preliminary identification of skin flora can be achieved using a Gram stain in combination with colony characteristics, such as color and shape. Understanding the typical microorganisms found in the throat and on the skin is essential for distinguishing between normal and pathogenic microbes during clinical evaluations.

TYPICAL THROAT FLORA AND PATHOGENS

Organism	Morphology
<i>Staphylococcus</i>	Gram-positive cocci in clusters
<i>Streptococcus</i>	Gram-positive cocci in chains
<i>Branhamella</i>	Gram-positive cocci in pairs
<i>Corynebacterium</i>	Gram-positive pleomorphic rods
<i>Haemophilus</i>	Gram-positive pleomorphic rods
<i>Actinomyces</i>	Gram-positive rods in filaments
<i>Bacteroides</i>	Gram-negative pleomorphic rods

TYPICAL SKIN FLORA

Organism	Morphology and Colony Appearance
<i>Staphylococcus epidermidis</i>	Gram-positive cocci in clusters, small white colonies
<i>Staphylococcus aureus</i>	Gram-positive cocci in clusters, yellowish colonies
<i>Propionibacterium acnes</i>	Gram-positive pleomorphic rods
<i>Corynebacterium</i> spp.	Gram-positive pleomorphic rods, waxy colonies
<i>Pityrosporum</i> spp.	Small budding yeasts
<i>Streptococcus pyogenes</i>	Gram-positive cocci in chains

MATERIALS (Per Student)

- 1 Nutrient agar plate
- 1 DI water tube
- 1 Sterile swab

METHODS/PROCEDURES

Choose a sampling area

Select an area of your skin approximately 2 inches by 2 inches. If the area you wish to test is not easily accessible (such as your back or upper thigh), you may take sterile swabs to the restroom for privacy.

2. Moisten the swab

Dip a sterile swab into the tube of sterile water to dampen it. Gently roll the swab against the inner wall of the tube to remove excess moisture. The swab should be damp but not dripping.

3. Swab the skin

Thoroughly swab the entire 2 x 2 inch skin area using firm but gentle pressure. Roll the swab between your fingers as you go to ensure all surfaces of the cotton tip make contact with the skin.

4. Inoculate the agar plate

Immediately after swabbing your skin, gently rub the swab across the entire surface of the nutrient agar plate. Roll the swab while streaking to transfer microorganisms from all sides of the swab to the agar surface.

5. Label and seal the plate

Write your last name, the date, and the area sampled on the bottom of the Petri dish (the smaller, deeper half). Use a small piece of tape to securely seal the lid to the base of the dish.

6. Incubate the plate

Place the labeled and sealed dish in the incubator as directed by your instructor.

UPPER RESPIRATORY FLORA

Important Safety Note: Only the instructor may perform throat swab collections.

Do not attempt to swab your own throat or another student's.

If you are interested in participating in a throat culture test, sign up on the board. This activity is optional, and not required for credit.

For those who choose to participate, a sterile swab will be used by the instructor to collect microorganisms from the back of your throat. The swab will then be streaked onto a blood agar plate. This medium is useful for detecting **hemolysis**—the breakdown of red blood cells—which can help differentiate between normal flora and potential pathogens.

NAME _____

EX 20 Skin and Respiratory Flora

EXPECTATIONS

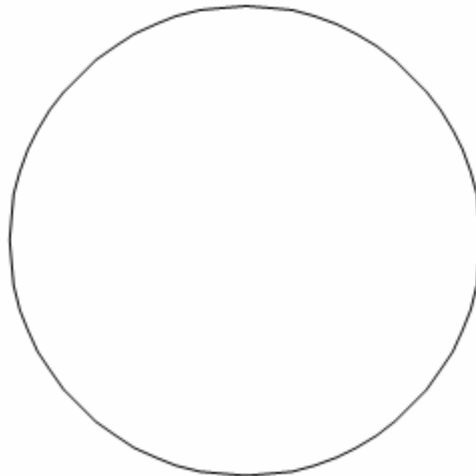
How many total colonies do you think will grow on your skin culture plate? _____

How many different types of colonies (based on appearance) do you expect to see? _____

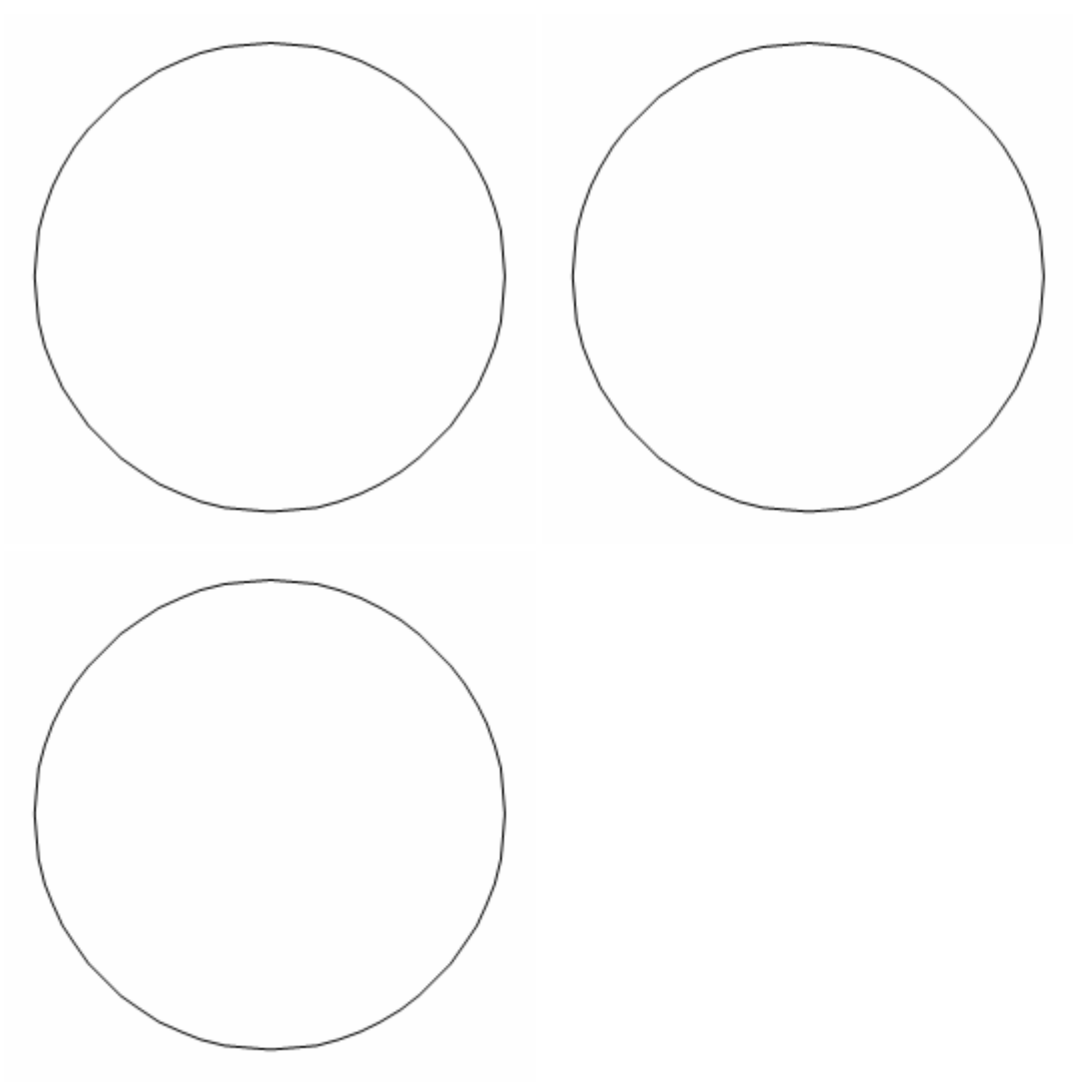
Which do you expect to show a greater variety of microorganisms—the skin cultures or the throat cultures? Briefly explain your reasoning.

RESULTS

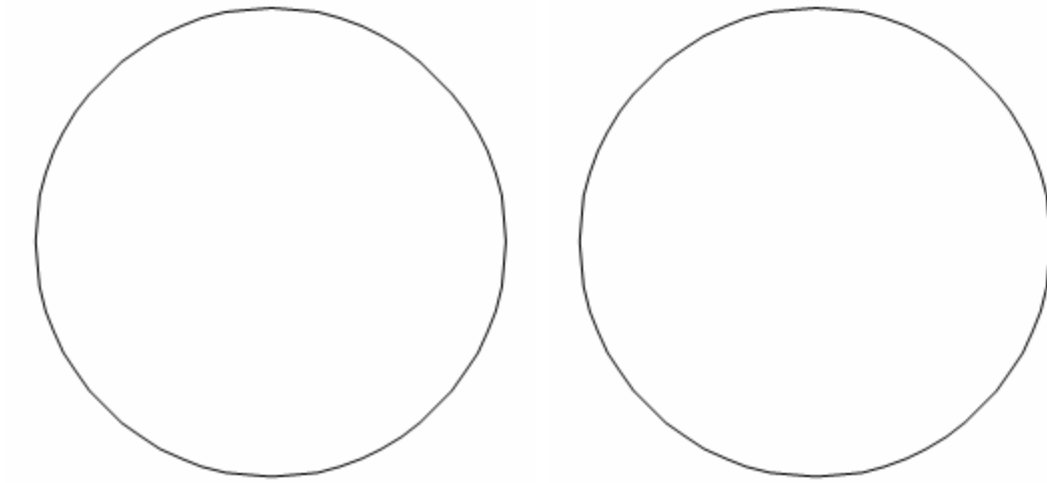
Draw your observations of the skin test plate after incubation.



Draw your Gram stain and controls below. Use accurate colors that reflect the colors seen on the slide below.



Alpha and Beta Hemolysis blood agar plates are on display at the instructor bench. Observe the growth on the plates then draw what you see below. Identify any hemolysis you see.



CONCLUSIONS

1. How accurate was your prediction about the number and types of colonies that grew on your skin culture plate? Explain how your results are compared to your initial guess.
2. How does alpha hemolysis differ from beta hemolysis in appearance and cause?
3. What does the term opportunistic pathogen mean in the context of normal flora?
4. Why is the ability to identify alpha, beta, and gamma hemolysis important in clinical microbiology?

21: ANTIBIOTIC PRODUCING MICROBES IN SOIL

LEARNING OBJECTIVES

- Differentiate between normal microbial colony growth and colonies that possibly produce antimicrobial compounds.
- Evaluate the effectiveness of antimicrobial compounds produced by soil microbes on known pathogens.

BACKGROUND

You might remember that the very first antibiotic, penicillin, was discovered by Alexander Fleming in 1928. Penicillin is made by a fungus in the genus *Penicillium*. Today, many antibiotics come from a group of thread-like bacteria called Actinomycetes (*a group of bacteria that form branching filaments and are commonly found in soil*). These bacteria are responsible for producing several well-known antibiotics, including streptomycin and neomycin.

Infections caused by bacterial strains that are resistant to antibiotics are becoming an increasingly serious global health concern. These *antibiotic-resistant* infections can be difficult—or even impossible—to treat with conventional medications. As a result, scientists are working hard to discover new types (or *classes*) of antibiotics and identify new organisms that produce them. Interestingly, some of the most promising sources of new antibiotics are quite unexpected! Researchers are now investigating tropical plants, marine organisms (like certain sponges and even sharks), and other unique environments to find microorganisms that naturally produce antibiotic compounds.

Today you will explore a more familiar environment—**soil**—in search of an *antibiotic-producing organism*. Soil is a rich and diverse ecosystem filled with bacteria, fungi, and other microorganisms—many of which engage in chemical warfare to survive. That chemical warfare includes producing antibiotics to inhibit the growth of nearby competing microbes. Because of this, soil is one of the most productive environments antibiotic-producing organisms.

In this exercise, you will collect a soil sample, culture it on nutrient agar plates, and observe whether any colonies are producing substances that inhibit the growth of other bacteria. If so, you may have found a natural antibiotic producer!

MATERIALS

Day 1

- 100 mL bottle (pre-measured for you)
- 1 gram soil sample (student-collected)
- 2 Nutrient agar plates
- 1 Alcohol burner
- 1 Cell spreader
- 1 Jar of alcohol (for sterilizing metal rods)
- 1 Turntable
- Balance
- Weighing paper or boats

Day 2

Soil culture plate (from previous lab)

2 Nutrient agar plates

Day 3

Isolated colony from soil culture (previous lab)

Test organisms:

Escherichia coli

Pseudomonas fluorescens

Staphylococcus aureus

METHODS/PROCEDURES

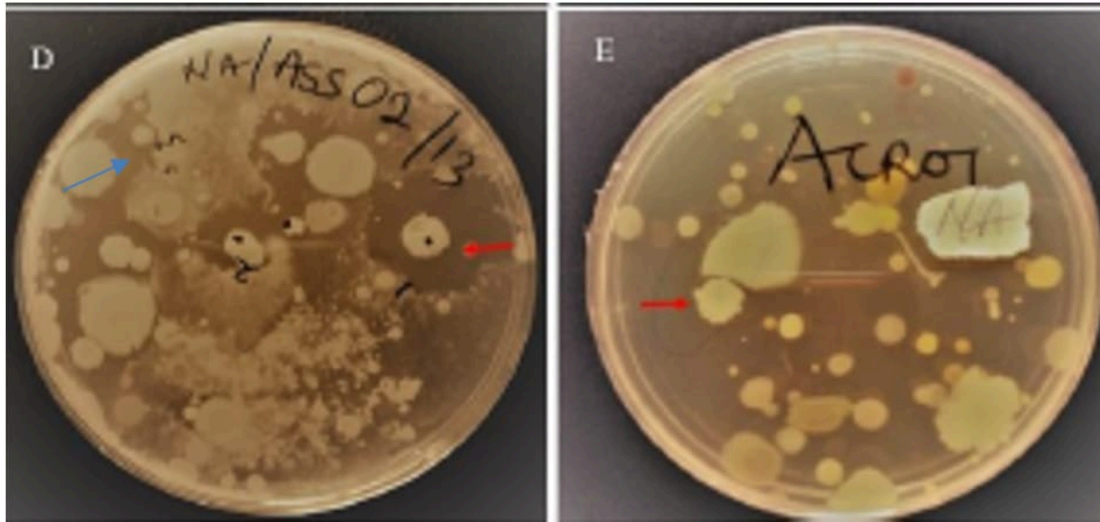
Day 1

1. Collect a soil sample from anywhere on campus.
 Tip: Typically slightly damp soil contains a higher number of microorganisms compared to very dry soil.
2. Use the balance to weigh approximately **1 gram** of your soil sample.
3. Place the 1.0g of soil in to the 100 mL bottle of water.
4. Secure the cap and shake vigorously for at least 30 seconds to mix well.
 This creates a *soil suspension* (a mixture of soil particles and microorganisms evenly dispersed in water).
5. Allow the particles to settle at the bottom of the bottle (about 10 minutes).
6. Label one of the plates with your name date, dilution amount (1.0 mL or 0.1 mL), and the source location of your soil sample.
(Your partner will label the other plate. Decide which dilution you will plate and which your partner will plate before labeling).
7. Student A: Transfer **1.0 mL** of the liquid from the top of the suspension onto the nutrient agar plate labeled 1.0 mL.
8. Use a sterilized cell spreader to spread the sample evenly across the surface of the agar.
 Tip: Let the cell spreader cool for a few seconds after flaming to avoid killing the bacteria.
9. Student B: Transfer **0.1 mL** of the suspension onto the second plate.
10. Use a sterilized cell spreader to spread the sample evenly across the surface of the agar.
 Tip: Let the cell spreader cool for a few seconds after flaming to avoid killing the bacteria.
11. Incubate the plates agar down at **room temperature** until the next lab session.

Day 2

12. Examine your soil plates carefully for colonies that seem to inhibit the growth of surrounding colonies.

👉 Remember: you are not looking for isolated colonies but rather colonies that appear to be hindering the growth of colonies around it. See example below.



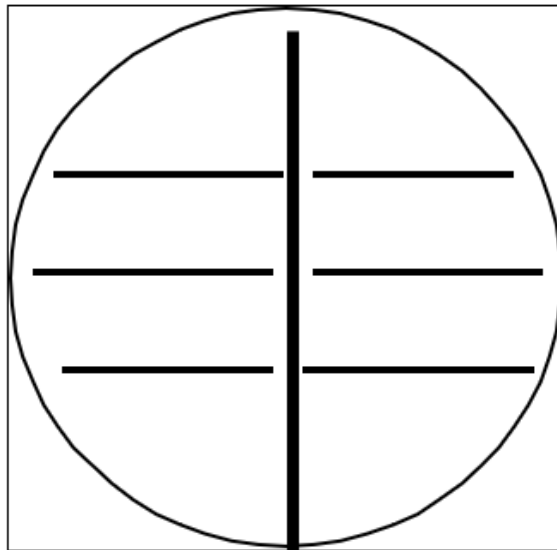
Draw your plate in the results section then indicate and label the colony that appears to be possibly producing an antibiotic.

1. Draw your plate in the results section then label the colony that appears to be possibly producing an antibiotic.
2. Show your drawing and plate to your instructor for approval of your colony selection.
3. Once approved use a sterile cooled loop to scrape the top of the chosen colony and inoculate a fresh plate by making a single streak down the center.
4. Label plate and incubate at room temperature for at least 2 days.

Day 3

1. After the streak line has grown, add the provided known cultures to the plate by making short streaks that are perpendicular on each side of the main streak line. Use the diagram below as a guide.

👉 Do not actually touch the original growth line - get as close to it as you safely can without touching.



2. Incubate at 30° C for 2-5 days.
3. Observe the test cultures for inhibition of growth where they are nearest to the original streak.
4. Draw what you see on the plate in the results section.

NAME _____

EX 21 Antibiotic Producing Microbes in Soil

EXPECTATIONS

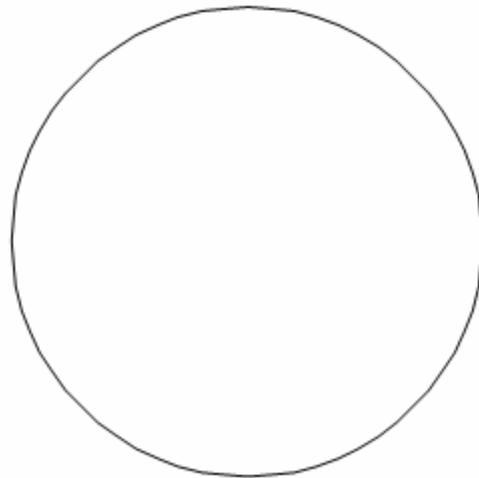
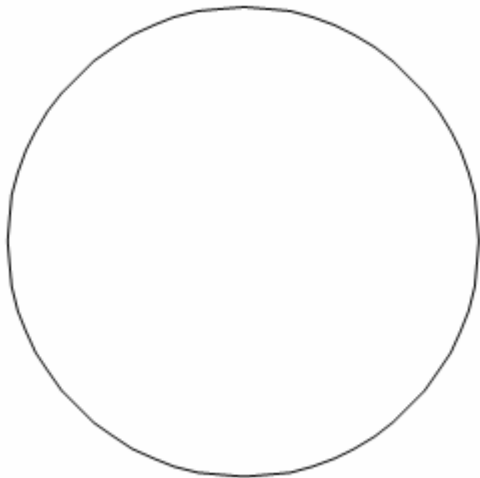
If the colony you choose secretes an antibiotic should you anticipate all test cultures to be inhibited equally? Explain your reasoning.

Describe what the growth might look like on the plate if a microorganism is producing an antibiotic?

RESULTS

Draw your soil plate after incubation

Draw your plate after incubation with the know pathogens



CONCLUSIONS

1. Did you find any inhibition by your chosen soil organism? If so, which test bacteria were inhibited?

2. Producing and releasing antibiotics requires significant energy and resources from a bacterial cell. What evolutionary

advantage does this ability provide? In other words, what benefit does the antibiotic-producing organism gain from investing in this process?

3. Compare the bacterial growth on your original soil plate and the 10^{-3} dilution plate. What does the difference in colony density tell you about the effectiveness of dilution in isolating individual colonies?

4. If no zones of inhibition were observed around any colonies, what are some possible explanations for this result? How would you modify the experiment to increase your chances of finding an antibiotic-producing organism?

This page titled [21: ANTIBIOTIC PRODUCING MICROBES IN SOIL](#) is shared under a [CC BY-NC 4.0](#) license and was authored, remixed, and/or curated by Emalee MacKenzie.

22: VIRAL PLAQUE ASSAY

LEARNING OBJECTIVES

- Perform a viral plaque assay using serial dilutions to quantify the number of infectious virus particles in a sample, and accurately calculate the plaque-forming units per milliliter (PFU/mL).
- Explain the relationship between bacteriophage replication cycles (lytic and lysogenic) and plaque formation, and evaluate the impact of bacterial growth phase and experimental technique on assay outcomes.

BACKGROUND

Viruses are not classified as living organisms because they lack the ability to reproduce independently. Instead, they must infect a **host cell** (a living cell that supports viral replication) and utilize its internal machinery to carry out the processes necessary for viral reproduction. Structurally, a virus consists of genetic material—either DNA or RNA—enclosed within a protein coat. Upon infection, the virus takes over the host cell's **metabolic processes** (the chemical reactions essential to sustaining life) to produce new viral particles.

In this laboratory exercise, we will be working with a **bacteriophage**, (a type of virus that specifically infects bacterial cells). In order to propagate a bacteriophage, we must provide it with actively growing bacterial cells and the appropriate nutrient conditions required for bacterial growth.

To accomplish this, a small volume of liquid culture containing millions of rapidly dividing bacterial cells will be spread onto the surface of a nutrient agar plate. Unlike experiments designed to isolate individual colonies, this method aims to produce a bacterial lawn (continuous, uniform layer of bacterial growth). While individual bacterial cells are not visible to the naked eye, their collective growth creates a hazy, opaque surface that resembles a grassy lawn.

Following the preparation of the bacterial lawn, bacteriophage is added to the plate. When a virus particle encounters a susceptible bacterial cell, it attaches to the cell surface and injects its DNA into the host. This initiates the bacteriophage **lytic cycle** (a viral replication process in which the bacteriophage commandeers the host's cellular machinery to synthesize and assemble new virus particles. Once the replication process is complete, the host cell undergoes **lysis** (rupturing of the cell membrane), releasing newly formed viruses into the surrounding environment. These viruses then infect neighboring cells, perpetuating the cycle for as long as the bacterial population remains in the **log phase** (the period of rapid cell division when resources are abundant).

Each successful infection initiated by a single virus particle produces a **plaque** (visible clearing in the bacterial lawn). These clear zones represent areas where host cells have been lysed by the virus. If the viral sample is viable and evenly distributed, individual plaques will be uniform in size and shape. However, if the sample contains an excessive number of viruses, the entire bacterial lawn may be destroyed, preventing proper plaque visualization.

Some bacteriophages are also capable of an alternative reproductive strategy known as the lysogenic cycle. In this pathway, the viral DNA is incorporated into the bacterial chromosome, forming a **prophage** (viral DNA integrated into the host genome). The infected bacterial cell, now referred to as a lysogen, continues to grow and divide without producing new viruses or undergoing lysis. The prophage is stably maintained and passed on to daughter cells during bacterial replication. Additionally, lysogenized cells typically become immune to reinfection by the same type of bacteriophage.

Under certain environmental stress conditions—such as exposure to ultraviolet (UV) light) or chemical agents—the lysogenic cycle can be disrupted. The prophage may be excised from the bacterial chromosome, initiating the lytic cycle and leading to viral production and eventual cell lysis. This reactivation results in plaque formation similar to a standard lytic infection.

To quantify the concentration of infectious virus particles in a sample, a plaque assay is performed. If a sample is overly dilute, plaques may be absent or too few to count accurately. Conversely, if the viral concentration is too high, the host cells may be entirely lysed, resulting in no visible lawn or plaques may merging together preventing accurate count. To achieve a countable range of plaques, a series of serial dilutions (systematic reductions in concentration) is prepared. A well-planned dilution series allows for accurate estimation of the number of viable virus particles present in the original sample

MATERIALS

- 1 5 mL of bacteriophage (obtain from instructor)
- 1 5 mL log phase bacterial culture (obtain from instructor)
- 6 Nutrient agar plates, (3 per student)
- 6 sterile transfer pipets
- 3 Nutrient broth dilution tubes, 9.0 ml each
- 1 Bent glass rod
- 1 Alcohol jar
- 1 Turntable
- 6 10 mL Serological sterile pipette

METHODS/PROCEDURES

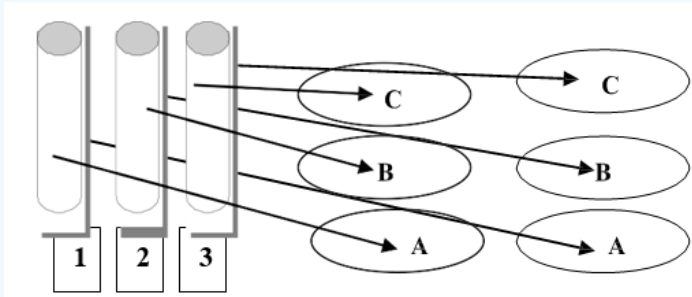
Gather and label your materials

1. Number the nutrient broth dilution tubes 1, 2, and 3 then make sure all three tubes have equal volume of broth. If the volume is not equal use a transfer pipette the transfer broth between tubes until equal
2. Each student should label their 3 plates with the required information along with A, B, and C along with the appropriate dilution factor.

2. Performing serial dilutions

1. Obtain the bacteriophage from your instructor then gently swirl the nutrient broth tube to distribute the phage in the medium
2. Using a sterile transfer pipette, transfer 1.0 mL of the virus sample into the first dilution tube then distribute the virus by gently by tapping or swirling the tube. Discard the used pipet
3. Using a fresh sterile pipet, transfer 1.0 mL from tube 1 into tube 2. Mix gently and discard the pipet
4. Using another fresh sterile pipette, transfer 1.0 mL from tube 2 into tube 3. Mix gently and discard the pipet

Be sure to use a new pipet for each transfer to prevent cross-contamination



3. Preparing the viral plaque assay plates

1. Obtain the host bacterial culture from your instructor
2. Gently mix the host bacterial culture to resuspend cells
3. Using a sterile transfer pipet, dispense 3–4 drops of the bacterial suspension onto the surface of plate A then immediately add 0.1ml of the first viral dilution tube
4. Use the alcohol jar, turntable and cell spreader cell to spread the virus and bacteria on the agar plate to form a lawn
 1. Flame the glass rod by dipping it in alcohol and briefly passing it through a flame
 2. Spread the mixture of bacteria and virus evenly across the surface of the agar
 3. Repeat steps b-f for each plate. Be sure to re-sterilize the rod between each plate

4. Absorption and incubation

1. Allow the plates to sit at room temperature, agar-side down, for at least 15 minutes to permit full absorption of the liquid into the agar.
2. After 15 minutes invert the plates (agar side up) and place them in the incubator as directed by your instructor.

NAME _____

EX 22 Viral Plaque Assay

EXPECTATIONS

Which plates do you think will have the highest number of viral plaques? Why?

RESULTS

Choose one of your plates that has clear plaques and draw it below. Remember to label fully.

Name of the bacterial host culture _____ Virus sample ID _____

Number of plaques			
	Plate A	Plate B	Plate C
Student 1 plates			
Student 2 plates			

Calculate the concentration of plaque forming units per mL in the original sample (PFU)

Viral concentration of original sample _____ PFU/mL.

CONCLUSIONS

1. Why is it important to perform plaque assays using bacteria that are in the log phase of growth rather than in the stationary or death phase?
2. If no bacterial lawn appears on a plaque assay plate, the cause could be either too few bacteria or too many viruses. Since both conditions produce similar-looking results (a plate with no visible lawn), virologists use a control plate to determine the actual cause. What should be added to the control plate to distinguish between these two possibilities, and why?
3. How would exposing a bacterial lawn carrying a lysogenic virus to a sub-lethal dose of ultraviolet (UV) light affect plaque formation? Would you expect the number of plaques to increase or decrease? Explain your reasoning based on the effect of

UV light on the lysogenic cycle.

4. Why is it important to spread the virus and bacteria mixture evenly across the agar surface when preparing a plaque assay?
How could uneven spreading affect your results?

23: ACID FAST STAIN

LEARNING OBJECTIVES

- Explain the purpose of acid-fast staining in microbiology.
- Differentiate between acid-fast and non-acid-fast bacteria based on observed staining results.

BACKGROUND

In microbiology, staining techniques are essential for observing and classifying bacteria based on their structural characteristics. While most bacterial species are effectively stained using the Gram stain, some species possess unique cell wall structures that make them resistant to standard staining techniques. One such group of organisms includes acid-fast bacteria, which are characterized by a thick, waxy cell wall rich in mycolic acids. These waxy lipids create a highly hydrophobic barrier that protects the cell from desiccation, disinfectants, and many antibiotics. This waxy coat also prevents traditional water-based stains from penetrating the cell wall. To visualize these bacteria, microbiologists use acid-fast staining—a differential staining method that distinguishes acid-fast organisms from non-acid-fast ones based on their ability to retain dye. The Kinyoun method, also called the 'cold' acid-fast stain, is a common variation of this technique. It uses a concentrated carbolfuchsin solution along with phenol to allow the dye to enter the waxy cell wall without the need for heat, making it safer and more practical in teaching labs. The Kinyoun stain is particularly important in identifying bacteria from the genus *Mycobacterium*. Two major species in this genus are *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), and *Mycobacterium leprae*, which causes leprosy (Hansen's disease). Tuberculosis is a serious infectious disease that primarily affects the lungs but can also spread to other organs. It is spread through airborne droplets when an infected person coughs or sneezes. Symptoms include a chronic cough, weight loss, night sweats, and fever. TB remains a leading cause of death in many parts of the world, especially in immunocompromised individuals such as those with HIV/AIDS. Leprosy, caused by *M. leprae*, affects the skin, peripheral nerves, mucosal surfaces, and eyes. It is a slow-progressing disease and is transmitted through prolonged close contact, likely via respiratory secretions. Symptoms include skin lesions, numbness, muscle weakness, and deformities if left untreated. Though rare in many countries, leprosy still affects thousands of people globally each year. During the Kinyoun staining process, the slide is first flooded with carbolfuchsin for several minutes. Acid-fast bacteria will retain this red dye even after being washed with acid-alcohol, the decolorizing agent. Acid-alcohol is used in acid-fast staining instead of regular alcohol because it provides the necessary strength to differentiate between acid-fast and non-acid-fast bacteria. The waxy lipids and extremely hydrophobic cell wall of the Acid-fast organisms make the cell wall extremely resistant to the penetration and removal of dyes by mild solvents like regular alcohol.

Acid-alcohol, which contains both alcohol and a small amount of acid (typically hydrochloric or sulfuric acid), is far more effective. The acid component increases the strength of the decolorizer, allowing it to strip the carbolfuchsin dye from non-acid-fast cells while leaving the acid-fast cells stained. A counterstain such as methylene blue is then applied, staining the background and non-acid-fast bacteria a contrasting blue color

- - Acid-fast bacteria → bright red/pink
- - Non-acid-fast bacteria → blue

MATERIALS (Per Group of 2)

- 1 Acid Fast organism
- 1 Non-Acid -Fast organism
- 2 Clean microscope slides
- Eye Protection (Goggles)
- Kinyoun Staining Reagents

Carbolfuchsin (Kinyoun stain)
Acid-alcohol (decolorizer)
Methylene blue (counterstain)
Distilled water (for rinsing)

METHODS/PROCEDURES

A. Smear Preparation

1. Label your slide *remember to include the organism names.
2. Aseptically transfer a small amount of each bacterial culture onto either side of the slide:
 - If using a solid culture, remember to add a loop or two of distilled water before transferring bacteria.
 - Mix gently with the loop to create a thin, even smear.

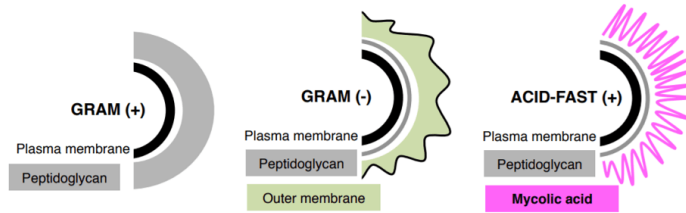
*Remember to draw a slide diagram of a paper towel with three inoculate circles

*Take care to stay within the boundaries of your diagram.

3. In the middle of the slide put one loop of each culture then mix them together.
4. Place the slide on the slide warmer to air dry completely.
5. Heat-fix the slide.

B. Kinyoun Staining Steps

6. Place the heat-fixed slide on a staining tray.
7. Flood the smear with carbolfuchsin. Let it sit for 5-10 minutes.
8. Rinse gently with distilled water.
9. Decolorize with acid-alcohol until the runoff is nearly clear (10-20 seconds).
10. Rinse again with distilled water.
11. Apply methylene blue for 1-2 minutes.
12. Final rinse with distilled water.
13. Place the slide on the slide warmer to air dry completely.
14. Once completely dry use a sharpie to draw landmarks
15. View the cultures under oil immersion
16. Draw the swears in the Results section.



Acid-fast	Non Acid-Fast	
		1. All bacteria are transparent prior to staining
		2. Steam is used to force the CARBOL FUCHSIN into all the bacteria
		3. Decolorization with acid removes the CARBOL FUCHSIN from the bacteria that do not have mycolic acid
		4. Methylene blue is the counterstain

Sourced from: [Lab 4: Acid-Fast, Spores, and Capsule Stains](#) is shared under a [CC BY-SA](#) license and was authored, remixed, and/or curated by [Nazzy Pakpour & Sharon Horgan](#).

EXERCISE 28 Acid Fast Stain

NAME _____

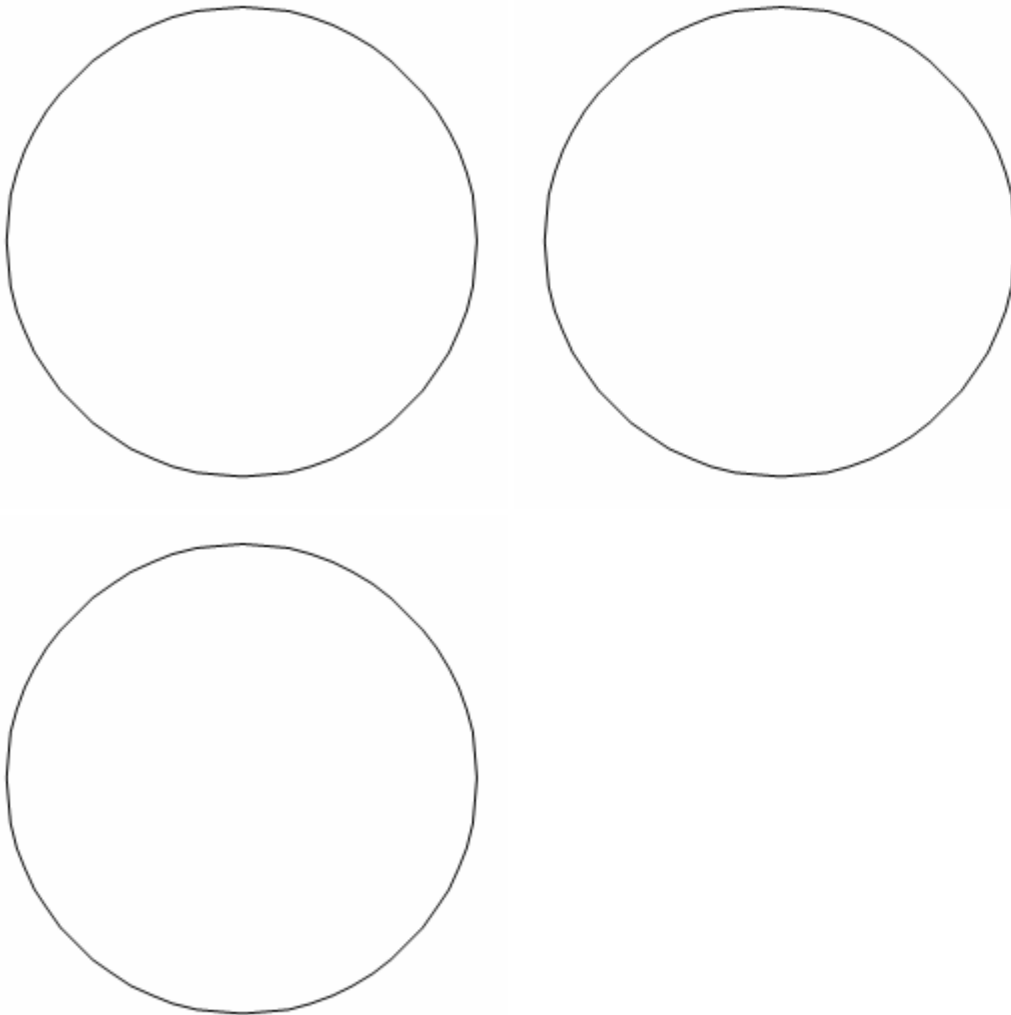
EXPECTATIONS

Which of the two cultures you used today do you anticipate being stained a bright red/pink?

Why is the acid-fast stain considered a differential stain?

RESULTS

Draw what you see on your acid-fast slide under oil immersion. Identify the acid fast and non-acid-fast cells. Remember to use color.



CONCLUSIONS

1. What color and shape were the acid-fast cells?
2. What color would acid-fast cells be if alcohol were used in place of acid alcohol? Explain your response
3. What color and shape were the non-acid-fast cells?
4. What color would non-acid-fast cells be at the end of the staining process if the acid alcohol wash was replaced with alcohol alone? Explain your response
5. Why are acid-fast bacteria often more resistant to disinfectants and antibiotics?

24: CAPSULE STAIN

LEARNING OBJECTIVES

- Correctly prepare a bacterial smear for capsule staining.
- Use a microscope to focus, observe, and record capsule stain preparations at the appropriate magnification.
- Distinguish between capsule-producing and non-capsule-producing bacteria through careful microscopic observation.

BACKGROUND

Some bacterial cells produce an additional outer layer called a capsule or slime layer. Both of these structures are composed primarily of polysaccharides (complex carbohydrates made of long chains of sugar molecules), although in some species, small amounts of protein or other molecules may also be present. The difference between the two is structural: a capsule is firmly attached to the cell wall and is well-organized, while a slime layer is loosely attached and irregular in shape. Regardless of the type, both structures offer significant survival benefits for bacteria.

These polysaccharide coatings are not always produced. Instead, they are often made only when environmental conditions support or demand their formation. For example, when nutrients are plentiful and growth is rapid, many bacteria do not waste energy producing a capsule. But under stressful conditions—such as dehydration, chemical exposure, or host immune response—these coatings provide an important form of protection.

The presence of a capsule or slime layer alters the **permeability** (ability to allow substances to pass through) of the bacterial cell membrane. This can prevent antibiotics and chemical disinfectants from entering the cell, making these bacteria harder to kill. Capsules also help protect bacteria from **desiccation** (drying out) by retaining moisture around the cell.

Importantly, capsules are considered a major **virulence factor** (a characteristic that enhances a microorganism's ability to cause disease). They can shield bacterial cells from detection and attack by the immune system. Under normal circumstances, the body's **phagocytes** (white blood cells that engulf and destroy invaders) recognize and bind to **antigens** (specific molecules on the surface of pathogens that trigger an immune response). But when a capsule covers the cell, these antigens are hidden. As a result, phagocytes may not recognize the bacteria as harmful, allowing the pathogen to survive, multiply, and cause infection over time.

Bacteria that produce capsules can remain undetected in a host for an extended period. This "stealth mode" gives them time to grow in number and establish infection before the immune system can respond effectively. Some pathogens known for having prominent capsules include *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Haemophilus influenzae*, all of which are capable of causing serious illness, especially in vulnerable patients.

Another interesting benefit of capsules is their use as a backup energy source. When nutrients are scarce, bacteria can **hydrolyze** (break down with water) their own capsule into simpler sugar molecules, which can then be used for energy. This gives capsule-producing bacteria an edge in surviving longer between meals—or between hosts—compared to those without this feature.

Despite their importance, capsules are difficult to visualize using traditional microbiological staining techniques. The high water content and delicate structure of capsules mean they shrink or disappear when subjected to heat fixing. In addition, capsules do not take up most common stains like crystal violet or methylene blue. To detect capsules, microbiologists use a special method called negative staining.

Negative staining is a technique that allows capsules to be seen clearly by staining everything except the capsule itself. Two common dyes used in this technique are India ink and Congo Red. These stains are repelled by the capsule and do not penetrate it, which leaves a clear, unstained area around each capsule-producing cell.

To perform a negative stain, a small drop of dye is mixed with a sample of live bacteria on a glass slide. The mixture is spread out thinly and allowed to air dry—without applying heat. As the sample dries, the dye coats the background and outlines the cells, but it cannot enter the capsule. This results in a striking image under the microscope: cells are visible against a dark or colored background, and any capsule appears as a clear "halo" surrounding the cell.

In many cases, a **counterstain** (a secondary stain that does enter the bacterial cell, such as crystal violet or safranin) is applied after the background stain has dried. This colors the actual cell body, making it easier to distinguish the cell from its surroundings and to verify the presence or absence of a capsule.

When viewed under the microscope, the differences are clear:

- Cells without capsules are surrounded directly by the dark stain.
- Cells with capsules are surrounded by a clear, unstained zone between the cell and the background.

Since heat fixing can destroy capsules, it is important to avoid using this step during negative staining. By preserving the natural structure of the capsule, this method allows for accurate observation and identification.

Capsule staining is often used in clinical laboratories to help identify pathogenic bacteria. It's a simple, rapid, and effective way to observe a structure that plays a critical role in bacterial survival and infection.

MATERIALS (Per Group of 4)

1 *Bacillus* culture grown for capsules

4 Clean slides (one per student)

Sheep serum (Instructors Desk)

1 Congo Red stain

1 Maneval's stain

METHODS/PROCEDURES

1. Place a small drop of sheep serum near one end of a clean glass slide.
2. Add a small drop of Congo Red stain next to the serum.
3. Using aseptic technique, add a small amount of bacterial culture to the mixture and gently blend using a sterile loop or stick. Set the slide down on a paper towel on the lab bench.
4. Prepare a smear using the slide spreader technique. Hold a second clean slide at a 30–45° angle. Touch it to the drop of stain until the liquid spreads along the edge. Gently push or pull the spreader slide across the surface to evenly distribute the mixture in a thin smear.
5. Allow the smear to air dry completely.

****Do not heat fix the slide. Air drying sets the Congo Red in place around the cells without destroying the capsule.**

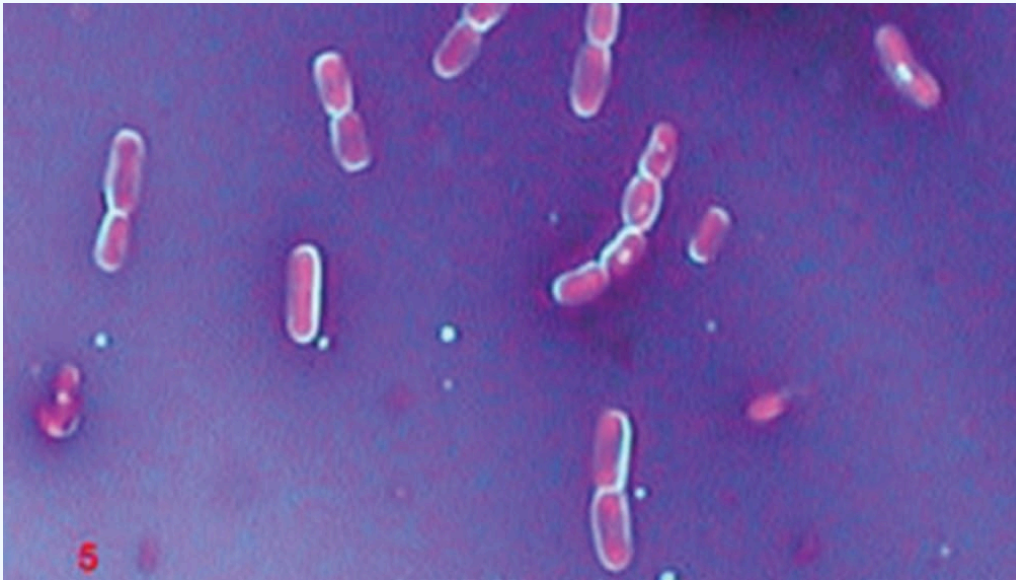
6. Cover the dried smear with Maneval's stain and let it sit for 1 minute. Maneval's stain is a solution that contains acetic acid and acid fuchsin:

* The acetic acid lowers the pH and causes the background (containing Congo Red, a pH indicator) to shift from red to blue.

* The acid fuchsin is a basic dye that penetrates the bacterial cell wall and stains the cells red to pink.

* The capsules remain clear and unstained.

7. Rinse the slide briefly and gently with water. Tilt the slide and let water run gently over it to remove excess stain. Avoid spraying directly or too forcefully.
8. Let the slide air dry completely.
9. View the slide under oil immersion using the 100x objective (final magnification 1000x).



- [2.4: Staining Microscopic Specimens](#) by OpenStax is licensed CC BY 4.0. Original source: <https://openstax.org/details/books/microbiology>.

EXERCISE 29 Capsule Stain

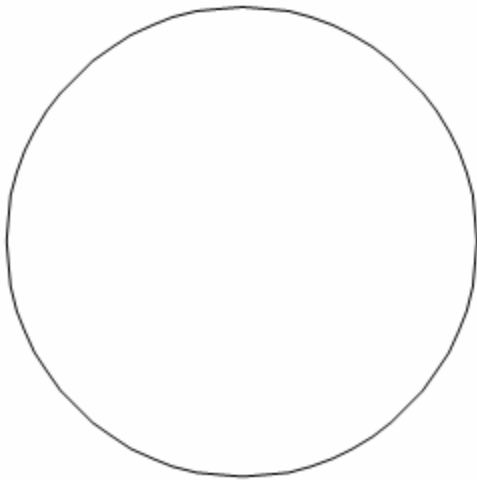
NAME _____

EXPECTATIONS

Describe what you expect to see under oil immersion if a bacterium produces a capsule.

RESULTS

Draw what you see on your capsule stain slide under oil immersion.



CONCLUSIONS

1. Describe what a capsule looks like under the microscope when using negative staining.
 2. How can you tell the difference between a capsule-positive and capsule-negative bacterial cell on a stained slide?
 3. Why might a bacterium that produces a capsule be more likely to cause a long-lasting infection?
-

Attributions:

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25: BACTERIAL COUNTS ON FOOD

LEARNING OBJECTIVES

- Differentiate between food infection and food intoxication, and explain how each causes illness.
- Perform serial dilutions and plate food samples accurately to obtain countable colony-forming units (CFUs).
- Observe, count, and calculate colony-forming units (CFUs) to estimate microbial load in different foods.

BACKGROUND

Microorganisms in food can cause illness in two main ways. In some cases, food carries live, disease-causing microbes that begin to grow and multiply inside the body after the food is eaten. This is known as a **food infection**. In other cases, microorganisms produce toxins as they grow in food, and these toxins can remain behind even if the microbes themselves are dead or removed. When illness is caused by these pre-formed toxins, it is called a food intoxication. Although both are often described as food poisoning, food intoxications and food infections differ in their underlying causes and the way they impact the body.

Most foods are not sterile. Canned and bottled items are designed to be free of microorganisms until they are opened, but fresh meats, vegetables, dairy products, seafood, and grains commonly contain some level of microbial life. Normally, the low pH of the stomach and digestive enzymes are enough to destroy these microbes. Illness becomes more likely when the number of organisms is unusually high, when the microbe is especially virulent, or when toxins have accumulated in the food before ingestion.

Proper handling and storage are critical to preventing microbial growth. A small number of bacteria can multiply into millions in just a few hours under the right conditions. Refrigeration, acidic environments, and foods with high salt or sugar concentrations help limit microbial growth. Contamination can also occur when food is handled by someone who is ill or asymptotically carrying an infectious agent. Because of these risks, public health agencies monitor food workers and routinely inspect food products for microbial contamination.

A well-known case that illustrates the risks of microbial contamination occurred during a community picnic in a rural town. Several attendees fell ill with symptoms including nausea, vomiting, and abdominal cramps just hours after eating. Public health officials traced the outbreak to a large bowl of potato salad that had been prepared the night before and left at room temperature for several hours before serving. Testing revealed the presence of *Staphylococcus aureus*, a bacterium commonly found on the skin and in the nasal passages. While the initial contamination likely occurred during preparation, the real danger came from the bacteria multiplying and producing toxins while the salad sat unrefrigerated. Even though reheating could have killed the bacteria, the heat-stable toxins remained in the food and caused the illness. Cases like this highlight the importance of proper preparation, handling and storage of food.

To reduce the risk of foodborne illness and food intoxication, the food industry is required to follow strict regulations that ensure safe food handling, storage, and preparation. These standards include guidelines for cleanliness, temperature control, and limits on the number of microorganisms allowed in food products. Microbial testing plays a vital role in determining whether food has been safely prepared, handled, and stored.

In this lab, you will investigate the total number of microorganisms present in various foods to explore how different items may support microbial growth. The focus will be on overall microbial levels rather than on identifying specific types of organisms. Gaining a better understanding of how and why microbial contamination occurs is essential for preventing future outbreaks and promoting public health.

MATERIALS (Per Group of 2)

- 1 Gram food sample (Brought by Student)
- 2 Nutrient agar plates
- 3 empty dilution tubes
- 4 Sterile 1.0 ml pipets
- 1 Graduated cylinder
- 1 2 x 2 piece of Parafilm

METHODS/PROCEDURES

1. Label two dilution tubes 1:100 and 1:1000 then add 9.0 ml of DI water to each.
2. Use a digital scale to measure 1.0 gram of your food sample.
3. Transfer the 1.0 gram of food into a clean 10 ml graduated cylinder.
4. Carefully add DI water to the cylinder until the total volume reaches exactly 10 ml.
5. Cover the top of the cylinder with Parafilm.
6. Mix the contents thoroughly by inverting or gently shaking the cylinder for one full minute. This is your first dilution (1:10).
7. Let the sample sit for a few minutes undisturbed to allow solid particles to settle.
8. Using a sterile transfer pipet, remove 1.0 ml of liquid from the top of the graduated cylinder (without disturbing the settled solids) and transfer it into the 1:100 dilution tube containing 9.0 ml of DI water.
9. Use a new pipet to transfer 1.0 ml from the 1:100 dilution tube to the 1:1000 tube with 9.0 ml of DI water. Mix thoroughly.
10. Label one nutrient agar plate with the following information: 1:100, name of the food tested, the date, your first initial, last name.
11. From the 1:100 dilution tube transfer 0.1 ml a nutrient agar plate using a sterile pipet.
12. Create a bacterial lawn on the agar plate by spreading the liquid evenly across the surface of the agar using a sterile spreader and turn plate. Make sure to spread the liquid evenly over the entire plate.
13. Label the second nutrient agar plate with the following information: 1:1000, name of the food tested, the date, your first initial, last name.
14. Using a sterile pipet from the 1:1000 dilution tube transfer 0.1 ml onto the second nutrient agar plate.
15. Create a bacterial lawn on the agar plate by spreading the liquid evenly across the surface of the agar using a sterile spreader and turn plate. Make sure to spread the liquid over the entire plate.
16. Incubate the plates **agar side down** at 30°C. until the next class meeting.

EXERCISE 30 Bacterial Counts on Food

NAME _____

EXPECTATIONS

Among the food items being tested today, which do you expect to have the highest microbial counts, and which do you expect to have the lowest? Explain your reasoning

RESULTS

After incubation, count the Colony Forming Units (CFU) on each of your plates. If the colonies are merging together there may be too many CFUs to count accurately. Ask your instructor how to estimate the CFUs or if the plate should be discarded.

Record the CFUs on each of your plates

CFU plate 1_____ Plate 1 dilution _____

CFU plate 2_____ Plate 2 dilution _____

Calculate the concentration of CFU/gram of food. Show your calculations.

CFU/Gram of food_____

Add your data and the class data from the board to the table below.

Class Data

Food Source	CFU/gram of food	Food Source	CFU/gram of food

CONCLUSIONS

1. What characteristics of the food samples such as moisture, pH, nutrient content etc, might explain the different levels of microbial growth? Explain how.
2. If two students tested the same type of food but obtained different CFU counts, what factors could explain the difference?
3. Explain the difference between a food infection and a food intoxication.

26: AEROBIC VS ANAEROBIC GROWTH

LEARNING OBJECTIVES

- Correctly set up and handle both aerobic and anaerobic culture systems.
- Inoculate and incubate bacteria under conditions that test their oxygen requirements.
- Interpret experimental data to classify organisms by oxygen requirement.

BACKGROUND

Microorganisms are found in nearly every environment on Earth, and their ability to grow often depends on the presence or absence of oxygen. These differences reflect how they generate energy and what type of metabolism they use.

Some bacteria grow only when oxygen is present. These are known as strict aerobes. They rely on aerobic respiration, where oxygen serves as the final electron acceptor in the electron transport chain. These organisms cannot ferment sugar and will not grow in oxygen-free environments. Strict aerobes are commonly found on skin, exposed surfaces, dust, and in the upper layers of soil—places where oxygen is readily available.

Other bacteria grow only when oxygen is absent. These are called strict anaerobes. They generate energy exclusively through fermentation and are often harmed or even killed by exposure to oxygen. Strict anaerobes thrive in environments such as deep soil, sediments, swamps, marshes, sewage, the gastrointestinal tract of animals, and deep tissue wounds. A related group, aerotolerant anaerobes, also use fermentation but are not damaged by oxygen exposure.

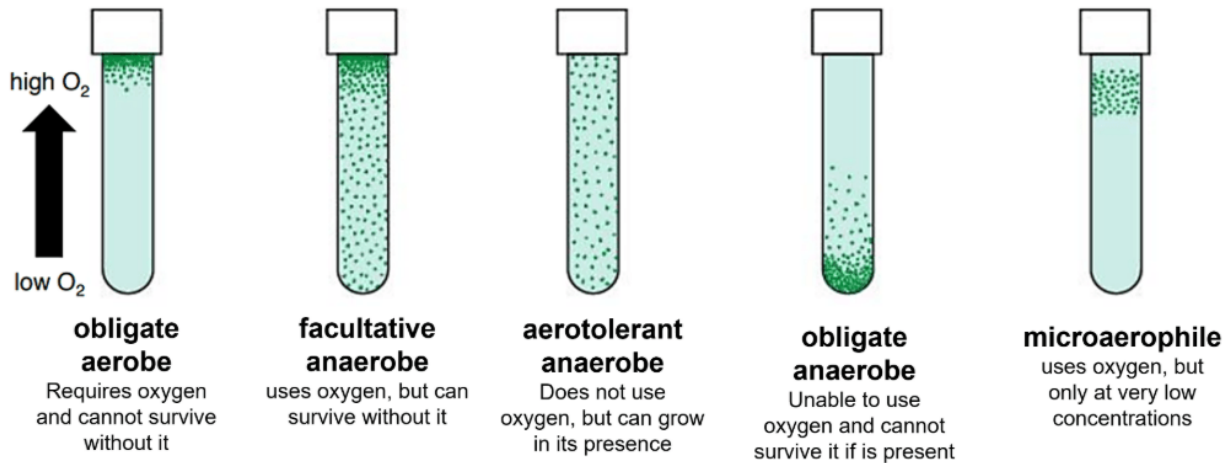
Some bacteria are more flexible. Facultative anaerobes can switch between aerobic respiration and fermentation depending on whether oxygen is available. When oxygen is present, they use it to carry out respiration, which allows them to extract more energy from their nutrients. When oxygen is not available, they switch to fermentation. Because fermentation is less efficient, organisms growing anaerobically typically grow more slowly and to lower population densities than they would in oxygen-rich conditions.

In a clinical setting, several important human pathogens are anaerobes. For example, *Clostridium tetani* (the causative agent of tetanus), *Clostridium botulinum* (which causes botulism), and *Clostridium perfringens* (a common cause of gas gangrene and foodborne illness) are all strict anaerobes. These organisms can flourish in deep wounds or tissues where oxygen supply is limited. Other anaerobic pathogens are part of the body's normal microbiota, particularly in the intestines or oral cavity, and may only cause disease when they enter normally sterile areas of the body or when the balance of normal microbes is disrupted.

Growing anaerobic organisms in the lab can be challenging, especially for strict anaerobes, because most lab procedures expose cultures to air. Even brief contact with oxygen may inhibit their growth or kill them. In research and diagnostic laboratories, anaerobic organisms are cultured using special oxygen-free environments, including sealed anaerobe jars, anaerobic glove boxes, or systems that use gas-generating chemical packets to remove oxygen. Some specialized liquid media, such as thioglycolate broth, contain reducing agents that bind oxygen and allow anaerobes to grow in deeper parts of the tube while limiting oxygen diffusion.

Understanding how microorganisms respond to oxygen is important not only for culturing bacteria in the lab but also for diagnosing infections, choosing the correct antibiotics, and predicting where pathogens may be found in the body or in the environment.

In this lab, you will investigate how different bacteria respond to the presence or absence of oxygen. You will work with three organisms: one that requires oxygen to grow (aerobe), one that is killed or inhibited by oxygen (anaerobe), and one that can grow with or without oxygen (facultative anaerobe). By inoculating all three onto both an aerobic and an anaerobic plate, you will observe how each organism grows under different conditions. This experiment will help you understand the connection between oxygen availability, microbial metabolism, and the classification of bacteria based on their oxygen requirements.



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MATERIALS

- 1 Anaerobic culture
- 1 Aerobic culture
- 1 Facultative culture
- 2 Nutrient agar plates

METHODS/PROCEDURES

1. Label your agar plate with your name, date, and the condition you are assigned (aerobic or anaerobic).
2. Divide the plate into three sections.
3. Spot inoculate one culture into each of three separate sections on the plate. Only one organism should be placed in each section, but all three organisms will be included on the same plate. Do not streak. Keep each spot of culture well within its own section to avoid mixing the organisms.
Cross-contamination will interfere with your results.
4. After inoculation, place the plate labeled “aerobic” directly into the incubator.
5. Place the “anaerobic” plate into an anaerobe jar or box with a gas-generating packet, then seal the container properly and incubate.

EXERCISE 26 Bacterial Oxygen Requirements

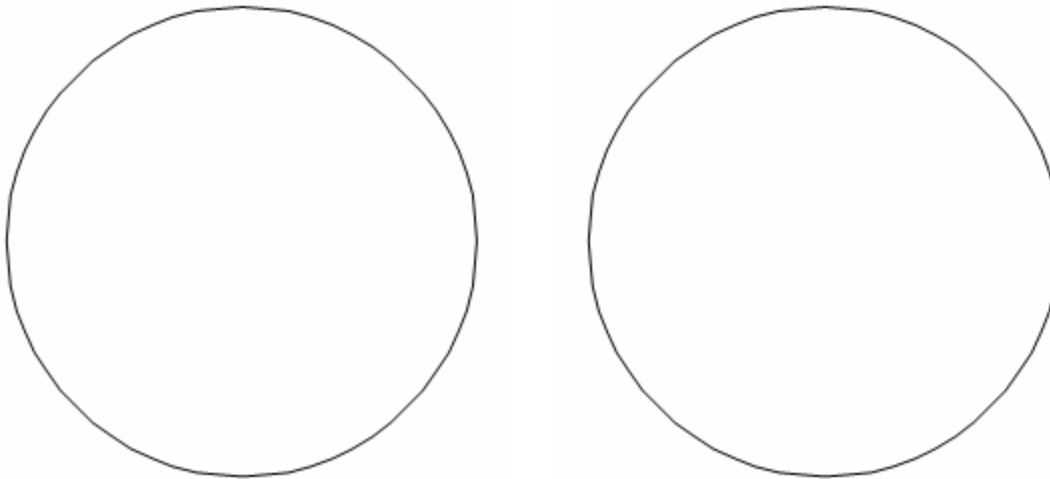
NAME _____

EXPECTATIONS

Do you think the facultative organism will look the same on both plates? Why or why not?

RESULTS

Draw your observations of the cultures on both the aerobic and anaerobic plates after incubation. Note any differences in growth, such as colony size, density, and pigmentation. Be sure to record any distinctive differences between the organisms under each condition.



Organism	Aerobic Plate Description	Anaerobic Plate Description	Oxygen Requirement (based on results)

CONCLUSIONS

1. What additional tests or media could you use to confirm the oxygen requirement of an unknown organism?
2. Why might facultative anaerobes be more commonly isolated from infections than strict aerobes or anaerobes?
- 3 Which of the three organisms texted do you think would be most likely to thrive in a deep puncture wound? Why?

4. In cases of suspected tetanus or botulism, what clues about the infection site or patient history might alert a clinician to suspect an anaerobic pathogen?

27: ENDOSPORE STAIN

LEARNING OBJECTIVES

- Accurately identify endospores and vegetative cells.
- Differentiate between primary stains and counterstains based on their purpose in the staining procedure.

BACKGROUND

Endospores are unique survival structures produced only by bacteria. Unlike the true spores of fungi or plants—which serve as reproductive units—endospores are not reproductive. They do not result in the production of new bacterial cells, but instead help a single bacterium survive harsh or unfavorable environmental conditions. In this way, endospores function more like cysts or resting forms seen in other organisms. They represent one of the most resilient forms of life known and can withstand extreme heat, cold, dryness, and even radiation.

Endospore formation is triggered when environmental conditions become unfavorable for growth. The specific trigger can vary between bacterial species. Common triggers include nutrient depletion, toxic waste accumulation, unfavorable pH, or extreme salt or water levels. However, the most common cause is starvation. In response, some bacteria begin to form highly durable structures capable of surviving extreme stress.

Endospores are formed inside the bacterial cell, typically around the nuclear material. They are highly desiccated (very low in water content) and surrounded by a thick, protein-rich outer layer called the endospore coat. This coat is nearly crystalline in structure, offering strong protection. Once the endospore is fully developed, the remainder of the bacterial cell breaks down, leaving the endospore as a free-floating, dormant structure. At this stage, it is extremely resistant to damage and death.

Free endospores can survive boiling, freezing, drying, and radiation. They are unaffected by most disinfectants and antiseptics, and because they are not metabolically active, they are resistant to antibiotics. This resilience makes endospores a serious public health concern. For example, the food canning industry measures the effectiveness of sterilization by its ability to destroy endospores from *Clostridium botulinum*, the bacterium responsible for botulism. If the sterilization process successfully prevents endospore growth, the food is considered safe and sterile, since endospores are the hardest form of life to kill.

Only five genera of bacteria can form endospores. Four of these are rod-shaped: *Bacillus*, *Sporolactobacillus*, *Clostridium*, and *Desulfotomaculum*. The fifth, *Sporosarcina*, is spherical. Among these, *Clostridium* species are commonly found in soil and are medically significant. They are responsible for diseases such as tetanus, gas gangrene, Buruli ulcer, botulism, and possibly some cases of sudden infant death syndrome (SIDS).

It's important to note that even endospore-forming bacteria cannot always make endospores on demand. If the cell lacks the necessary nutrients to form the endospore coat, it cannot complete the process. In addition, the bacterium must have time to complete endospore development. For example, if a culture containing only vegetative cells (non-spore forms) is suddenly exposed to boiling, the cells will die before they can form protective endospores. However, if the culture already contains mature endospores, it can survive boiling for up to four hours.

Endospores are barely visible under a light microscope, but they are notoriously difficult to stain. Their tough outer coat protects them from common dyes, and they do not stain during a typical Gram stain. Instead, they appear as clear, glassy bodies inside stained bacterial cells. These may be in different positions within the cell—central (middle), terminal (end), or sub-terminal (off-center). Free endospores can be difficult to see unless viewed with a phase contrast microscope, where they appear as bright, glowing structures.

To effectively visualize endospores, microbiologists use a special structural staining technique called the Schaeffer-Fulton method. This method uses malachite green, a powerful dye that can be forced into the endospore coat by applying heat. Heat allows the dye to penetrate the tough structure and stain the endospore green.

Use caution: Malachite green is a permanent dye. If the dye contacts clothing, it cannot be removed. If it contacts skin, it may take several days to fade. After the staining procedure discard the run off in the hazardous waste.

After staining the endospore green, a counterstain (usually safranin) is used to color the surrounding vegetative cells red or pink. The result is a striking image:

- Green free endospores
- Red or pink vegetative cells
- Red or pink cells with green endospores inside

This technique produces a vivid contrast that makes it easy to identify both the presence and location of endospores within a bacterial sample. It remains a classic and powerful example of a differential stain used to study bacterial structure and survival strategies.

MATERIALS (Per Group of 2)

1 Culture with endospores

1 Hot plate

1 Small glass beaker

Strips of paper towel

2 Microscope slides

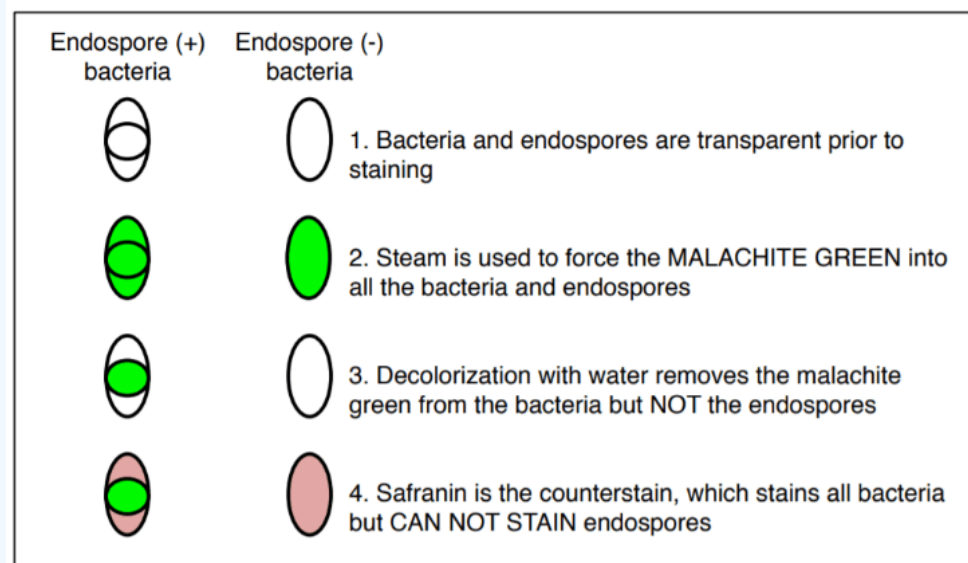
Malachite green stain

Safranin stain

METHODS/PROCEDURES

1. Ensure your slides are very clean before beginning. Any residual soap or oil from your fingers can affect stain absorption and alter the final colors. If needed, wipe the slide gently with a lens wipe and 95% ethanol. Allow it to dry completely before use.
2. Prepare a bacterial smear of *Bacillus* on the slide. Label the frosted edge of the slide in pencil. Allow the smear to air dry completely, then heat-fix the slide by quickly passing it through a flame.
3. Place a small beaker filled with approximately 100 mL of water on a hot plate and bring it to a gentle boil. Monitor the water level carefully—do not let it boil dry. Once boiling, lay the heat-fixed slide across the top of the beaker. Two slides may fit across one beaker if needed.
4. Tear a strip of paper towel slightly smaller than the slide in both length and width. This prevents stain from dripping over the edges. Place the paper strip directly over the bacterial smear.

5. Completely saturate the paper towel with malachite green stain. As the stain begins to steam and evaporate, add more malachite green as needed—just a few drops at a time. Avoid spilling stain onto the beaker or hot plate. The paper towel must remain moist and never be allowed to dry out.
Continue steaming the stain into the slide for 15 minutes.
6. After 15 minutes, carefully remove the slide using a clothespin and place it onto a staining rack over a stain tray. Hold a folded paper towel under the slide as you move it to catch any drips. If any stain spills, clean it immediately with paper towels and alert your instructor.
7. Using forceps, carefully remove the saturated paper towel from the slide. Wrap the used paper in a dry paper towel and dispose of it in the regular trash—not in the stain tray or chemical waste container.
8. Tilt the slide and gently rinse with distilled water until no more green stain runs off the surface.
9. Flood the slide with safranin and allow it to sit for 10 minutes to counterstain the vegetative cells.
10. Rinse briefly again with distilled water. Blot gently with bibulous paper or clean paper towels, and allow the slide to air dry completely.
11. Examine the slide under the microscope. Look for green endospores and pink/red vegetative cells. If endospores are still inside cells, you should see red cells with green centers.



EXERCISE 27 Endospore Stain

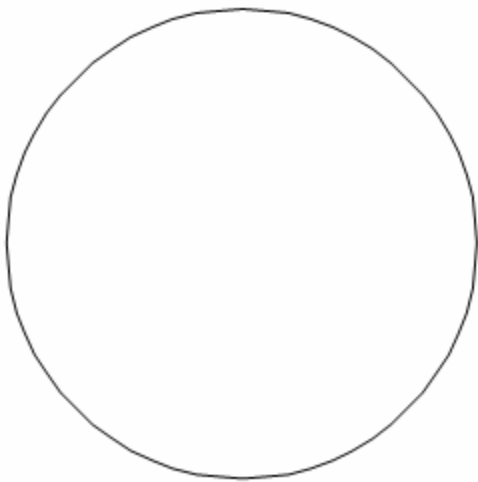
NAME _____

EXPECTATIONS

Describe what a correctly stained slide should look like under the microscope. What colors will indicate endospores versus vegetative cells?

RESULTS

Draw what you see on your slide under oil immersion. Identify endospores and vegetative cells.



CONCLUSIONS

1. Why is it important to use an older culture (24–48 hours) of *Bacillus* when staining for endospores?
2. Why are endospore-forming bacteria a concern in clinical or food safety settings?
3. Ultraviolet (UV) radiation is often used in hospitals and research labs to clean work surfaces. Do you think this would be an effective method for killing *Bacillus anthracis*? Why or why not?

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28: EFFECTS OF UV LIGHT ON BACTERIA

LEARNING OBJECTIVES

- Compare the effects of different UV exposure times on bacterial survival and analyze how sunscreen protection (SPF 15 vs. SPF 50) influences bacterial survival under UV radiation.
- Relate experimental results to real-world issues such as human skin protection, the importance of sunscreen, and risks of overexposure to UV radiation.

BACKGROUND

Mutations are heritable changes in the base sequence of **DNA** (deoxyribonucleic acid). While some mutations are neutral or even beneficial to an organism, most tend to be harmful because they can disrupt essential cellular functions. In bacteria, mutations occur spontaneously during DNA replication at a natural rate of approximately 1 in 10^8 to 10^9 nucleotides base pairs. However, exposure to a **mutagen** (an agent that increases the mutation rate) can cause this rate to rise significantly. Mutagens may be chemical—such as nicotine—or physical, such as certain forms of electromagnetic radiation.

There are two primary types of mutagenic electromagnetic radiation: ionizing radiation and non-ionizing radiation.

- **Ionizing radiation** (e.g., X-rays and gamma rays) carries enough energy to eject electrons from atoms and molecules, forming unstable ions known as **free radicals**. These free radicals can damage a wide variety of cellular components, including DNA, RNA, and essential enzymes, through oxidative reactions.
- **Non-ionizing radiation**, such as **ultraviolet (UV) light**, lacks sufficient energy to ionize atoms but can still damage DNA by exciting electrons. This excitation often leads to the formation of **pyrimidine dimers**, which are abnormal covalent bonds between adjacent pyrimidine bases—usually thymine—on a DNA strand. Pyrimidine dimers distort the DNA structure and interfere with transcription and replication, frequently resulting in mutations if not repaired.

Radiation is classified by its wavelength, frequency, and energy. Generally, the shorter the wavelength, the higher the energy.

- **Ionizing radiation** has wavelengths typically shorter than 1 nanometer and energies greater than 100 electron volts (eV).
- **Non-ionizing radiation** spans wavelengths from about 100 to 390 nanometers and has lower energy levels (a few to 100 eV).

Because of its high energy, ionizing radiation is considered more hazardous and is capable of deeply penetrating cells and structures. For example, X-rays require protective lead shielding, while UV light can be blocked by clothing or plastic covers.

Both ionizing and non-ionizing radiation are widely used to control microbial growth in clinical, food, and laboratory settings. Due to its strong penetrating power, ionizing radiation is effective for sterilizing medical supplies and food products. In contrast, only specific forms of non-ionizing UV light are suitable for microbial control.

There are three types of UV radiation classified by wavelength:

- **UV-A** (longest wavelength, lowest energy)
- **UV-B** (medium wavelength and energy)
- **UV-C** (shortest wavelength, highest energy within the UV spectrum)

UV-C, typically around 254 nm, is the most effective type for microbial control and is considered germicidal. It is used in hospitals, food service areas, and HVAC systems to reduce microbial contamination. UV-C radiation damages microbial DNA, and if the exposure is sufficient, it can lead to cell death.

Sub-lethal UV exposure may not immediately kill bacterial cells but can induce enough DNA damage to interfere with their ability to reproduce. Some bacteria may repair this damage through **photoreactivation** (light-dependent enzyme repair) or nucleotide excision repair mechanisms. However, if the damage overwhelms these systems, cells may die or accumulate mutations.

UV light can also harm human cells. Prolonged exposure to UV-B and UV-C rays can damage skin cell DNA and may impair the cell's ability to regulate division, potentially leading to skin cancers such as melanoma. For this reason, sunscreen products are widely recommended. Sunscreens are labeled with a Sun Protection Factor (SPF) rating, which indicates their effectiveness at blocking UV-B radiation. SPF 15, 30, and 45 are common formulations, with higher values theoretically offering longer protection.

In this experiment, we will evaluate how bacterial survival is affected by UV exposure over various time intervals, with and without protection from two different SPF-rated sunscreens. By comparing the number of surviving bacteria, we will assess how effectively SPF 15 and SPF 50 sunscreens prevent UV-induced damage in a model microbial system.

MATERIALS

1 Nutrient agar plate

1 Assigned culture (per table)

Assigned sunscreen

Plastic wrap

UV lamp

METHODS/PROCEDURES

1. Label the nutrient agar plate with the following: your name, organism name, and assigned exposure time.
2. Use a sharpie to divide the plate into two quadrants. Label one quadrant exposed (exp). Label the other quadrant with your assigned sunscreen SPF.
3. Aseptically pipet 0.10 mL of the bacterial culture onto the surface of the nutrient agar.
4. Using a sterile cell spreader gently and evenly spread the bacteria over the entire surface of the agar plate to create a uniform bacterial lawn.
5. Allow the bacteria to soak in to the agar for at least 10 minutes.
6. Remove the lid and quickly cover the plate with plastic wrap. Take care to avoid contact between the plastic wrap and the agar surface. The wrap should be smooth and tight enough so that the plate sits flat on the bench. Once the agar plate is covered with plastic, invert the lid and place it under the plate.
7. Using a clean finger, gently apply a thin, even layer of your assigned sunscreen SPF only over the quadrant you labeled with your assigned sunscreen SPF. Only half of the plate should be covered with sunscreen. Do not allow the plastic wrap to touch the surface of the agar. Spread the sunscreen evenly to simulate realistic application on skin. Over time the sunscreen will absorb into the plastic and no longer be visible. If you can still see the sunscreen on the plastic after 15 minutes there is too much, remove some.
8. When your exposure time is announced, bring your plate to the UV exposure station.

9. After UV exposure is complete, carefully remove the plastic wrap and immediately replace the lid.
10. Incubate the plate, agar side up, until the next lab period.

EXERCISE 28 Effects of UV Light on Bacteria

NAME _____

EXPECTATIONS

Do you expect to observe a difference in bacterial growth between the two quadrants? Describe what differences, if any, you anticipate and explain your reasoning.

RESULTS

Your assigned SPF _____

Your assigned exposure time _____

After incubation your instructor will arrange the plates into a class data set. Record your observations of the plates below.

Time (seconds)	15 SPF	No SPF	50 SPF	No SPF
0				
20				
60				
120				
300				

CONCLUSIONS

1. Describe any trends observed in the class data.
2. What can you conclude from the data?

3. Based on the data above, how might sunscreen affect UV-induced damage in human skin cells?
4. Would UV light be an appropriate method to sterilize a syringe packaged in plastic wrap? Explain your reasoning based on the properties of UV light and its ability to penetrate materials.
5. Ionizing radiation is often used to treat fresh fruits and vegetables before they are sold to consumers. Would UV light be equally effective for this purpose? Explain your reasoning based on the properties and limitations of UV radiation.

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29: Probiotics

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30: BLOOD TYPING

LEARNING OBJECTIVES

- Differentiate between the four ABO blood groups based on the presence or absence of specific antigens and antibodies.
- Record and interpret agglutination patterns to determine an individual's ABO and Rh blood type.

BACKGROUND

The human immune system has many ways of responding to infection. One important defense is the production of proteins called **antibodies** (protective proteins made by the immune system that recognize and neutralize harmful substances). Antibodies are highly specific to the infectious agent they target. They help stop infections from spreading and assist the body in removing bacteria or viruses.

Antibodies can also recognize and destroy “foreign” cells such as tumor cells. Each antibody binds tightly to only one type of foreign structure, known as an **antigen** (a molecule that triggers an immune response). Some antigens are found on the surface of red blood cells. This antibody–antigen interaction is the basis for identifying blood types, which is especially important when preparing for a blood transfusion.

The major blood group system in humans is the **ABO system**. A person's blood type is determined by the presence or absence of two antigens on the surface of red blood cells: **Antigen A** and **Antigen B**. There are four possible blood types:

- **Type A** (has A antigens; produces B antibodies)
- **Type B** (has B antigens; produces A antibodies)
- **Type AB** (has both A and B antigens; produces no A or B antibodies)
- **Type O** (has neither antigen; produces both A and anti-B antibodies)

In addition to the ABO system, red blood cells may also have another marker called the **Rhesus (Rh) antigen**. If present, the person is considered **Rh positive**; if absent, **Rh negative**. Combining ABO and Rh results, there are eight possible human blood types. Type O negative is called the “universal donor” because it can be given safely to anyone, while Type AB positive is the “universal recipient” because people with this blood type can receive blood from any donor.

Blood typing is performed using antibody reagents that react with A, B, and Rh antigens. To test, three drops of blood are placed on a microscope slide. A drop of anti-A serum is added to one, anti-B to the second, and anti-Rh to the third. The slide is gently rotated and observed for clumping, called **agglutination** (clumping of red blood cells caused by antibodies binding to their specific antigens). The pattern of agglutination indicates the blood type. For example, if clumping is seen with anti-B and anti-Rh but not anti-A, the person's blood type is B positive.

Interestingly, the antibodies against A and B antigens are unusual. Unlike most antibodies, which are produced only after exposure to a foreign antigen, anti-A and anti-B antibodies are produced naturally—likely because of exposure to bacterial antigens in the gut that resemble blood group antigens. This “accidental training” of the immune system explains why a person can have antibodies against blood types they have never been directly exposed to. For example, a person with Type A blood naturally makes anti-B antibodies even without ever receiving Type B blood.

The **Rh factor** plays a particularly important role in pregnancy. If an Rh-negative mother is carrying an Rh-positive fetus, her immune system may recognize the fetus's red blood cells as foreign and begin producing anti-Rh antibodies. This condition, called **hemolytic disease of the newborn (HDN)**, can cause anemia, jaundice, or even life-threatening complications for the baby. Fortunately, modern medicine can prevent this reaction by giving Rh-negative mothers a special injection (Rh immune globulin, commonly known as RhoGAM) during pregnancy and after delivery. This treatment stops the mother's immune system from making harmful antibodies and protects future pregnancies.

Receiving the **wrong blood type** during a transfusion can also cause serious complications. If the donor blood contains antigens that the recipient's antibodies recognize as foreign, the antibodies will immediately attack the transfused red blood cells. This reaction, known as a **hemolytic transfusion reaction**, can cause rapid destruction of the donor cells, releasing hemoglobin into the bloodstream. Symptoms may include fever, chills, chest or back pain, difficulty breathing, and dark-colored urine. In severe cases, the reaction can lead to shock, kidney failure, or even death. Because of these risks, careful blood typing and cross-matching are always performed before transfusions.

MATERIALS (Per Group of 4)

4 Patient synthetic blood samples – one per student
12 Toothpicks (3 per student)
4 test plates (1 per student)
1 Anti-Rh serum bottle
1 Anti-A serum bottle
1 Anti-B serum bottle

METHODS/PROCEDURES

1. Label the wells on your test plate as shown in the diagram below.
2. Place a few drops of the patient's blood sample into each well of the plate. Replace the cap right away to prevent cross-contamination.
3. Add a few drops of anti-A serum (A Antibody) to the well labeled **A**, then replace the cap.
4. Add a few drops of anti-B serum (B Antibody) to the well labeled **B**, then replace the cap.
5. Add one drop of synthetic anti-Rh serum (Rh Antibody) to the well labeled **Rh**, then replace the cap.
6. Using a separate clean toothpick for each well, gently stir the contents for about 30 seconds. Discard the toothpick after each use.
7. Examine the liquid in each well for agglutination (clumping).
8. Record your observations in the results section.

*If the liquid remains smooth and uniform, **no agglutination** has occurred.

*If the liquid appears clumpy or granular, **agglutination** is present.

Agglutination Reaction			
Blood type	Anti-A	Anti-B	Anti-Rh
A+	Yes	No	Yes
A–	Yes	No	No
B+	No	Yes	Yes
B–	No	Yes	No
AB+	Yes	Yes	Yes
AB–	Yes	Yes	No
O+	No	No	Yes
O–	No	No	No

EXERCISE 29 Blood Typing

NAME _____

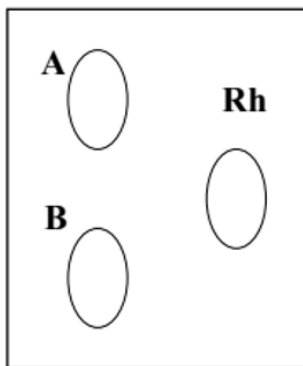
EXPECTATIONS

Which blood type should show agglutination in all three wells (anti-A, anti-B, and anti-Rh)? Explain your reasoning.

RESULTS

Draw the test results below

Patient Sample Number _____



	Agglutination Reaction		
Blood Type	Anti-A	Anti-B	Anti-Rh

CONCLUSIONS

1. If your patient needed a blood transfusion what blood type(s) could they safely receive? Explain why.
2. If a person with Type AB negative blood needs a transfusion, which blood type(s) could they safely receive? Explain why.
3. Explain what would happen if type B positive blood was transfused into a person with type A blood.

4. Explain the concept of “universal donor” and “universal recipient”. Be sure to include what the blood types are of each type of donor.

5. Explain why a person with type A blood has type B antibodies even though they have never been exposed to type B blood.

Attributions:

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31: TESTING YOUR KITCHEN

LEARNING OBJECTIVES

- Recognize common unsafe kitchen practices that can lead to food borne illness.
- Evaluate your own kitchen habits through a self-assessment quiz to determine strengths and areas for improvement.

BACKGROUND

When most people imagine a clean kitchen, they often picture polished floors, gleaming stainless-steel sinks, spotless countertops, and neatly organized cupboards. While these features create the impression of cleanliness, appearance alone does not guarantee that a kitchen is truly sanitary. A genuinely clean kitchen is one that reduces the risk of food borne illness by consistently preventing the growth and spread of microorganisms through proper cleaning, disinfection, and safe food-handling practices.

Microorganisms such as bacteria, molds, and yeasts exist everywhere in the environment. They are present on hands, utensils, food packaging, and even in the air. Although many are harmless or beneficial, others can cause food spoilage or illness if they contaminate food or food-contact surfaces. Kitchens provide ideal conditions for microbial growth because they are often warm, moist, and rich in organic material from food preparation. Even a countertop or sink that appears spotless may harbor millions of microscopic organisms invisible to the naked eye.

Maintaining true kitchen hygiene therefore requires more than simple tidiness. It involves consistently applying safe practices during food storage, preparation, and cooking. Proper storage keeps perishable items at safe temperatures to slow bacterial growth. Careful handling prevents cross-contamination between raw and cooked foods, and thorough cooking ensures harmful microorganisms are destroyed before consumption. These practices form the foundation of food safety and are essential to protecting health in the home.⁴

MATERIALS

1 Nutrient Agar plate

1 Sterile swab

Tape

3 Tablespoons bleach

[Self assessment quiz](#)

METHODS

1. Select a surface in your kitchen that appears to be clean (for example, a countertop, cutting board, refrigerator handle, or sink area).
2. Using the sterile cotton swab provided, gently rub the tip of the swab over a small section of the chosen surface. Be sure to rotate the swab so that all sides make contact with the surface.
3. Carefully lift the lid of your petri dish containing nutrient agar just enough to insert the swab—do not fully remove the lid to minimize airborne contamination.

4. Lightly streak the agar surface in a zigzag pattern to distribute any microorganisms collected on the swab. Avoid gouging or tearing the agar.
5. Replace the lid immediately and securely tape the edges of the petri dish closed to prevent accidental opening.
- 6 Label the bottom of the plate with your name, date, and the location swabbed.
7. Place the plate in a safe location at room temperature, away from direct sunlight and areas where it may be disturbed. Do not open the dish once it has been sealed.
8. While the plate is incubating complete the Kitchen cleanliness
9. After four or five days, observe the plate. Record your observations in the results section.

EXERCISE 30: Testing Your Kitchen

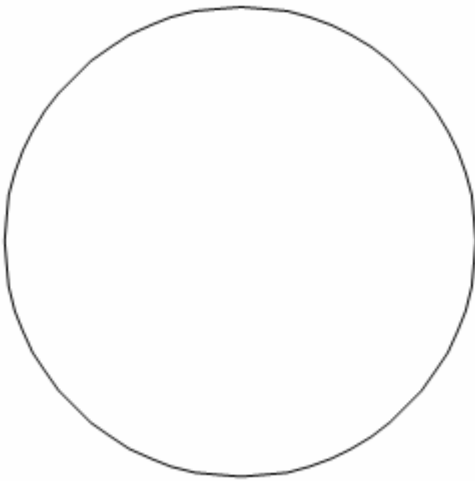
NAME _____

EXPECTATIONS

Do you anticipate any bacterial growth on your plate? Explain your reasoning.

RESULTS

Draw what you saw on the agar plate after four or five days of growth



CONCLUSIONS

1. What was the location of the surface you swabbed, and why did you choose this area?
2. Do your results match your expectations? Explain why or why not.
2. What was your score on the Kitchen cleanliness quiz _____.
3. Which areas of food safety (storage, handling, or cooking) did you perform best in on the quiz?

4. Describe two habits or practices you are planning to change after taking the kitchen quiz or this class.

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32: METABOLIC TESTING

Learning Objectives

- Explain the principles behind bacterial metabolic testing and how enzymatic pathways produce different metabolic end products.
- Integrate metabolic test results with Gram stain and colony morphology data to determine the probable genus and species of an unknown bacterium.
- Summarize and communicate metabolic test principles, methods, results, and interpretations in written reports.
- Create and apply flowcharts to select the most relevant metabolic tests for identifying an unknown bacterium.
- Maintain the viability and purity of assigned organisms throughout testing.

BACKGROUND

Some of the most valuable tools for identifying microorganisms are tests based on their metabolic end products (the chemical compounds produced when organisms break down nutrients). Even when different organisms are provided with the same medium, they may metabolize the nutrients through entirely different pathways. This is because not all bacteria use the same enzymatic steps to process a given compound. As a result, the final products—or waste products—of metabolism can vary between species. These end products represent substances the organism cannot use further, or byproducts it must eliminate because they may be toxic if accumulated.

For example, animals eliminate excess nitrogen in the form of urea. Some bacteria, however, can use urea as a nutrient source. The ability to metabolize such compounds is determined by the specific enzymes an organism produces, which in turn are encoded by its DNA. Therefore, examining the metabolic waste products of a microorganism is, in effect, a way to study its genetic blueprint. Over the next several weeks you will

Metabolic tests are often performed using specialized culture media. Selective media are designed to support the growth of certain organisms while inhibiting others. This selection is achieved by including nutrients that only target organisms can utilize, or by incorporating compounds that are toxic to non-target species. If a microorganism cannot grow due to its inability to use the available nutrients or withstand inhibitory agents, it will not appear as a colony on the plate.

Selective Media

Selective media are highly sensitive to contamination. Introducing nutrients or other chemicals when transferring the inoculum can produce misleading results—appearing weakly positive or negative when the true reaction is more clear-cut. To avoid this, it is best to suspend a sample of the pure culture in sterile water before inoculating the test media. This reduces the risk of transferring additional nutrients or overwhelming the test with too many cells.

Simmons Citrate Agar is a common example of a selective medium used in metabolic testing. In this medium, citrate is the only available source of carbon. Only bacteria capable of transporting and breaking down citrate can survive and grow. As citrate is metabolized, the medium releases ammonia as a byproduct, which raises the pH. A pH indicator dye in the agar changes from green to blue when this alkaline shift occurs. Bacteria that cannot metabolize citrate will not grow and will not cause any color change in the medium.

By observing growth and color change, metabolic tests like the citrate test allow microbiologists to infer the presence or absence of specific enzymes and pathways, providing important clues for microbial identification.

Differential Media

Differential media are designed to support the growth of a wide variety of organisms while distinguishing between them based on their metabolic properties. Unlike selective media, which inhibit the growth of certain organisms, differential media contain a full complement of nutrients that allow most organisms to grow. What makes them useful for identification is the inclusion of indicators—compounds that visibly change in response to metabolic byproducts produced by the growing organisms.

As bacteria grow and metabolize nutrients in the medium, they may release acids, bases, gases, or other compounds. These byproducts can interact with the indicator, producing visible changes such as a color shift or changes in the appearance of the colony or the medium around it. If an organism does not produce the specific metabolic product being tested for, no change occurs.

A classic example is Eosin Methylene Blue (EMB) agar, which is both selective and differential. It allows the growth of Gram-negative organisms while inhibiting most Gram-positive bacteria. The differential component lies in its ability to detect lactose fermentation. Organisms that ferment lactose and produce significant acid will appear as colonies with a distinctive metallic green sheen. Those that do not ferment lactose, or do so with little acid production, form white, cream, or pale pink colonies without a green sheen. The dye itself responds to the acid level, creating this visual contrast.

Selective and Differential Media

Some media combine both selective and differential functions. A widely used example is Mannitol Salt Agar (MSA). This medium contains: A high concentration of salt (7.5%), which inhibits most organisms except for salt-tolerant species such as those in the genus *Staphylococcus*. The sugar mannitol, which may be fermented by certain bacteria. A pH indicator (phenol red), which changes color in response to acid production. If a bacterium can tolerate the high salt concentration and ferment mannitol, the acid produced during fermentation will turn the agar from red to bright yellow. If the bacterium grows but does not ferment mannitol, the agar remains red. If the organism cannot tolerate high salt, it will not grow at all, and no change will be observed.

MSA is commonly used to help identify *Staphylococcus aureus*, a clinically significant human pathogen. *S. aureus* can grow in the high-salt environment and ferment mannitol, causing a yellow color change in the agar. Therefore, the appearance of yellow growth on MSA from a clinical sample is a strong presumptive indication of *S. aureus* infection.

By combining metabolic profiling with selective growth conditions, selective and differential media provide powerful tools for identifying and differentiating microbial species based on their physiological characteristics.

Cell/Water Suspension

Some bacterial metabolic tests require making a cell and water suspension before performing the reaction. This step helps ensure that results reflect the bacteria's enzyme activity based on only the nutrients in the test media without interference from nutrients from the nutrient agar. When bacterial cells are mixed in sterile water instead of nutrient media, the environment stays chemically neutral and doesn't supply extra nutrient sources that could interfere with the test. The water suspension also helps standardize the number of cells making results easier to compare. To make a cell/water suspension use a sterile loop to select an isolated colony from your T-streak plate then inoculate the cells into a test tube of sterile water. Only inoculate cells into the water once. Use the same sterile water suspension to set up all the metabolic tests.

Exoenzymes

Many microorganisms have developed an efficient strategy for accessing nutrients that are too large to be transported across the plasma membrane. Some energy-rich substances in the environment—such as starches, proteins, and lipids—exist as polymers (large molecules made by joining many smaller building blocks). These large molecules are often too big to be directly absorbed by the cell.

To solve this problem, some bacteria possess the genetic ability to produce exoenzymes—enzymes that are secreted into the environment and function outside the cell. These enzymes are encoded by the bacterial DNA, synthesized within the cell, and then released into the surrounding medium. Once outside the cell, exoenzymes break down complex macromolecules into smaller subunits that can be transported through the membrane and used by the cell.

For example, starch is a carbohydrate polymer made of many linked sugar molecules. Starch cannot pass through the bacterial membrane in its full form. However, certain bacteria produce amylase, an exoenzyme that breaks the bonds between the sugar units, releasing individual glucose molecules that are small enough to be taken up by the cell and used as a carbon and energy source.

Bacteria can produce various exoenzymes to degrade other naturally occurring organic polymers:

Proteases break down proteins into amino acids

Lipases degrade fats and oils into fatty acids and glycerol

DNases and RNases digest nucleic acids

These enzymes enable bacteria to thrive in nutrient-rich environments by breaking down a wide range of organic materials. Some bacteria are even capable of degrading more complex or synthetic compounds. For instance, certain species have been shown to break down petroleum and toxic industrial chemicals, and research is ongoing to explore or engineer bacteria that produce exoenzymes capable of degrading plastics and other man-made polymers.

The production and activity of exoenzymes are important tools in microbial metabolism testing. By providing specific substrates in or on the culture media—such as starch, casein, or triglycerides—microbiologists can determine whether a bacterium is capable of producing the corresponding exoenzyme. The presence or absence of a visible reaction, such as a clearing zone around bacterial growth, helps identify metabolic capabilities and can contribute to bacterial classification and identification.

Before using metabolic tests to identify an unknown organism, you will first perform these tests on an assigned known organism. A Gram stain is completed first to determine the organism's cell wall type, which helps guide the selection of appropriate tests. You will conduct all the metabolic tests outlined below on the assigned organism. Once you have recorded the metabolic test results for your assigned organism, you will complete a written summary for each test. The class data for all the metabolic test results for all the assigned organisms will be compiled into two excel sheets, one for Gram negative and one for Gram positive bacteria. The data will then be used to create two flow charts—one for Gram-negative organisms and one for Gram-positive organisms. These charts will serve as road map to help you decide which tests to perform on the unknown organism in order to determine its genus and species. Not all the metabolic tests will be conducted on the unknown organism. Only the tests indicated in the flow chart you create.

Your top priority is to maintain the viability and purity of your assigned organism. If you notice anything unusual in your Gram stain or streak plate that suggests contamination or a mixed culture, consult your instructor immediately.

32.1: Starch Hydrolysis Test

32.2: Skim Milk Test

32.3: Spirit Blue Agar Test

32.4: Phenol Red Fermentation Test

32.5: Methyl Red / Voges-Proskauer (MR/VP) Test

32.6: Nitrate Reduction Test

32.7: Kligler Iron Agar (KIA) Test

32.8: Mannitol Salt Agar (MSA) Test

32.9: Eosin Methylene Blue (EMB) Test

32.10: Indole Broth Test

32.11: Urease Test

32.12: Simmon's Citrate Agar Test

32.13: Motility Test

32.14: Oxidase

32.15: Catalase Test

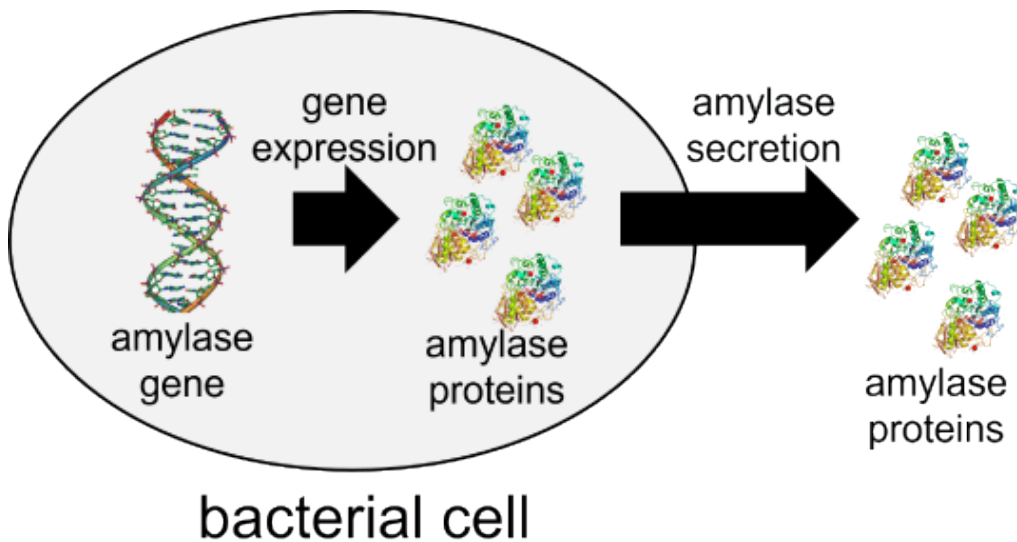
32.16: Flow Chart

32.1: Starch Hydrolysis Test

Background

The starch agar test determines whether a microorganism produces the exoenzyme amylase, which hydrolyzes starch into smaller sugars that can be absorbed and metabolized for energy.

Starch agar contains starch, a complex carbohydrate, as the primary source of energy. Some microorganisms possess the gene that codes for amylase, an exoenzyme secreted into the surrounding environment. Amylase breaks down starch into simpler sugar molecules, which the cell can then absorb and utilize. Because the enzyme works outside the cell, starch is removed from the medium in the area surrounding the growth if the organism is positive for this enzyme. Starch is largely transparent in the agar, so it is not possible to visually determine where starch remains or has been degraded just by observing the plate. To detect starch breakdown, the medium must be treated with iodine after the organism has incubated for at least 48 hours. Iodine binds to starch and produces a blue-black color in areas where starch is still present. In contrast, areas where starch has been broken down by amylase will remain yellowish to clear after iodine is applied. This visual contrast allows for interpretation of the test result.



[DNA double helix horizontal.png](#) by Jerome Walker is in the [public domain](#)

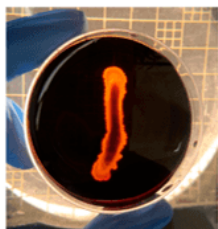
Procedure

1. Inoculate the surface of the Starch Agar plate with a single straight line in the middle of the plate. Take care not to touch the sides of the plate with the test organism.
2. Incubate the plate for 48 hours at 30°C.
3. After incubation, flood the plate with iodine solution.
4. Observe and record the results within 5 to 10 minutes, as the color will begin to fade after approximately 20 minutes.

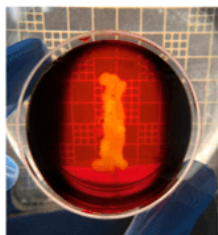
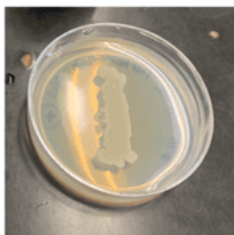
Interpretation of Results

Before addition of iodine

After addition of iodine



Negative for Starch hydrolysis



Positive for Starch hydrolysis

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32.2: Skim Milk Test

Background

Skim Milk Agar is used to detect the production of caseinase, an exoenzyme that breaks down casein, the primary protein found in milk. The medium contains skim milk, which gives it a white, opaque appearance due to the presence of intact casein.

If the test organism produces caseinase, the enzyme is secreted into the surrounding environment and hydrolyzes the casein in the agar. As the casein is broken down, the white color disappears, and a clear zone appears around the bacterial growth. This clearing indicates that the casein has been digested by the enzyme. The clearing must extend beyond the edges of visible bacterial growth, as the enzyme must act in the medium surrounding the cells.

If the organism does not produce caseinase, the casein remains intact, and the agar around the colony remains white and opaque. The clearing should occur outside the area of growth, as the enzyme acts externally in the medium.

Procedure

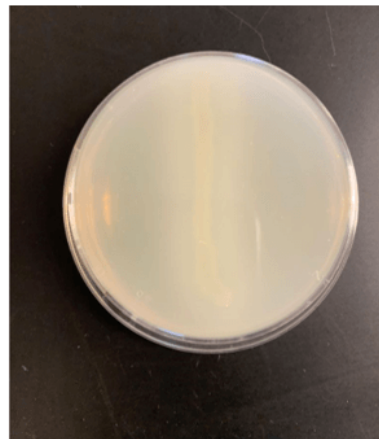
1. Inoculate the surface of the Skim Milk Agar with a single straight line in the middle of the plate. Take care not to touch the sides of the plate with the test organism.
2. Incubate the plate for 48 hours at 30°C.
3. Observe the area surrounding bacterial growth for signs of casein hydrolysis.

Interpretation of Results

Positive for casein activity



Negative for casein activity



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32.3: Spirit Blue Agar Test

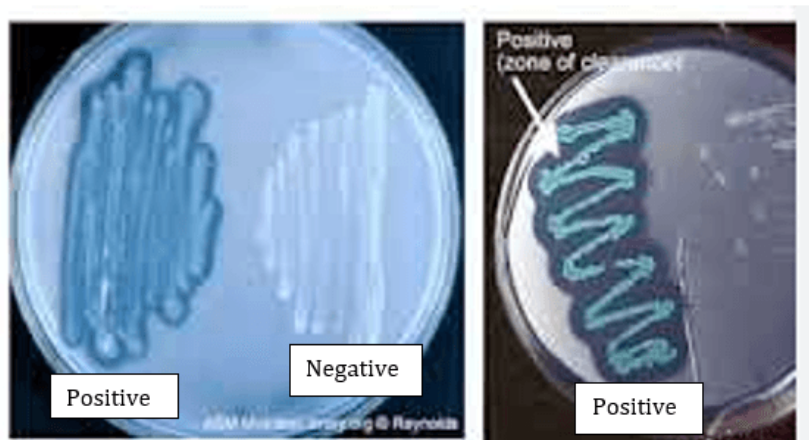
Background

The Spirit Blue Agar test is used to detect the production of lipase, an exoenzyme that hydrolyzes lipids into fatty acids and glycerol. In addition to enzymes that degrade carbohydrates and proteins, some bacteria produce enzymes that break down lipids—another major class of macromolecules. The medium contains a lipid source, typically tributyrin oil, and a dye that gives the agar its characteristic opaque light blue color. If a bacterium produces lipase, the enzyme is secreted into the surrounding medium and breaks down the lipids. As the lipids are digested, the medium becomes transparent around the bacterial growth. This may appear as a clear halo or a dark blue ring, depending on how the dye redistributes during lipid breakdown. The clearing in the medium, not just a color change, is the critical indicator of a positive result. It reflects the breakdown of lipids by enzymes acting outside the bacterial cell.

Procedures

1. Inoculate the surface of the Spirit Blue Agar with a single straight line in the middle of the plate. Take care not to touch the sides of the plate with the test organism.
2. Incubate the plate for 48 hours at 30°C.
3. Observe the area surrounding bacterial growth for signs of lipase activity.

Interpretation of Results



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<https://www.youtube.com/watch?v=Sqmiw0XTFIQ&list=PLjJMtdYdh3VASgW60OJYPqjsNIVvILrXW&index=3>

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32.4: Phenol Red Fermentation Test

Background

The Phenol Red Fermentation Test is used to determine whether a microorganism can ferment specific carbohydrates—such as glucose, lactose, or sucrose—and to detect the production of acid and gas as end products of fermentation. Because different bacteria possess different metabolic capabilities, not all organisms can ferment the same sugars. If an organism lacks the genes coding for the necessary enzymes, it will be unable to metabolize a particular sugar.

The test medium contains a carbohydrate (glucose, lactose, or sucrose), nutrients to support bacterial growth, the pH indicator phenol red, and an inverted Durham tube to detect gas production. Phenol red is red at neutral pH but turns yellow if the pH drops due to the accumulation of acidic byproducts from fermentation. If the organism ferments the sugar, acid is produced, lowering the pH and turning the medium yellow. A bright yellow color indicates strong acid production and a positive fermentation result.

If the organism cannot ferment the sugar—or does not ferment at all—the pH will remain unchanged, and the medium will stay red. This is often the case for strict aerobes, which rely solely on respiration and do not produce organic acids as fermentation byproducts.

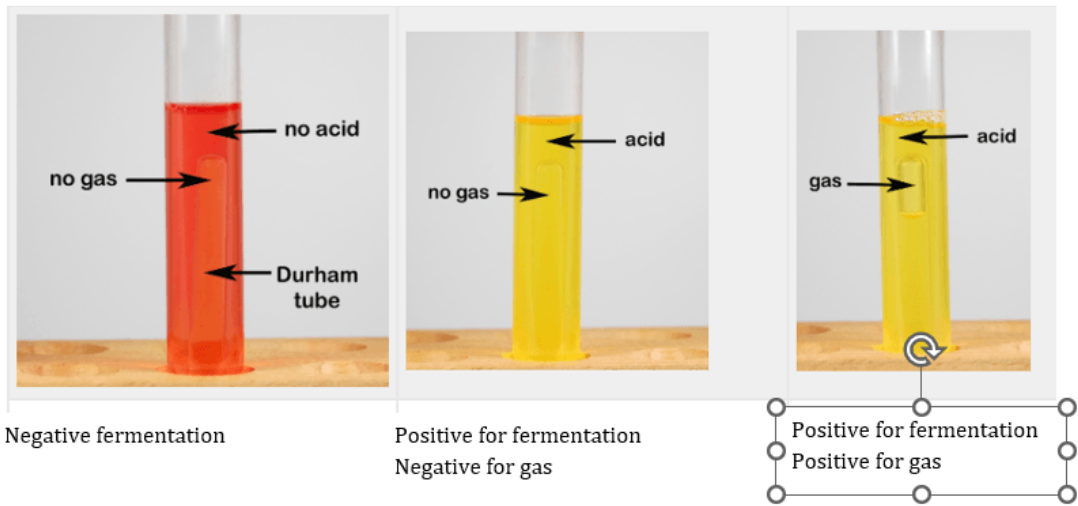
In addition to acid production, some fermentation pathways also produce carbon dioxide (CO₂) gas. To detect this, a small Durham tube is placed inverted in the bottom of the test tube. If gas is produced, it becomes trapped in the Durham tube and appears as a visible bubble. A true positive for gas production includes both a yellow color change (acid) and a clearly visible gas bubble. Very small bubbles may result from displacement of air during inoculation or production of other gases and should be interpreted with caution. There should never be CO₂ gas production without accompanying acid production. A gas bubble is only considered significant if the broth also turns yellow. Read results after incubation and before prolonged growth alters pH conditions.

Procedures

1. Verify a small, inverted Durham tube is present in each of the phenol red broth tubes and is completely filled with liquid (no bubble). If there is a bubble, consult with your instructor.
2. Using a sterile loop inoculate each Phenol Red broth tube with one loopful from the cell/water suspension.
3. Leave the cap a quarter-turn loose for gas exchange.

Incubate at 36°C for 48 hours.

Interpretation of Results



32.5: Methyl Red / Voges-Proskauer (MR/VP) Test

Background

The Methyl Red (MR) and Voges-Proskauer (VP) tests are performed together to assess two alternative fermentation pathways that begin with the same glucose-based medium but produce different types of end products. These tests help determine how an organism ferments glucose and what byproducts result.

Some bacteria use the mixed acid fermentation pathway, which produces a variety of stable organic acids such as lactic acid, formic acid, and acetic acid. These acids cause a significant drop in pH. The Methyl Red test detects this acid production using the pH indicator methyl red, which turns red in acidic conditions (pH below 4.4). A red color after adding the indicator indicates a positive result for mixed acid fermentation.

Other organisms may metabolize glucose using the butanediol fermentation pathway, which results in more neutral end products like ethanol, acetoin, and 2,3-butanediol. These compounds do not significantly lower the pH, so the Methyl Red test would yield a negative result. However, the Voges-Proskauer test detects the presence of acetoin, an intermediate in this pathway.

The VP test requires two reagents: alpha-naphthol (Reagent A) and potassium hydroxide (KOH, Reagent B). When these reagents are added to the culture and oxygen is present, acetoin is converted to a red-colored compound. A rosy red color developing within 15 to 30 minutes indicates a positive result. The reaction requires vigorous mixing to introduce oxygen, and caution should be used when handling the reagents, as they are toxic. A small amount of the media is transferred into a separate test tube before evaluating any results.

Do not confuse the red color observed in the MR test with the red color seen in other tests such as Durham tubes. In the MR test, red indicates strong acid production and a low pH, while in Durham tube tests, red is often the original color of the pH indicator and does not necessarily indicate acidity.

Procedures

1. Inoculate the MR/VP medium with the test culture.
2. Incubate at 30°C for 48 hours.

Interpretation of Results

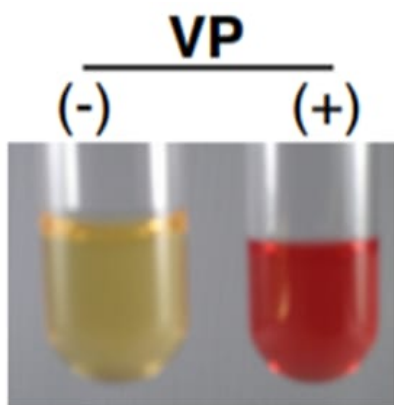
After incubation, use a transfer pipette to remove approximately 1 mL of the culture from the MR/VP broth and transfer it into a small clean test tube.

Label the small test VP.

The remaining culture is now the MR.

Voges-Proskauer (VP) Test (small test tube)

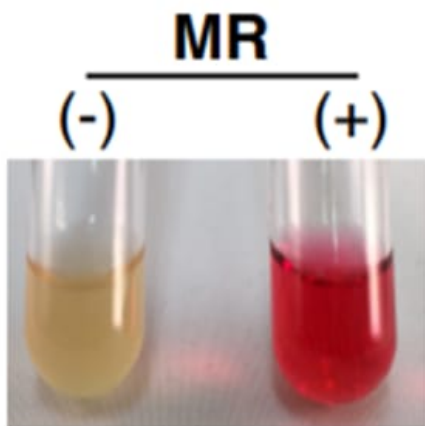
1. Add 9 drops of alpha-naphthol (Reagent A) and mix thoroughly.
2. Add 3 drops of potassium hydroxide (Reagent B) and mix vigorously for at least 30 seconds to introduce oxygen
3. Allow the tube to stand undisturbed for 15 to 30 minutes
 - o A rosy red or pink color indicates a positive VP result
 - o No color change or a copperish color after 30 minutes indicates a negative result



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Methyl Red (MR) Test

1. Add 6 to 8 drops of methyl red indicator to the MR broth
2. Observe the color change
 - o An orange or yellow color indicates a negative result
 - o Red color throughout the broth indicates a positive MR result



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32.6: Nitrate Reduction Test

Background

In many environments, especially those low in oxygen, some bacteria can switch from aerobic respiration (which uses molecular oxygen as the final electron acceptor) to anaerobic respiration. In anaerobic conditions, certain microorganisms can use alternative terminal electron acceptors such as nitrate (NO_3^-). The nitrate reduction test is designed to identify organisms that can use nitrate in this way.

During nitrate reduction, bacteria use nitrate reductase enzymes to convert nitrate into nitrite (NO_2^-). Depending on the metabolic pathway and the organism, this process may stop at nitrite or continue through a series of reductions to produce nitrogen gas (N_2), ammonia (NH_3), or other nitrogen-containing end products.

After incubation, the broth is tested for the presence of nitrite using two reagents: Sulfanilic acid (Reagent A) and Dimethyl-alpha-naphthylamine (Reagent B).

If nitrite is present, it will react with the reagents and produce a red or pink color within 1 to 2 minutes. This result indicates that the organism can make the nitrate reductase enzyme to reduce nitrate to nitrite, and the test is considered positive.

If no color change occurs, the result is inconclusive. There are two possibilities: the nitrate was not reduced at all (a true negative) or the nitrate was reduced beyond nitrite to nitrogen gas or another compound (a positive result that cannot be detected with the initial reagents). To resolve this, zinc powder is added. Zinc chemically reduces any remaining nitrate in the medium to nitrite. Zinc is flammable - handle zinc powder carefully and follow safety guidelines. If unreduced nitrate is still present, the zinc-induced nitrite will react with the previously added reagents and turn the broth red. This red color confirms that the nitrate was not reduced by the organism, resulting in a negative test. If no color change occurs after the addition of zinc, it means that no nitrate remains in the medium—because the organism already reduced it entirely to nitrogen gas or other compounds. This is interpreted as a positive result for complete nitrate reduction. The zinc step is therefore essential to differentiate between a true negative and an undetectable positive result. Be sure to interpret color changes only within the specified time frame. Do not rely on color changes that appear after 20 to 30 minutes.

Procedure

1. Inoculate the nitrate broth with one loopful of the test culture.
2. Incubate 48 hours at 30°C. Remember to leave the cap slightly loose.

Interpretation of Results

1. After incubation, add 6 drops of sulfanilic acid (Reagent A) and gently mix.
2. Add 6 drops of dimethyl-alpha-naphthylamine (Reagent B).

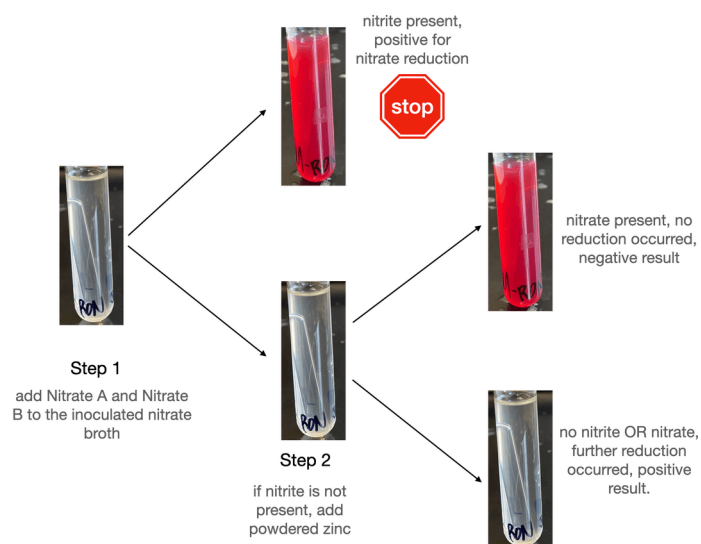
*Red color within 1 to 2 minutes indicates a positive result (nitrate reduced to nitrite).

*No color change requires further testing.

3. If no red color after adding reagents A and B add a pinch of Zinc powder.

*Red color within 10 minutes confirms negative result (nitrate was not reduced by the organism).

*No red color after addition of reagents A, B and zinc powder confirms a positive results (nitrate reduced to nitrite then further reduced).



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32.7: Kligler Iron Agar (KIA) Test

Background

The **Kligler Iron Agar (KIA) test** is a widely used diagnostic tool in microbiology designed to distinguish bacteria based on their ability to ferment sugars and produce hydrogen sulfide (**H₂S**) and gas. It is particularly useful for identifying members of the *Enterobacteriaceae* family, a diverse group of Gram-negative bacteria that inhabit the human gut and include many important pathogens. Because the test provides information about several metabolic processes in a single culture tube, it is considered one of the most efficient biochemical tests in a clinical or teaching laboratory.

KIA medium is prepared in a way that allows observation of both aerobic and anaerobic bacterial activity. After being poured into a test tube, the medium solidifies at an angle, forming a **slant** on top and a deeper section called the **butt** at the bottom. The slant is exposed to oxygen and supports aerobic metabolism, while the butt, buried deeper in the agar, remains low in oxygen and allows anaerobic growth. Proper inoculation is essential: the slanted surface is streaked with a sterile loop to introduce bacteria into the aerobic zone, and then the butt is stabbed straight down with a sterile inoculating needle to deliver bacteria into the anaerobic environment. This two-step inoculation ensures that both metabolic conditions can be observed.

The medium contains two sugars, **lactose** and **glucose**, at different concentrations. Lactose is present in a relatively high amount, while glucose is present at a much lower concentration. Bacteria that can ferment both sugars will produce a substantial amount of acid, causing the pH indicator, phenol red, to turn the medium **yellow** throughout both the slant and the butt. When an organism can ferment only glucose, the small amount of acid produced initially lowers the pH and turns the medium yellow everywhere. However, once the glucose is exhausted, bacteria growing on the oxygen-rich slant begin metabolizing proteins instead, releasing alkaline byproducts that reverse the color change. In such cases, the slant reverts to **red** while the butt remains **yellow**, reflecting continued acid production in the anaerobic zone. Bacteria that cannot ferment either sugar rely solely on protein metabolism, resulting in alkaline byproducts throughout. These tubes remain **red** on the slant and usually unchanged in the butt, indicating no significant acid production.

In addition to detecting sugar fermentation, the KIA medium also reveals whether a bacterium produces **hydrogen sulfide**. Some bacteria can reduce sulfur-containing compounds during their metabolism, releasing H₂S gas as a byproduct. The medium contains ferrous sulfate, which reacts with H₂S to form **black ferrous sulfide**. The appearance of black precipitate, typically in the butt where anaerobic conditions prevail, indicates a positive result for H₂S production. If no blackening is seen, the organism does not produce hydrogen sulfide.

Another important observation comes from the detection of gas produced during fermentation. When bacteria metabolize sugars, they may release **carbon dioxide (CO₂)** and other gases. Because the agar is solid, these gases become trapped, causing visible physical changes such as bubbles, cracks, or even lifting of the agar from the bottom of the tube. In some cases, large amounts of gas can push the entire agar column upward toward the cap.

Because the KIA test simultaneously reveals information about sugar fermentation, gas production, and hydrogen sulfide generation, it is a powerful multipurpose tool. Accurate interpretation requires careful observation of several features: the color of the slant and butt, the presence or absence of black precipitate, and any visible evidence of gas production. Taken together, these results help microbiologists classify unknown organisms and understand their metabolic capabilities. The ability to interpret KIA results is a foundational skill in microbiology, as it illustrates how metabolic diversity among bacteria can be used to identify and differentiate them in both clinical and environmental settings.

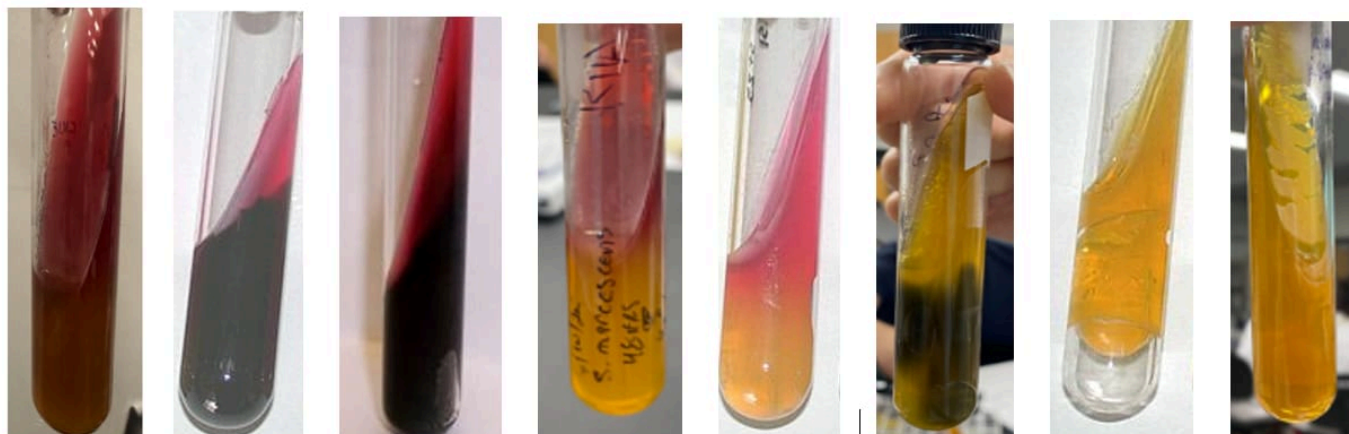
Procedures

1. Using a sterile loop inoculate surface of the KIA slant with one loopful from of the cell/water suspension.
2. From the cell/water suspension use a sterile needle to stab the butt of the KIA media: insert the needle straight down through the center of the medium. Withdraw along the same path.
3. Leave the cap a quarter-turn loose for gas exchange.
4. Incubate at 30°C for 48 hours. Avoid over-incubation beyond 72 hours to prevent slant re-alkalinization that can give

false results.

Interpreting Results

1. Negative fermentation, negative H₂S, negative gas
2. Positive glucose fermentation, positive H₂S, Negative gas
3. Positive glucose fermentation, positive H₂S, positive gas
4. Positive glucose fermentation, negative H₂S, negative gas
5. Positive glucose fermentation, negative H₂S, positive gas
6. Positive glucose, positive either lactose and/or sucrose fermentation, positive H₂S, negative gas
7. Positive glucose, positive either lactose and/or sucrose fermentation, negative H₂S, positive gas
8. Positive glucose, positive either lactose and/or sucrose fermentation, negative H₂S, negative gas



1 2 3 4 5 6 7 8

32.8: Mannitol Salt Agar (MSA) Test

Background

Mannitol Salt Agar (MSA) is a specialized growth medium that is both selective and differential, making it a valuable tool for isolating and identifying certain types of bacteria, particularly among Gram-positive cocci.

The medium is selective because it contains a high concentration of sodium chloride (7.5 percent), which inhibits the growth of most bacteria that are not salt-tolerant which includes most Gram-negative bacteria. This environment favors the growth of halotolerant organisms, especially species in the genus *Staphylococcus*, while preventing the growth of many others.

MSA is also differential due to the presence of the sugar mannitol and the pH indicator phenol red. Organisms that can ferment mannitol produce acidic byproducts, which lower the pH and cause the medium to change color from red to bright yellow. Organisms that grow but do not ferment mannitol will not significantly affect the pH, and the agar remains red or slightly pink.

This test is especially useful for distinguishing *Staphylococcus aureus*, which tolerates salt and ferments mannitol, from other staphylococci like *Staphylococcus epidermidis*, which tolerates salt but does not ferment mannitol.

To ensure accurate results, the MSA plate should be inoculated using a cell/water suspension. This helps prevent carryover of nutrients or pH-altering compounds from other media that could interfere with interpretation. The results should be evaluated based on both the presence or absence of growth and any associated color change.

If no growth is observed, it is important to consider the possibility of improper inoculation before concluding that the organism cannot tolerate high salt concentrations.

The MSA test is time-sensitive and should be read within 48 hours of incubation. Over-incubation can lead to the organism fermenting all the sugar in the medium then reverting to breakdown the peptones in the medium, resulting in alkaline byproducts that may neutralize or reverse the acid-induced color change. This can make a mannitol-fermenting organism appear as if it does not ferment mannitol, resulting in a false-negative result. Additionally, prolonged incubation may allow slow-growing, salt-sensitive organisms to grow, potentially leading to misinterpretation. To ensure reliability, record results at the recommended time point and do not interpret changes that appear after extended incubation.

Procedure

1. Using a sterile inoculating loop, obtain a loopful of the cell/water suspension then streak the MSA slant.
2. Incubate at 30°C for 48 hours.

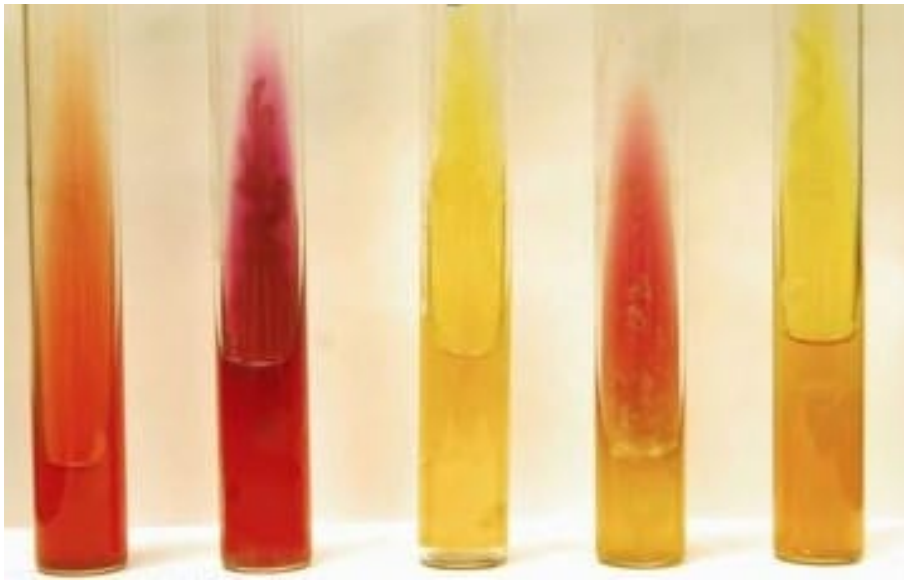
Interpretation of Results

After incubation, examine the slant plate and record results based on both growth and any color change in the agar and surrounding the colonies.

Growth with yellow color: organism is salt-tolerant and ferments mannitol

Growth with no color change: organism is salt-tolerant but does not ferment mannitol.

No growth: organism is not salt-tolerant.



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32.9: Eosin Methylene Blue (EMB) Test

Background

Eosin Methylene Blue (EMB) agar is a selective and differential medium used primarily for the isolation and identification of Gram-negative enteric bacteria, particularly those in the family Enterobacteriaceae. The Enterobacteriaceae family is a large and diverse group of Gram-negative, rod-shaped bacteria that are facultatively anaerobic, meaning they can grow with or without oxygen. Members of this family are typically found in the intestinal tracts of humans and animals, as well as in soil, water, and other environments. They are commonly referred to as enteric bacteria. These bacteria are medically important because they are frequently associated with gastrointestinal infections, urinary tract infections, wound infections, and hospital-acquired infections, and many have developed resistance to multiple antibiotics. Diagnostic labs use tests like EMB agar and other metabolic assays to identify and differentiate members of this family.

EMB agar contains two dyes, eosin Y and methylene blue, which serve dual functions. First, they inhibit the growth of most Gram-positive bacteria, making the medium selective for Gram-negative organisms. Second, the dyes act as pH indicators, allowing the medium to differentiate between organisms based on their ability to ferment lactose and produce acid.

Lactose-fermenting organisms lower the pH of the medium, causing dye uptake and resulting in distinct colony colors. Strong acid producers, such as *Escherichia coli*, typically form dark purple to black colonies with a characteristic metallic green sheen. Weaker lactose fermenters may produce pink to purple colonies without a sheen. Non-lactose fermenters will form colorless or light beige colonies, as they do not acidify the medium.

Procedure

1. Using a sterile loop, obtain a small amount of the test organism from a pure culture.
2. Streak the surface of the EMB agar plate with a single straight line. Do not touch the side of the plate.
3. Incubate agar up at 30°C for 48 hours.

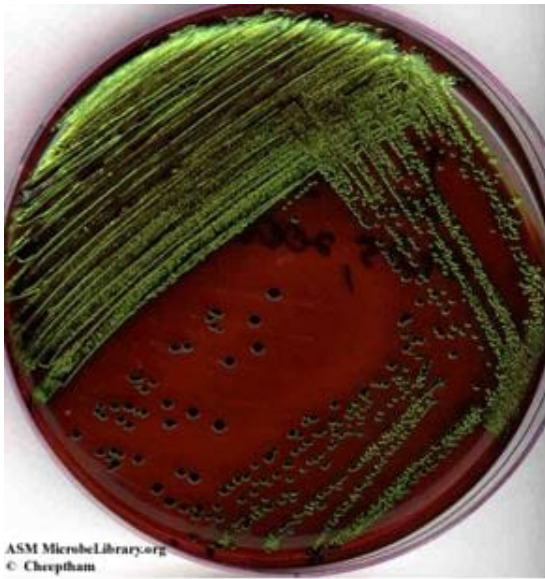
Interpretation of Results

Growth with dark purple to black colonies and a metallic green sheen: strong lactose fermentation with acid production, characteristic of *Escherichia coli*

Growth with pink or purple colonies (no sheen): weak or slow lactose fermentation

Growth with colorless or beige colonies: no lactose fermentation

No growth: organism is likely Gram-positive and inhibited by the dyes in the medium



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32.10: Indole Broth Test

Background

The indole test is used to determine whether a bacterial organism can break down the amino acid tryptophan to produce indole. This biochemical reaction helps identify certain Gram-negative bacteria, especially within the Enterobacteriaceae family. It is commonly used in clinical and environmental microbiology to help differentiate between closely related species, including those responsible for typhoid fever (*Salmonella*) and bacterial dysentery (*Shigella*).

The test medium, tryptone broth, contains tryptophan as a substrate. Bacteria that produce the enzyme tryptophanase can hydrolyze tryptophan into three products: indole, pyruvate, and ammonia. Indole, if present, can be detected by adding Kovac's reagent, which contains para-dimethylaminobenzaldehyde, amyl alcohol, and hydrochloric acid.

When indole is present, it reacts with the aldehyde component of the reagent in the acidic and alcohol-containing environment to form a rosindole dye. This compound is visible as a bright pink or fuchsia color in the top layer of the tube. Because indole is soluble in amyl alcohol and tryptophan is not, the color forms only in the upper alcohol layer. Any pink color observed in the aqueous lower layer is not considered a positive result and may be due to unreacted tryptophan.

Procedure

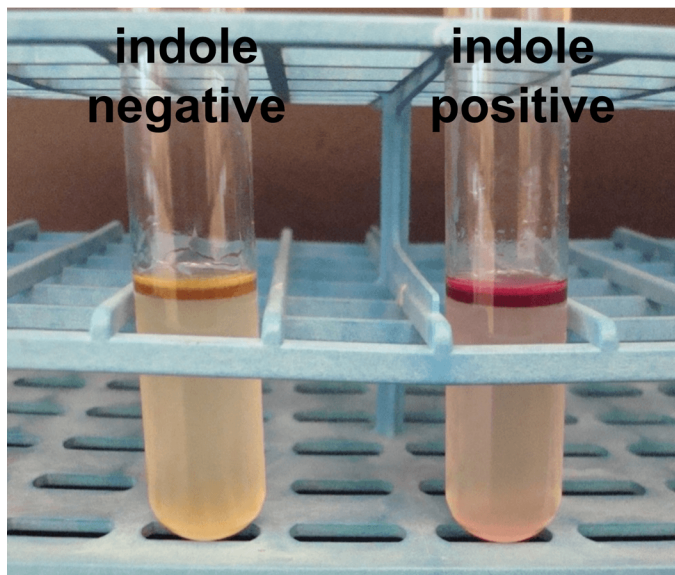
1. Inoculate the tryptone broth with a loopful of your culture
2. Incubate at 30°C for 48 hours
3. After incubation, carefully add 10 drops of Kovac's reagent down the side of the tube
4. Do not shake or swirl the tube to mix the contents
5. Allow the tube to stand undisturbed for a few moments
6. Observe the upper layer for a color change.

Interpretation of Results

A bright pink or fuchsia color in the top (amyl alcohol) layer indicates a positive result, confirming indole production

No color change, or a yellow layer at the top, indicates a negative result (no indole produced)

Color in the bottom aqueous layer is not used for interpretation.



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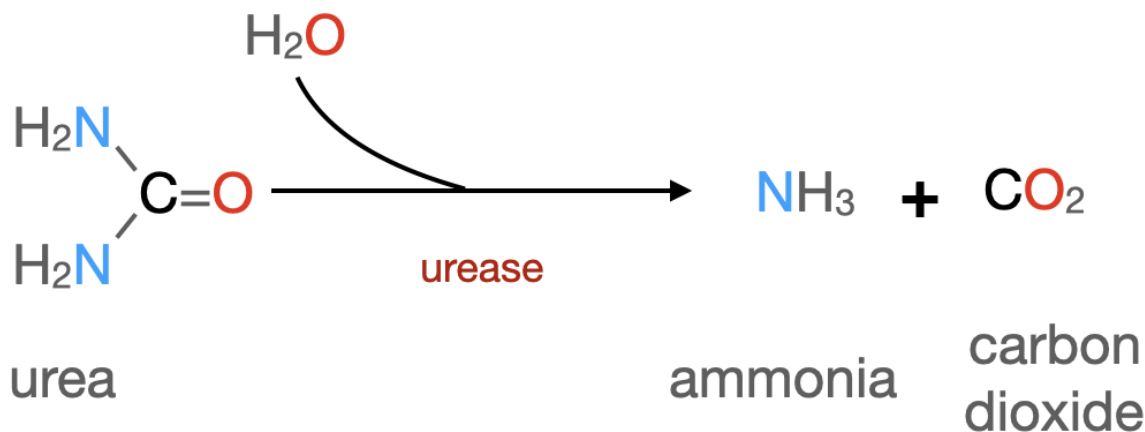
32.11: Urease Test

Background

The Urease test is used to determine whether a bacterial organism produces the enzyme urease, which catalyzes the hydrolysis of urea into ammonia and carbon dioxide. This test is especially useful in identifying certain enteric bacteria and is often included in diagnostic workflows to distinguish among members of the Enterobacteriaceae family.

Urease activity results in the accumulation of ammonia, a basic compound that raises the pH of the medium. The urea broth contains a pH indicator, phenol red, which is yellow-orange under neutral conditions and turns bright pink or violet in an alkaline environment. A positive test is indicated by a color change from the original pale yellow to bright pink or magenta, reflecting the presence of ammonia and confirming urease activity.

Organisms that produce urease rapidly, such as *Proteus* species, can cause a noticeable color change within just a few hours. Slower urease producers may require a longer incubation period to achieve a visible result. The test is particularly useful in clinical settings, as urease-producing bacteria are often associated with urinary tract infections. These organisms can thrive in the urinary environment by alkalinizing their surroundings, contributing to the characteristic ammonia odor of stale urine.



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Procedure

1. Inoculate a **small** amount of the test culture into the urea medium. Avoid using much inoculum. Inoculating too much of the inoculum may overwhelm the system resulting in an inaccurate result.
2. Incubate at 30°C for 48 hours.

Interpretation of Results

- Bright pink or magenta broth: positive result, indicating urease production and ammonia accumulation
- No color change (yellow or light orange broth): negative result, indicating no urease activity or activity below detectable levels



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32.12: Simmon's Citrate Agar Test

Background

The Simmons citrate test is used to determine whether a bacterial organism can produce the transport enzyme citrate permease. Citrate permease enables the organism to bring citrate into the cell and utilize citrate as its sole source of carbon. This metabolic capability is helpful in identifying and differentiating members of the Enterobacteriaceae family, particularly when distinguishing between closely related species.

The medium, Simmons citrate agar, contains sodium citrate as the only available carbon source and ammonium Dihydrogen phosphate as the sole nitrogen source. Most bacteria cannot grow unless they possess the specific transport system and enzymes required to take in and metabolize citrate. Organisms that can produce citrate permease are able to grow and multiply in this otherwise limited environment.

As citrate is utilized, the organism also metabolizes the ammonium salt, producing alkaline byproducts such as ammonia. These byproducts raise the pH of the medium. The agar contains the pH indicator bromthymol blue, which is green at neutral or slightly acidic pH and turns blue in alkaline conditions. A visible color change from green to blue indicates that the organism is capable of using citrate as its sole carbon source and has produced alkaline end products during growth. The Simmons citrate test is inoculated using a cell suspension in sterile water rather than a loopful of culture from a nutrient-rich medium in order to prevent accidental carry over of the nutrient agar thereby preventing false-positive results.

A positive result is demonstrated by either a deep blue coloration of the slant or obvious growth on the slant, even if the color change is limited. Growth alone on Simmons citrate agar, in the absence of other nutrients, is considered a positive result. A negative result is indicated by no growth and no color change, with the slant remaining green.

Procedure

1. Using the cell/water suspension previously prepared from the test culture, streak the surface of the Simmons citrate agar slant with a zig-zag motion. Avoid stabbing the butt of the slant.
2. Incubate at 30°C for 48 hours with the cap slightly loose to allow for gas exchange.

Interpretation of Results

- Blue slant (with or without visible growth): positive result, organism can use citrate as the sole carbon source and produces alkaline byproducts
- Growth on green slant (no color change): also considered positive, as growth alone indicates citrate utilization
- No growth and no color change (slant remains green): negative result, organism cannot use citrate as the sole carbon source

32.13: Motility Test

Background

The motility test is used to determine whether a bacterial organism is capable of self-directed movement, typically through the use of flagella. Motility is a valuable characteristic in the identification of unknown bacterial isolates, particularly among members of the Enterobacteriaceae family and other rod-shaped bacteria. Motility agar provides a more reliable alternative for detecting bacterial motility. The medium contains a reduced agar concentration (usually around 0.4 percent), which creates a soft consistency that allows motile bacteria to move through the medium. Non-motile bacteria will remain restricted to the line of inoculation. To enhance visibility of growth, the redox-sensitive dye tetrazolium salt is included. This indicator turns bright pink or red in areas where bacterial metabolism has occurred, helping to clearly show the pattern of movement.

Procedure

1. Inoculate the medium by stabbing the sterile needle straight down through the center of the agar to approximately two-thirds of the tube's depth
2. Withdraw the needle along the same stab line, being careful not to disturb the surface
3. Incubate at 30°Celsius for 48 hours

Interpretation of Results

Hold the tube up towards the light then observe the growth pattern in the tube

- Diffuse, hazy growth that extends outward from the stab line (often with pink or red coloration if an indicator is used) indicates a positive result for motility
- Growth restricted to the stab line, with no lateral spread, indicates a negative result for motility
- If an indicator is present, color change throughout the medium enhances visibility of the result but is not required to confirm motility

32.14: Oxidase

Background

The oxidase test is used to determine whether a bacterial organism produces the enzyme cytochrome c oxidase, which plays a key role in the electron transport chain during aerobic respiration. This enzyme facilitates the transfer of electrons from cytochrome c to molecular oxygen, serving as the final electron acceptor in the process. The presence or absence of cytochrome c oxidase helps differentiate bacterial groups, particularly among Gram-negative species.

For example, genera such as *Pseudomonas*, *Vibrio*, and *Neisseria* are typically oxidase positive, while members of the Enterobacteriaceae family are usually oxidase negative. This distinction makes the oxidase test an important tool in microbial identification.

The test is based on a redox reaction involving the reagent p-phenylenediamine dihydrochloride. When this reagent is oxidized by cytochrome c oxidase, it turns from colorless to dark purple or blue. A color change within one minute indicates a positive result. If the organism does not produce the enzyme, the reagent remains colorless or shows only the natural pigmentation of the cells.

It is essential to perform the test under controlled conditions. A color change that appears after one minute should not be interpreted as a positive result, as it may be due to atmospheric oxidation rather than enzymatic activity. Additionally, using a fresh, actively growing culture is important because older or stressed cells may not express the enzyme, leading to a false-negative result.

*Do not perform this test on cultures you need to preserve, If you have more tests to do prepare a fresh nutrient agar slant first.

Procedure

1. Using a sterile wooden stick (not a metal loop or needle), collect a small amount of the organism
2. Smear the colony onto a piece of clean filter paper, forming a visible spot. Avoid picking up any agar or growth medium
3. Add one to two drops of oxidase reagent directly onto the smear
4. Start a timer immediately. Results must be read within 15-30 seconds

Interpretation of Results

- Purple or dark blue color within one minute: positive result, cytochrome c oxidase is present
- No color change or a color that appears after one minute: negative result, or not producing detectable enzyme under current conditions
- Purple color developing after one minute should be ignored, as this is likely due to oxidation by air, not enzyme activity

32.15: Catalase Test

Background

The catalase test is used to detect the presence of the enzyme catalase in bacterial cells. This enzyme plays a critical role in protecting aerobic and facultatively anaerobic organisms from oxidative damage caused by reactive oxygen species. During aerobic respiration, bacteria that use oxygen inevitably produce toxic byproducts, such as superoxide radicals and hydrogen peroxide (H_2O_2). These molecules can damage cellular components, including proteins, DNA, and membrane lipids.

To survive in oxygen-rich environments, bacteria must have mechanisms to neutralize these toxic compounds. Superoxide radicals are first converted into hydrogen peroxide by the enzyme superoxide dismutase. Hydrogen peroxide, although less reactive, is still harmful and must be broken down further. Catalase facilitates the decomposition of hydrogen peroxide into water (H_2O) and oxygen gas (O_2), both of which are non-toxic to cells. The overall reaction catalyzed by the enzyme is: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$

The release of oxygen gas during this reaction forms visible bubbles, which serve as the basis for the catalase test. A positive result is indicated by the rapid production of bubbles when hydrogen peroxide is applied to a bacterial culture. The presence or absence of this reaction helps differentiate catalase-positive organisms, such as staphylococci, from catalase-negative organisms, such as streptococci.

It is important to note that this test should not be performed on cultures you need to preserve, as the addition of hydrogen peroxide can damage or destroy the cells. The test should also be performed on actively growing cultures, as older cells may lose enzyme activity and produce false-negative results.

Once H_2O_2 is added to the culture the culture is no longer viable.

Be sure to make a new slant if there are more tests to be done.

Procedure

1. Add one to two drops of 3 percent hydrogen peroxide (H_2O_2) directly onto the bacterial smear
2. Observe the reaction immediately for the formation of bubbles

Interpretation of Results

- Immediate and vigorous bubbling: positive result, indicating the presence of catalase
- No bubbling or only a few scattered bubbles: negative result, indicating the absence of detectable catalase activity

32.16: Flow Chart

Learning Objectives

- Select appropriate laboratory tests for identifying an unknown bacterium based on previous test results.
- Construct a logical and efficient flow chart that uses test outcomes to progressively narrow down possible bacterial identities.

BACKGROUND

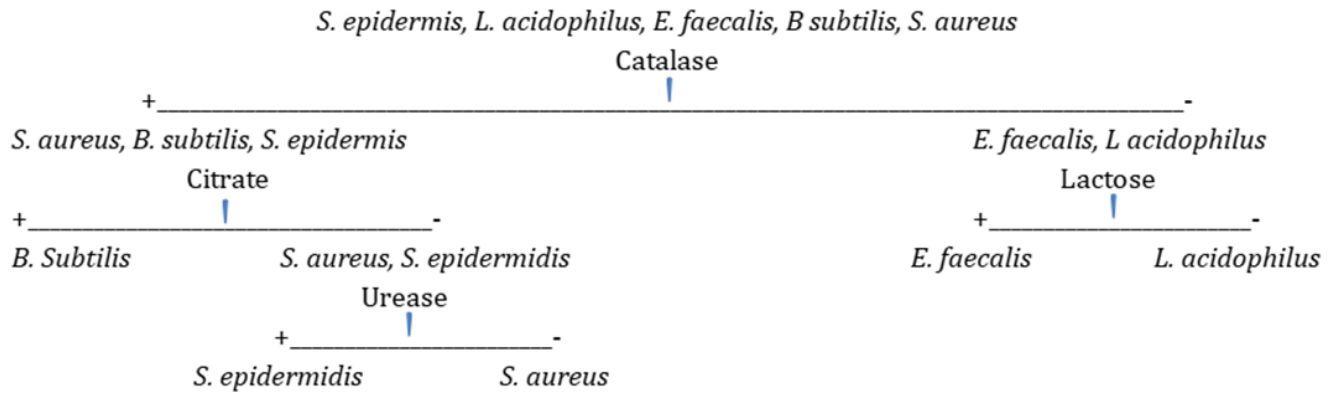
One of the most important skills in microbiology is the ability to identify an unknown bacterium based on laboratory testing. In clinical, research, and public health settings, accurate identification of microorganisms is critical for diagnosing infections, choosing the correct treatments, tracking disease outbreaks, and conducting scientific investigations.

Because bacteria cannot be reliably identified by appearance alone, microbiologists use laboratory tests to gather clues about an organism's identity. Some tests reveal structural features, such as the Gram stain, which divides bacteria into Gram-positive and Gram-negative groups based on differences in their cell wall. Other tests detect metabolic traits, such as the ability to ferment sugars, produce specific enzymes, or utilize certain nutrients. These biochemical tests can reveal important information about how a bacterium functions and help narrow down its identity.

However, not every test is helpful for every organism. Running every possible test on an unknown would be wasteful and slow down the identification process. Instead, microbiologists use a flow chart (a step-by-step decision-making guide that helps you choose which tests to run based on the previous result). Although not technically a metabolic test, typically, a flow chart begins with the Gram stain. Each test should separate approximately half of the possible organisms until each organism is isolated. Consistently utilizing tests that only separate one organism from the group at a time will result in more testing than necessary in order to identify the unknown.

In this exercise, you will learn how to select appropriate tests from a set of data then practice building a flow chart using a small set of organisms and sample data.

Example Flow Chart



METHODS

1. Only use tests that are included in the table below.
2. Review the list of possible organisms and their test results.
3. In the space provided in the Results section below write the names of all the organisms in the Gram negative sample data table below in a row across the top of the paper.
 *Remember the genus can be abbreviated but the species cannot.
4. Find a metabolic test in which approximately half of the organisms have one result, and half have a different result. Write the name of the test underneath the row of possible organisms then draw a line under the name of the test that is a little shorter than the width of the paper. Above the line add the result possibilities. Write the organisms in a row under the correct result.
5. Continue to separate the organisms based on their test results until each organism is isolated.

Ex 31.16 FLOW CHART

NAME _____

EXPECTATIONS

If there are 10 possible organisms, how many tests do you think it would take to identify what organism you have?

Use the data below to build a Gram-negative flow chart in the results section.

SAMPLE DATA: Biochemical Test Results for Known Gram-Negative Bacteria

Organism	Gram Reaction	Catalase	Citrate	Glucose Fermentation	Lactose Fermentation	Urease
<i>Escherichia coli</i>	Negative	+	-	+	+	-
<i>Proteus vulgaris</i>	Negative	+	+	+	-	+
<i>Pseudomonas aeruginosa</i>	Negative	+	+	-	-	-
<i>Salmonella enterica</i>	Negative	+	+	+	-	-
<i>Klebsiella pneumoniae</i>	Negative	+	+	+	+	+
<i>Shigella sonnei</i>	Negative	+	-	+	-	-
<i>Enterobacter aerogenes</i>	Negative	+	+	+	+	-

RESULTS

Gram Negative Flow Chart:

CONCLUSIONS

SAMPLE DATA: Biochemical Test Results for Known Gram-Positive Bacteria

Organism	Gram Reaction	Catalase	Citrate	Glucose Fermentation	Lactose Fermentation	Urease
<i>Staphylococcus aureus</i>	Positive	+	-	+	+	-
<i>Bacillus subtilis</i>	Positive	+	+	+	-	-
<i>Enterococcus faecalis</i>	Positive	-	-	+	+	-
<i>Lactobacillus acidophilus</i>	Positive	-	-	+	-	-
<i>Staphylococcus epidermidis</i>	Positive	+	-	+	+	+

Use the sample Gram-positive data above to answer the following questions.

1. What metabolic test can be used to separate *B. subtilis* from *S. epidermidis* other than the Citrate test?
2. What metabolic test (or tests) should not be used to separate *E. faecalis* from *L. acidophilus*, and *S. epidermidis*?
3. What test, other than Catalase, would be appropriate to start a flow chart with? Explain your reasoning.
4. Why do microbiologists use flowcharts when performing metabolic tests to identify an unknown organism?

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GLOSSARY

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Sample Word 1 | Sample Definition 1

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