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Basic Microbiology Skills Part 1

TEACHER'S MANUAL WITH STUDENT INSTRUCTIONS

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Basic Microbiology Part 1

Teacher's Manual

Photocopy Masters

About the authors

Priya and Shil DasSarma have studied *Halobacterium* sp. NRC-1 for over 20 years. They are enthusiastic about making these safe, convenient microbes accessible to educators and students at all levels. Visit their website at http://www.carolina.com/life_science/halobacteria for more information, ideas, and contact information.

Basic Microbiology Skills Part 1

Student Objectives

- To learn and practice sterile technique
- To learn how to streak plates
- To learn to isolate and examine individual colonies

Summary

In this laboratory exercise, students will practice sterile technique and will learn the basic plating technique of streaking. They will streak a plate in a pattern they select from three options illustrated in the Student Instructions and will compare results from the three different patterns. They will also observe their colonies for different phenotypes.

Background

Microbiology, the study of single-celled microscopic life, is full of unknowns and questions to explore. Also, microbiology is critical in today's food, medical, and biotechnical industries. Students should know basic microbiology and microbiology skills in order to comprehend the world they live in.

There is no better way for students to learn about microorganisms than to study them in the laboratory. Many microbiology questions can be investigated in your classroom or laboratory. Your students could do community analysis to discover which microbes are present in their environment, or they could characterize isolated strains. They may also investigate the role of microbes in material cycling as a way to learn about the vital role of microbes in the natural environment.

Since microbes are everywhere—on our hands, our clothes, and our laboratory work surfaces—a problem with many microbiology studies in the classroom or school lab is the risk of contamination of cultures by unwanted species. Avoiding contamination is everyday work for those who use microbiology in their professions. In medicine, researchers and technicians grow microbes from sick patients in order to identify the **pathogen** (disease-causing agent), or to test a pathogen's antibiotic resistance. This work with known or potential pathogens requires special laboratory procedures for handling, containment, and disposal. These procedures are also used in the food and biotechnology industries, where workers carefully monitor microbe strains and populations (e.g., the milk supply is regularly tested for harmful microbes.)

To deal with the problem of contamination, microbiologists follow a basic set of procedures known as **sterile** or **aseptic technique**. Sterile technique is used for culturing and transferring cultures, and for streaking plates to isolate and purify strains. In this lab, students will learn and practice sterile technique. Basic sterile technique is described in the Student Instructions.

While learning sterile technique, students will practice another important microbiology skill, streaking plates. The goal of streaking agar plates is to generate individual colonies for examination or study. The technique is also used to isolate individual microbial species from a mixture of microbes in a

sample. In order to generate single colonies on a plate by streaking, you must have good streaking technique. Consider that an inoculating loop dipped into a pure culture of *Halobacterium* may pick up 10,000,000 cells. If a plate is streaked correctly, some of these cells will be spread out enough to grow individual colonies.

For this exercise, students will use a **pure culture** of *Halobacterium* sp. NRC-1. *Halobacterium* sp. NRC-1 is a member of the **Archaea** and grows in extremely salty environments including salt marshes, hypersaline lakes and ponds (e.g., the Great Salt Lake), **solar salt production** facilities, and brine inclusions in salt crystals in salt mines. In these environments, the salt concentrations are between 3 and 5 M (nearly saturated). *Halobacterium* is grown on medium so salty that few other organisms would survive it. In fact, *Halobacterium* will **lyse** at total **salinities** below 1.0–2.0 M. (For comparison, sea water has a total salinity of 0.6 M, while human blood contains only 0.14 M NaCl). For these reasons and the fact that *Halobacterium* is not known to cause any human disease, these microbes are ideal for beginning practice of sterile technique. Using these microbes allows students to practice the hand motions of sterile technique without the concern that they will culture a pathogen or contaminate their workspace with the microbe they are using. However, emphasize to your students that the point of the exercise is to learn good sterile technique, so they should use the same care they would use if they were working with a known pathogen.

Additional Background

In addition to discussing sterile technique with your students, you may wish to review other basic information about how microbes are grown and isolated in the lab. In this review you may wish to include the following information.

In order to identify microbes, laboratory workers initially examine the size, shape, color, and consistency of colonies formed by the microbe. To reliably and easily do this and to study other identifying and interesting aspects of a given microbe, workers must be able to culture the microbe in the laboratory. In the laboratory, specific microbes are grown in a **medium** containing the nutrients that they need to grow, often including certain sugars, amino acids, vitamins, and minerals. The medium may be a liquid, or it may be a solid such as an agar plate. In addition, the nutritional content of media may vary depending upon what kind of microbe a worker wants to grow.

Once they are placed into or on the appropriate media, microbes are then **incubated** (grown) at a particular temperature and under specific conditions depending upon their growth requirements. For example, some microbes are grown in the presence of oxygen (**aerobically**), others, in the absence of oxygen (**anaerobically**). The organism your students will use, *Halobacterium* sp. NRC-1, generally grows aerobically, using oxygen, but it also has facultative anaerobic and phototrophic capabilities and so can grow without oxygen in the presence of light.

Generally, microbes divide by **binary fission**, the splitting of one parent cell into two daughter cells. Each doubling represents a new generation. The amount of time required for a single cell to go through the division cycle to form a new generation is called the **doubling time** or **generation time**. The

generation time of various microbes ranges from a few minutes (e.g., 20 minutes for common laboratory *E. coli* in rich medium) to a year or more for slow-growing species. For *Halobacterium* sp. NRC-1, the generation time under optimal growth conditions is about 6 hours.

Usually, after 25 generations, a single cell has spawned a colony visible to the unaided eye. In most cases, this single colony is composed of millions of clones of the original parental cell. The parental cell of a colony is sometimes referred to as a **colony-forming unit** or **cfu**. The number of viable cells in a culture is often quantified as cfu/volume.

Materials

Included in the kit

40 petri dishes

8 bottles *Halobacterium* Agar, 135 mL each

Halobacterium liquid culture

128 sterile inoculating loops

autoclavable disposal bag

Teacher's Manual with photocopy master for Student Instructions

Needed, but not supplied

hand lenses, or dissecting microscope(s)

wax markers or lab pens

Bunsen burners

resealable plastic bags or plastic food-storage boxes

*Incubator, 42° or 37°

Disinfectant for benchtop (e.g., alcohol or bleach solution)

*If an incubator is not available, the plates may be stored on the lab bench. If the plates are incubated at 42°C, results may be obtained in 7 days, and if at 37°C, 7–14 days. If the plates are incubated at room temperature on the bench, it may take 2 weeks or longer to see the results, depending on the temperature of your lab.

Teacher Preparation

You will need to

- pour plates.
- read through the entire booklet to familiarize yourself with the information and instructions given to the students.
- photocopy sets of Student Guides for individuals or teams.
- decide how and when to share the Additional Background information and the Explanation of Results with the students.

Preparing Media Plates

1. To melt the medium, slightly loosen the cap(s) and set the bottle(s) of medium in a pot of water and bring it to a boil. Make sure the water level is even with the level of the medium in the bottle(s). Leave the bottle in the boiling water until the medium has completely melted. This will take approximately 30 minutes.

2. Allow the medium to cool to 55°C either by allowing the pot of water to cool to that temperature or by letting the bottle(s) sit for several minutes at room temperature. The bottle(s) should feel comfortably hot to the touch when around 55°C.
3. Disinfect the work surface. Wash your hands thoroughly. Unpack the petri dishes, being careful not to disturb their sterility. Align the sterile plates along the edge of a clean, level tabletop away from any draft or breeze.
4. Remove the cap and flame the mouth of a bottle of medium. Lift the lid of a petri plate just enough to pour in the molten medium. Carefully, pour to a depth of about 5 mm per plate. Replace the lid immediately to prevent contamination.
5. Repeat Step 4 with the other bottle(s) of medium.
6. Let the petri plates stand undisturbed until they solidify (about 1 hour). Let the plates sit out until any condensation on the lid evaporates.
7. Dispose of the empty bottles in an autoclave disposal bag.

For the lab procedure, see the Student Instructions.

Examination of Colonies and Explanation of Results

Have your students compare results with each other and then draw conclusions about whether they would use the same or a different streaking technique the next time they do the exercise.

In addition, have your students watch for the development of the following three possible **phenotypes** (all represented on the plate shown on the cover of this manual):

- Vac+, or wild-type, pink colonies. In the cells in these colonies, *Halobacterium*'s red pigmentation is somewhat altered by the presence of gas vesicles, internal oganelles that refract light and enable the cells to float in a liquid culture. *Halobacterium* converts light energy to chemical energy. Thus, this ability to float is advantageous, because it allows *Halobacterium* cells to float near the surface of natural lakes and ponds where the light is most intense.
- Vac– , or red colonies. *Halobacterium*'s red color is due to its pigments-**carotenoids** and **bacteriorhodopsin**. Bacteriorhodopsin enables the cells to convert light energy to chemical energy in a method similar to, but different from photosynthesis.
- Sectored colonies. These colonies result from mutations that occur in the founder cells during a colony's growth. A sectored colony consists of a mix of Vac+ and Vac– cells and can resemble a pie with different-colored (red or pink) slices.

Why do these colonies look so different from one another? It is because of the presence of **Insertion Sequences (IS elements)**, or "jumping genes," which "jump" into other genes at random. The insertion of the IS element often interrupts a gene's coding sequence in a way that causes the gene to become nonfunctional. In a *Halobacterium* cell, when an IS element jumps into one of the genes that normally code for the creation of gas vesicles, the disruption can render the cell and its progeny unable to produce gas vesicles. The absence of gas vesicles results in a

phenotypic (observable) change that is due to a **genotypic** (genetic) change—a change from pink to red cells as a result of a change in the DNA sequence.

Further Investigation

If you or your students want to investigate further with *Halobacterium*, you could try to keep the culture going. One option is to pick individual colonies from the streaked plates with a sterile loop or toothpick and use them to initiate individual 5-mL liquid cultures. Grow these cultures in a shaking incubator. BSYC medium (recipe follows) may be used for the cultures.

Potential questions to look into include: What is the appearance of cultures obtained from pink versus red or sectored colonies? What is the effect of temperature on the growth rate of the culture? Will the liquid culture grow in the absence of shaking? Why or why not?

Once a test-tube culture appears turbid, you can use it to streak a fresh plate, as you did initially. The plate colonies should be clones (identical progeny cells) of the original cell that you picked. In the case where the original colony was pink (wild type), there may be a few red or sectored colonies indicating the mutations discussed earlier.

Recipe for BSYC Medium (makes 1 L)

BSYC medium is inexpensive and is suitable for most educational purposes using *Halobacterium* sp. NRC-1.

Materials

1-L beaker or flask
1-L graduated cylinder
2 1-L bottles
autoclave tape
deionized water
stir bar
stir plate (preferably heatable)
weigh boats
250 g sodium chloride (NaCl)
20 g magnesium sulfate ($MgSO_4 \cdot 7H_2O$)
3 g (Tri) sodium citrate ($Na_3C_6H_5O_7$)
2 g potassium chloride (KCl)
5 g yeast extract
5 g casamino acids
5 M sodium hydroxide (NaOH) for adjusting pH
Concentrated HCl for adjusting pH

1. Place a beaker with a stir bar and 700 mL of deionized water onto a stir plate and set the speed to low.
2. Add NaCl and turn on the heat to low-medium. Warm to 50°C in order to help dissolve the salt (this may take up to 15 minutes).
3. Once the salt has fully dissolved, add the $MgSO_4 \cdot 7H_2O$, $Na_3C_6H_5O_7$, KCl , yeast extract, and casamino acids.

4. Once all the ingredients have dissolved, adjust the pH to 7.2 using 5 M NaOH or concentrated HCl.
5. Bring the volume to 1 L with deionized water. Mix.
6. Split the 1 L into two 1-L bottles to prevent overflow during autoclaving.
7. Autoclave at 15 lbs/in^2 and 121°C for 35 minutes.

Storage Conditions

Cultures can be stored for 2–3 months on plates or as liquid cultures at 4°C. Store the plates in a sealed plastic food container to prevent them from drying out.

The DasSarma Team would be happy to discuss projects with students and educators and can be reached by email: dassarma@comcast.net.

Glossary

Aerobic using oxygen for metabolism.

Anaerobic not using oxygen for metabolism. Some anaerobes cannot survive in the presence of oxygen.

Agar plate a medium made solid by the addition of agar, a seaweed derivative. The medium contains specific nutrients for culturing specific microbes. A plate is most often prepared in a petri dish.

Archaea a domain of life believed to be the most ancient and to contain organisms believed to be similar to ancient prokaryotes. This domain of unicellular organisms includes the halophiles, organisms that grow in high-salt environments; methanogans, anaerobes that produce methane; and thermophiles, which thrive in high-temperature environments.

Bacteriorhodopsin a red pigment that enables some microbes to turn solar energy into chemical energy.

Binary fission splitting of a parent cell into two daughter cells.

Carotenoids a group of pigments ranging from yellow to red that occur widely among a variety of organisms. They help protect *Halobacterium* from the harmful effects of UV radiation.

Clone a genetic duplicate of an organism. A microbial colony consists of clones of the founding cell.

Colony a visible cluster of millions of microbial cells resulting from binary fission of a founder cell and its daughters and so on.

Colony-forming unit (cfu) a microbial cell capable of undergoing binary fission and founding a colony.

Generation time length of time for a particular microbe to complete its division cycle to form two new daughter cells.

Genotype the genetic makeup of an organism, either in total or in terms of one or a few sets of alleles.

Incubate to furnish appropriate conditions for an inoculated solid or liquid medium to produce colonies or larger populations of the inoculated microbes.

Inoculum living material used to initiate a culture of microbes

Insertion sequences (IS elements) sections of DNA that insert at random into the genome, causing mutations that often bring about phenotypic change; also known as "jumping genes."

Lyse to cause the breaking open of a cell wall and membrane such that the contents are released and the cell dies (the breaking open itself is referred to as "lysing").

Medium substrate or material on or in which microbes are grown for study.

Microbiology the study of single-celled life forms.

Pathogen a disease-causing agent.

Phenotype observable characteristic of an organism, e.g., brown, blue, or green eyes in people.

Pure culture colony or collection of microorganisms containing only one type. A pure culture is usually obtained after a series of cultures has been performed to isolate a particular microbe.

Salinity measure of the proportion of salt in a substance. Aquatic halophiles live in water of high salinity.

Solar salt production collection of sea salt by means of flooding shallow pools and allowing the sun to evaporate the water. The brine is moved to pools of successively higher salt concentration until crystals form.

Sterile technique (aseptic technique) accepted laboratory practices geared toward preventing contamination of cultures and the laboratory and surroundings. Sterile technique covers procedures regarding lab preparation, transfer of cultures, and cleanup and disposal.

Streak to spread a microbial sample over the surface of a plate in order to isolate colonies. Usually, streaking is done as a pattern of zigzags.

Further Reading and Information

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http://www.carolina.com/life_science/halobacteria/index.asp

Name _____

Date _____

Basic Microbiology Skills Part 1

Introduction

Microbiology, the study of single-celled microscopic life, is full of unknowns and questions to explore. Also, microbiology is a critical component of today's food, medical, and biotechnical industries.

There is no better way to learn about microorganisms than to study them in the laboratory. Even so, since microbes are everywhere—on our hands, our clothes, and our laboratory work surfaces—special precautions must be taken to avoid contamination of cultures with unwanted species. Avoiding contamination is everyday work for those who use microbiology in their professions. In medicine, researchers and technicians grow microbes from sick patients in order to identify the **pathogen** (disease-causing agent) or to test a pathogen's antibiotic resistance. This work with known or potential pathogens requires special laboratory procedures for handling, containing, and disposing of cultures. The same rule applies in the food and biotechnology industries, where workers carefully monitor microbe strains and populations (e.g. the milk supply is regularly tested for harmful microbes.)

To deal with the problem of contamination, microbiologists follow a basic set of procedures known as **sterile or aseptic technique**. Sterile technique is used for culturing and transferring cultures, and for streaking plates to isolate and purify strains. In this lab, you will learn and practice sterile technique while developing another microbiology skill—streaking a plate.

The goal of streaking agar plates is to generate individual colonies for examination or study. The technique is also used to isolate individual microbial species from a mixture of microbes in a sample. In order to generate single colonies on a plate by streaking, you must have good streaking technique. Consider that an inoculating loop dipped into a pure culture of *Halobacterium* may pick up 10,000,000 cells. If a plate is streaked correctly, some of these cells will be spread out enough to grow individual colonies.

For this exercise, you will use a **pure culture** of *Halobacterium* sp. NRC-1. *Halobacterium* sp. NRC-1 is a member of the **Archaea** and grows in extremely salty environments. Because it causes no known disease in humans and because most other microbes cannot even survive in the high salt concentration of its growth medium, *Halobacterium* is a good microbe for practicing sterile technique. You can practice the hand motions of sterile technique without concern that you might unintentionally culture a pathogen or contaminate your workspace with the microbe you are using. Still, since the point of the exercise is to learn good sterile technique, you should use the same care as if working with a known pathogen.

In this laboratory exercise, you will practice sterile technique, learn basic plating techniques, and streak a plate in a pattern that you select from three options illustrated on page S-4. You will examine your culture plates and compare your results with those of other students.

Sterile Technique

1. Before beginning, wipe your work area with a disinfectant such as alcohol or bleach and wash your hands with soap and water.
2. Light your burner. Openings (lips) of test tubes and flasks must be flamed during the transfer of media or cultures. Heating the container directs air convection currents upward and away from the opening, momentarily preventing airborne contaminants from entering. It is essential to perform the transfer quickly, before the opening cools. Hold test tubes at a 45° angle in your left hand if you are right-handed, or in your right hand if you are left-handed. Use your other hand to hold your transferring instrument (in this case a loop) and to remove and hold the cap with your little finger.

Flame the top of the tube. Take out the inoculum (culture you are transferring), reflame the opening, and replace the cap.

3. When working with petri plates, always hold the lid over the plate to prevent contaminants from landing on the surface of the agar. Always place the lid back on as soon as possible. Realize that the lid is designed to fit loosely to allow diffusion of air around the edges, while minimizing the possibility of contamination. When you lift the lid, hold it directly over the plate. Do not place the lid on the benchtop.
4. When finished, turn off the burner and again wipe your work area with a disinfectant and wash your hands.
5. Dispose of microbial cultures in a safe manner. This usually involves either autoclaving all labware that comes in contact with cultures or soaking it in a bleach solution before disposal.

Name _____

Date _____

Pre-Lab Worksheet

Define the following terms:

media _____

agar _____

aerobic _____

anaerobic _____

colonies _____

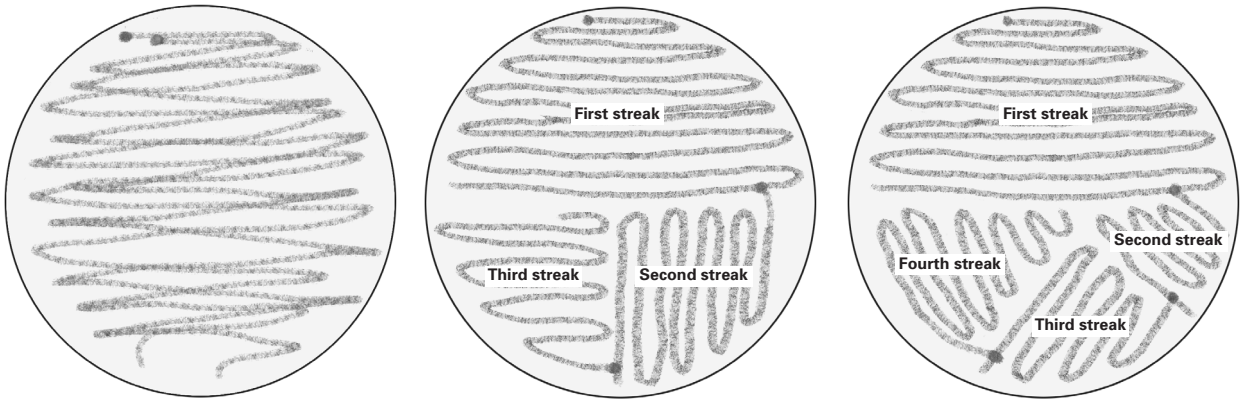
pathogen _____

sterile/aseptic technique _____

List the important steps of sterile technique in microbiological work.

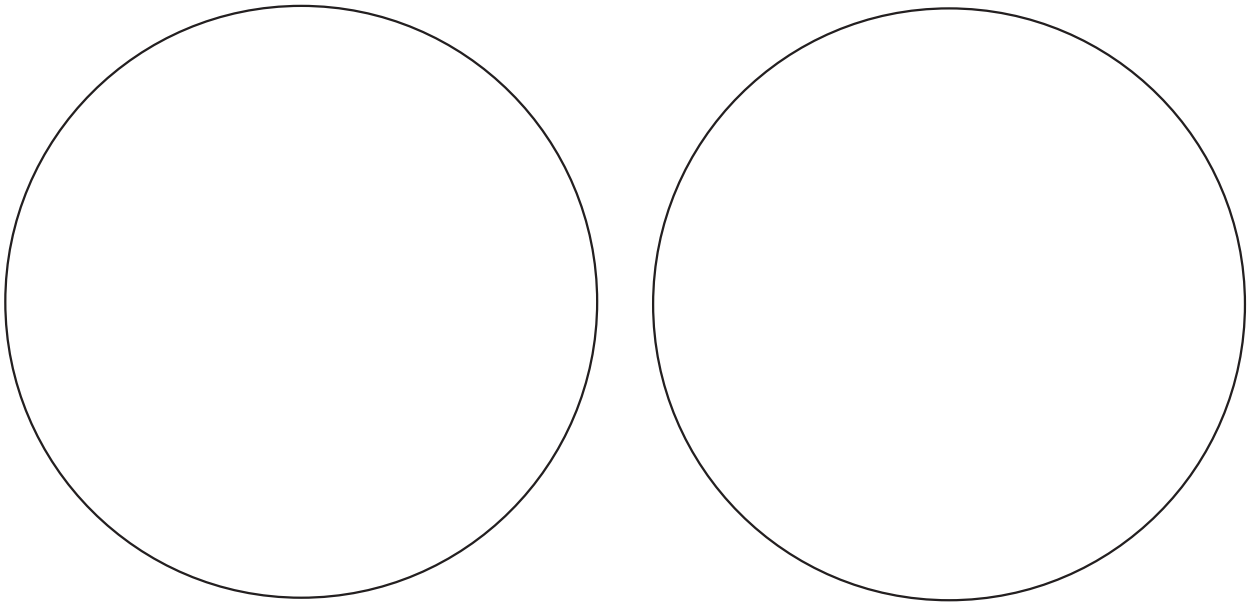
Name _____

Date _____



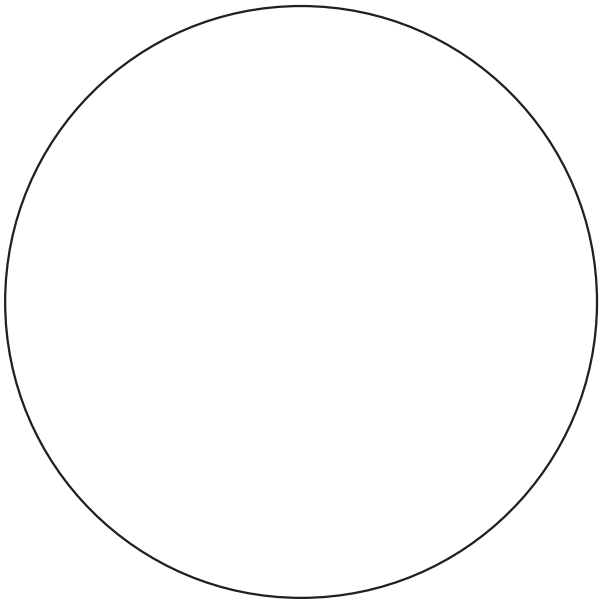
Pick out the streaking technique from above that you think will result in the best spacing of colonies on an agar plate. Explain why you think that streaking pattern will work best.

Try all three streaking techniques in the circles that follow, using a pencil or pen to draw the streaks. After you have tried the streaks on paper, decide which one of the three patterns you want to use on your agar plate.



Name _____

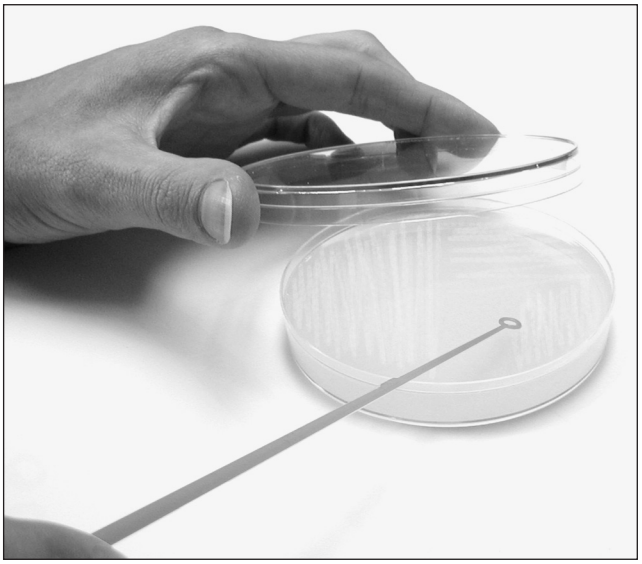
Date _____



Describe the materials and methods you will use (including conditions for incubation).

Procedures

1. Review sterile technique and plate-streaking procedure. Since live microbes will be used, it is vital that you use good laboratory technique.
2. Start with a clean, organized, and disinfected work surface. Wipe down the laboratory benchtop with a disinfectant such as bleach or ethanol.
3. Label your plate on the bottom of the smaller half of the petri dish. Make sure the date, type of organism plated, and your initials are clearly written along the edge, so as not to impede viewing the culture as it grows.
4. Remove the top of the petri dish and hold it in your left hand (if right-handed, or the other way around if left-handed), face down.
5. Dip your sterile loop into the liquid culture.
6. You have already selected the streak pattern to use for your plate. First touch the agar plate gently on the surface, and without lifting the loop from the surface, move it in a zigzag manner for streak #1. Be gentle with the streaking. If you dig into the agar, the cells will be deposited in a less aerobic environment, which reduces growth. At the end of streak #1, lift the loop. Discard the old loop and unwrap a new sterile loop to make streak #2 (If you were using a metal loop, you would flame the loop to sterilize it, and then let it cool enough to continue with the next streak.) Using a new sterile loop for each streak is important in order to spread the cells enough to get individual colonies.



Streaking a plate

7. If additional streaks are needed to complete your chosen pattern, continue in the same manner for streak #3 and/or streak #4 as you did for streak #2.
8. Place the cover on the petri plate and let the plate sit on the lab bench for 20 minutes to allow the cells to adhere to the agar surface.
9. Now, turn the petri plate upside-down to prevent condensation from dripping onto the agar surface and possibly causing contamination. The inverted plates will now be placed in airtight plastic boxes or bags and incubated as directed by your teacher. The boxes or bags will keep the plates from drying out during the extended incubation time. The plates should be checked after 2 hours to make sure that there is not any condensation on the agar surface or on the lid of the petri plate. If there is condensation, put the lid on at a slant and allow the plate to dry out for 10–15 minutes. Then, replace the lid fully.
10. After the number of days designated by your teacher, use a hand lens or stereomicroscope to examine your plate for colonies. Continue your observations of colony development and growth over several days. Pay particular attention to any apparent difference between colonies. Note: If you are incubating plates at room temperature rather than in an incubator, it may be 2 weeks before you see any results.

Name _____

Date _____

Post Lab Analysis

Draw the results of your plate streaking. Describe the appearance of the colonies on your plate.

Observe your classmates' plates and record your observations here. Look for any correlation between streaking method and results.

Would you choose a different streaking method next time? Why or why not?

Do you notice any differences between the different colonies regarding their phenotype? Can you come up with any explanations for any differences you see among the colonies on your plate?

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