Exercise 6
STREAKING FOR ISOLATION

(ed. F 2010)

INTRODUCTION:

Student Learning Objectives: At the end of this exercise, student will be able to:

a. Demonstrate the principle of streaking for isolation.
b. Define the different types of media (Differential, Selective, Supportive).
c. Describe the use of differential and selective media in isolating bacteria.
d. Demonstrate the isolation of *E. coli* and *S. aureus* from a mixed culture.
e. Successfully perform a Gram stain.

Materials

- Mixed culture of *E. coli* and *S. aureus*
- Inoculating loop
- Bunsen burner
- Blood agar, MacConkey agar, Rose agar (or PEA), and MSA plates
- Microscope and slides
- Gram staining kit
- Bibulous paper
- Wax pencils

Introduction:

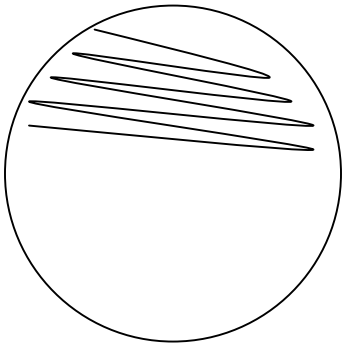
To separate Bacteria in a mixed culture, often a clinical specimen, such as urine or fecal specimens, the most common method is the streak plate technique. This is done to obtain well isolated colonies, separating bacterial species on an agar plate. A small amount of the mixed culture is transferred aseptically to the agar plate, and using a sterile loop, the organisms are spread (streaked) across the surface. The most common streaking pattern used to effectively obtain well isolated colonies, also known as Colony Forming Units (CFUs), is the quadrant method, which uses four streaks. Other methods are also used, but in this lab, we will use this method for practical purposes.

The idea behind the quadrant method is to obtain well isolated colonies, and to do this, you must streak the agar plate in a way to decrease the density of organisms with every streak cycle. Before steaking the agar plate, two very important observations must be made; first, the agar plate must be sterile, free of any contamination, and second, the agar surface must not have any moisture or condensation which could turn the streaks into "smears", thus no isolated colonies could be obtained.

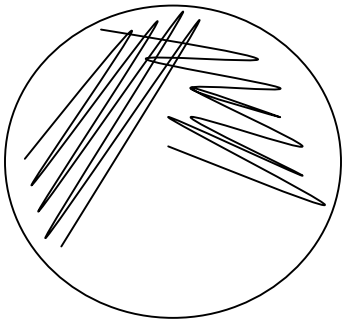
So when receiving the agar plates, make sure there is no suspected growth or contamination on the surface, especially at the edges where it is most likely to occur. If the plate is contaminated, let the instructor know about it, discard the plate, and obtain another one. If the plate surface appears to have water droplets or condensation, let the instructor know about this, and allow the plates to dry for some time in the incubator. Plates with moisture can also increase the chance of contamination, especially if the moisture is excessive and is around the edges of the agar. The instructor will demonstrate the 4 streak, quadrant method.

Streaking for Isolation

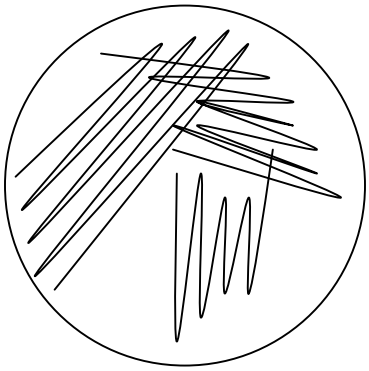
Important: The best results are obtained if you flame/sterilize the loop and cool it between each streak, by touching the agar near the edge of the plate (heat sink).



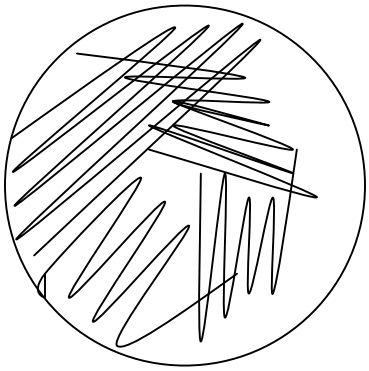
First zone- Sterilize your loop by flaming. Allow it to cool down. Use only a small amount of bacteria on your sterile, cooled loop. Make approximately 8-10 streaks.



Second zone- Sterilize your loop again and cool it as above. Turn your plate about 45 degrees and streak the second zone. Streak at an obtuse angle and don't go back into the first zone too many times.



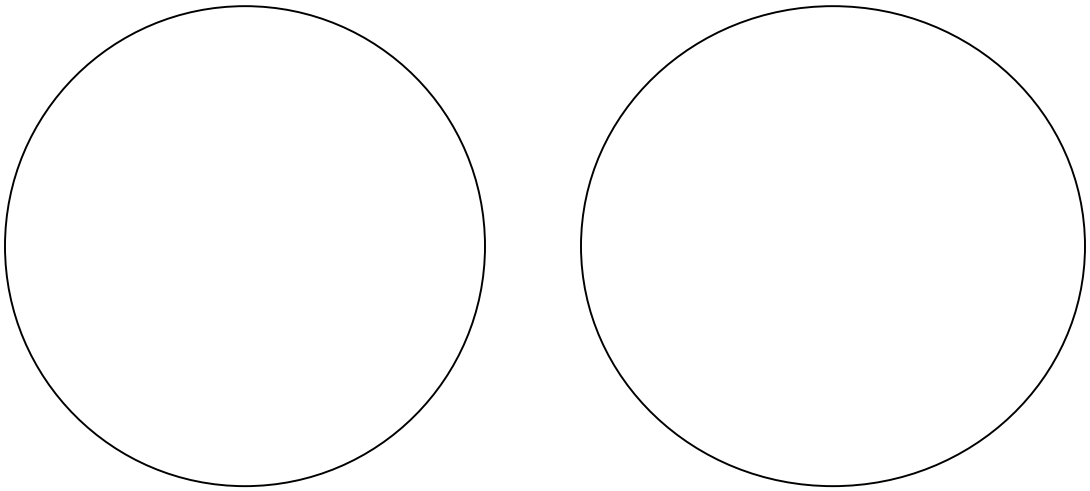
Third zone- Burn and cool as above.
Turn your plate another 45 degrees and streak another 4-8 streaks.



Fourth zone- Burn and cool as above. Turn your plate again and streak the last available area. **Be careful not to touch the first zone.**

Activities for Day 1:

1. **Practicing streaking for isolation** Pretend your pencil is a loop on which a small amount of bacteria has been placed. Make sure your drawing indicates heating the loop between quadrants and cooling the loop before streaking to insure isolation and the prevention of aerosol formation.



Streaking for isolation.

2. You are given a mixture of two bacteria. Your mission is to separate the two types of bacteria. Why do you think it would be useful to know how to do this?

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Based on what we have learned so far figure out at least one other method you could use to separate the two bacterial species growing in the same culture from each other:

_____

_____

3. Streaking for isolation. Streak the broth containing *Staphylococcus aureus* (a gram positive cocci) and *Escherichia coli* (a gram negative rod) onto a blood agar, MacConkey, and Rose agar plate (or MSA). Remember to "burn and cool" your loop between quadrants. Incubate at 37°C.

4. Predict what results we expect to observe on Day 2.

	AGAR			
	Blood	MacConkey	Rose	Mannitol Salt
Media classification: Supportive, Selective, Differential				
Prediction: ***Staphylococcus aureus*** **(gram positive cocci)** Growth or No Growth				
Prediction: ***Escherichia coli*** **(gram negative rod)** Growth or No Growth				
Inhibiting agents				
Differential agents				

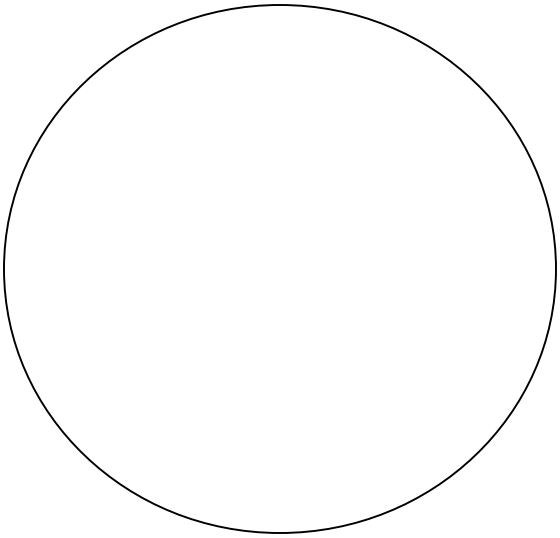
Activities for Day 2:

1. Reading the *Staphylococcus aureus* and *Escherichia coli* plates

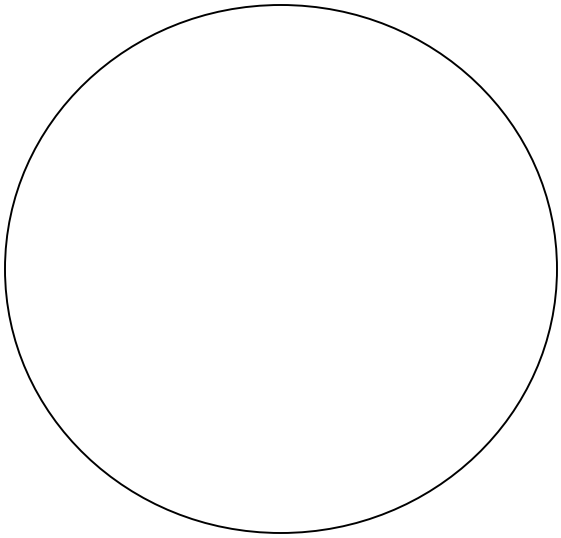
a. Instruction on reading plates for colony morphology.
b. Read the three plates and determine whether you were successful isolating both colony types.
c. Draw a picture of the blood agar plate in the circle below, showing the two different colony types.
d. Describe both colony morphologies on the blood agar plate on the chart provided.
e. For the MacConkey, Rose (or PEA) Agar, and MSA decide what you are looking for and then describe your results on the chart provided.

AGAR				
	Blood	MacConkey	Rose	MSA
RESULTS: ***Staphylococcus aureus*** **(gram positive cocci)** Growth/No Growth Describe any growth				
RESULTS: ***Escherichia coli*** **(gram negative rod)** Growth/No Growth Describe any growth				

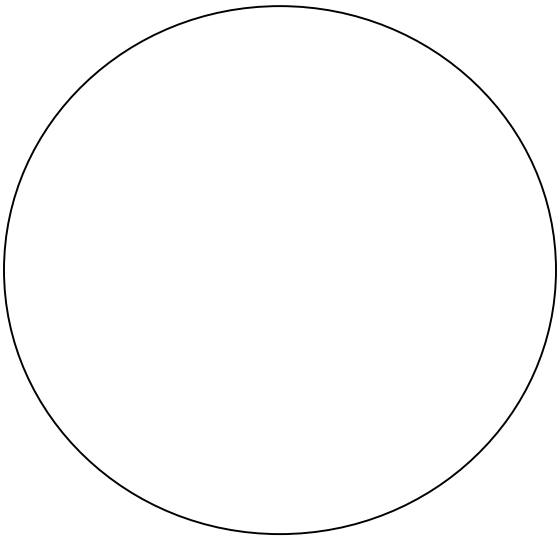
Results on Blood Agar



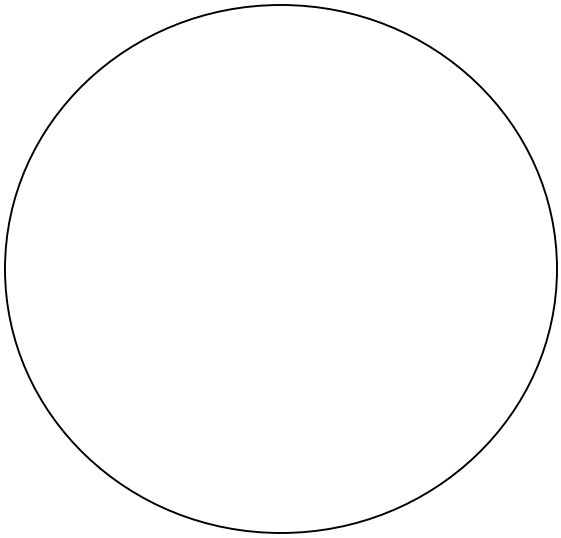
Results on MacConkey Agar



Results on Rose Agar/PEA



Results on Mannitol Salt Agar



2. The Gram stain

Instruction notes:

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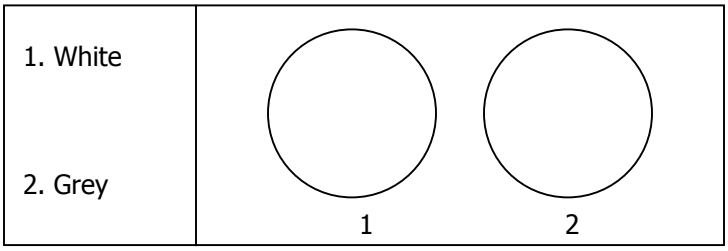
Procedure:

a. Selecting Colonies and Preparing the Slide.

From the blood agar plate, mark the two colony types by poking the agar next to it once and twice respectively. Concentrate on the area where colonies are isolated.

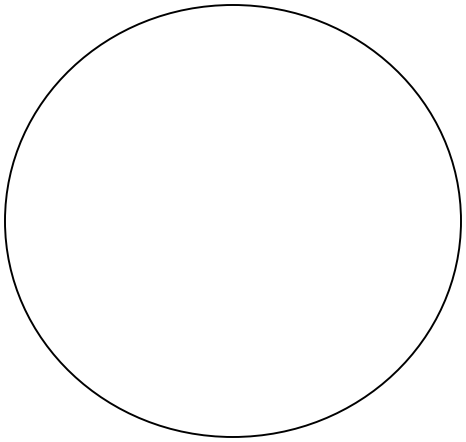
On a clean slide, make a wax circle for each colony type. Sometimes it is easier to make the circles if you warm the slide by putting it on the staining hot plate. The object of the circle on the slide is twofold. First, the circle serves to contain the specimen, so it doesn't fall off the slide if it is a liquid specimen. Second, the circle will help you focus your slide.



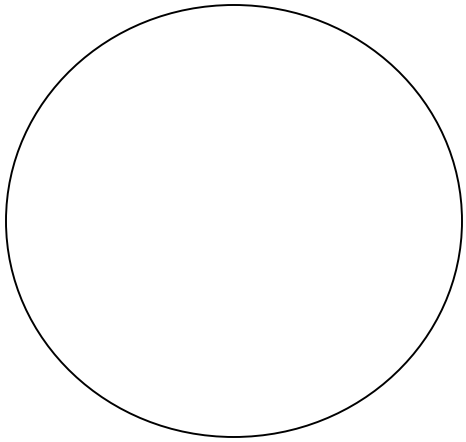
Label your slide on the frosted area with pencil; you can use colony description, i.e. 1. White , 2. Grey. You can use a small drop of distilled water in each well. This will make a smoother suspension of the bacteria but it is time consuming. After you become good at gram staining you may want to skip the water. Then with a sterile toothpick touch colony #1 and spread it in well #1. Repeat with colony #2. Remember how many cocci fit on the diameter of a period. Don't over do it or your slide will be too thick and hard to read.

b. Viewing the Gram Stain.

Look at your gram stains at 100x and then at 1000x magnifications. Draw what you see under oil immersion.



1000X
Colony #1 (White)



1000X
Colony #2 (Grey)

State which colony is *Staphylococcus aureus* and which is *Escherichia coli.*

Grey colony: _____

White colony: _____

Assignement due next week:

Make a table to present the following information: media name (list will be provided by instructor), media type (selective, differential, supportive), differential agents, selective or inhibiting agents, organisms grown, organisms inhibited, color changes if any.