Exercise 14
THE GRAM POSITIVE COCCI
Part 1

INTRODUCTION:

Student learning objectives: Upon completion of this exercise students will be able to:

a. Successfully set up the following tests: novobiocin disc, optochin disc, bacitracin disc, CAMP test, bile esculin agar, salt broth, catalase, slide coagulase.
b. Demonstrate understanding of each test in the write up.
c. Describe the key tests, typical gram stain morphology and colony morphology of Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus saprophyticus. (Family Album)
d. List the chief test (and principle) used to identify Staphylococcus saprophyticus.
e. List and describe the principle of the test that differentiates the genus Staphylococcus from Streptococcus.
f. Name the three types of hemolytic reactions of the streptococci and list one species of each.
g. Describe the typical gram stain and colony morphology of *Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus pneumoniae*, alpha strep. viridans, Group D Non-enterococcus and *Enterococcus* species.
h. Provide the underlying principle for each of the following tests commonly used to differentiate the beta-hemolytic streptococci: Bacitracin "A" disk, CAMP test.
i. List tests to differentiate specifically between the following pairs of streptococci: Streptococcus pyogenes (Group A) from Streptococcus agalactiae (Group B), Group D Non- enterococcus from Enterococcus species.
j. List the test commonly used to differentiate the alpha-hemolytic viridans streptococci from Streptococcus pneumoniae.
k. Provide the underlying principle for each of the following biochemical tests that are commonly used for the initial workup of alpha or gamma hemolytic streptococci: NaCl tolerance, Bile Esculin hydrolysis, and Optochin susceptibility.
l. Correctly draw an identification flowchart for the Staphylococci and Streptococci. (Family Tree)

Activities for Day 1:

- Perform Exercises #1 through #6.

- Read the material in your text regarding *Staphylococcus* species and *Streptococcus* nomenclature (Lancefield Grouping, other nomenclature)

Materials

Work in groups of 4 per table. This is a group learning activity.
Each group needs the following

Mueller Hinton Agar (MH) plate
Blood Agar Plates
Bile Esculin Agar Slants
6.5% NaCl Broth
Antibiotic impregnated paper discs of the following:
Bacitracin, Novobiocin, Optochin
3% hydrogen peroxide
Rabbit plasma for coagulase test
Clean microscope slides
Sterile cotton swabs
Sterile saline tube
Loop
Sterile toothpicks
Forceps
Slant cultures of:
Staphylococcus aureus (also *S. aureus* strain for the CAMP test)
Staphylococcus epidermidis
Staphylococcus saprophyticus
Streptococcus pyogenes
Streptococcus agalactiae
Enterococcus faecalis
Group D Non-enterococcus *(S. bovis, S. equinus)*
Streptococcus pneumoniae
Alpha Strep. viridans

Review lab exercise instructions and the material in the Atlas.

a. Exercises marked with ► are to be done as a group; one set per table. For best results, the whole team should do each test. If you assign each team member one test, you will be done much faster. However, YOU ARE RESPONSIBLE TO KNOW HOW TO SET UP EACH TEST. You will be setting these tests up individually for the identification of your unknown, so you need to know the procedure, purpose, principle, results, and conclusion for each test. You are building your reference manual. You are learning how to use the different identification tools. Later on, when you are working on your unknown, you will use your lab notebook to help you set up and interpret needed tests. Don't just go through the motions! UNDERSTAND THE REASONSING BEHIND EACH TEST. Ask yourself: Why am I doing this? What will this test identify? How will I use it?

b. YOUR WORK MUST BE YOUR OWN AND BE IN YOUR OWN WORDS.

c. Unless otherwise noted all exercises are incubated at 37^0 C.

d. Example Laboratory Exercise Report Format.

Date:
Title/Exercise #: Done for you.
Lab Procedure: Done for you.
Purpose: Describe what the purpose of this test is. Why do we do this test?
Principle: Describe what the test is about. What is it detecting and by what mechanism?
Results: Describe what you observed- example: The tube turned from pink to yellow, turbidity, growth or no growth, etc.
Conclusion: Based on your results, state your conclusion-example: *Staphylococcus aureus* ferments mannitol, *Staphylococcus epidermidis* does not.
Further Notes and Discussion: If there were any unexpected results you can discuss them here.

How do I label my culture plate (Petri dish)?

Print legibly on the **BOTTOM** side of the plate toward the outer edges and not center, using a black or blue Sharpie pen the following information:

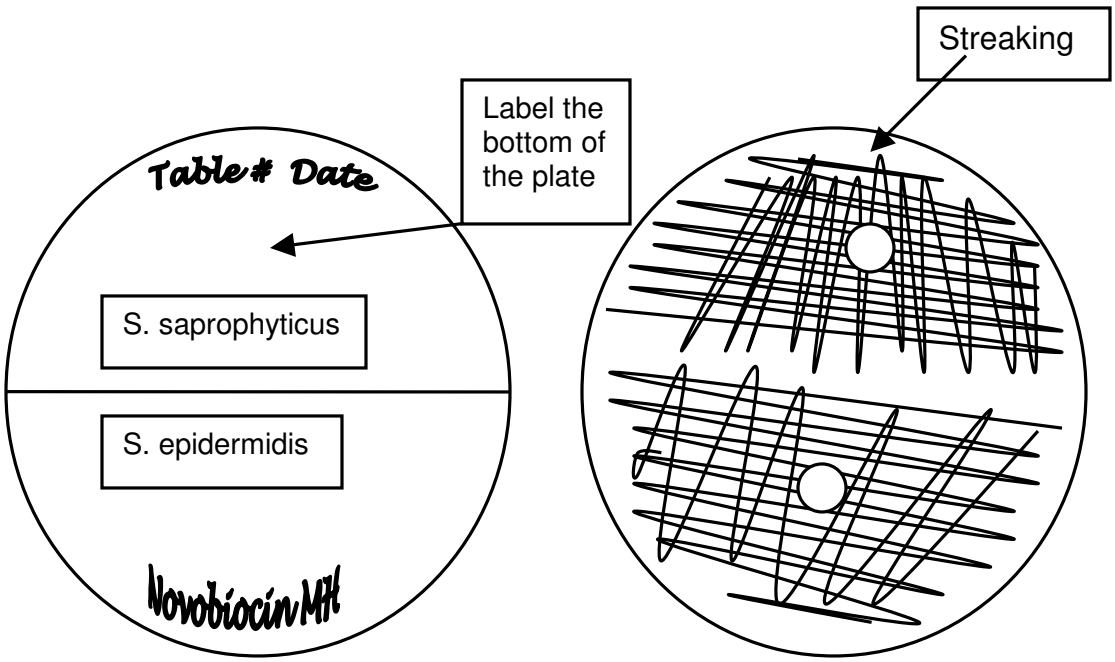
1. Name, lab section, table number
2. Date of inoculation
3. Type of plate (MC, MSA, DNA, etc.)
4. Other pertinent info

Date:
Title: ►Exercise #1 Novobiocin Disc.

Lab Procedure:

Pause to recall what you have learned about labeling plates and inoculating them.

a. Obtain a **Mueller Hinton (MH)** agar plate. We put our name or table number, the date, and what test we are doing. Since the plate looks similar to other plates, we also write the name of the plate. For example, the Mueller Hinton looks exactly like the Tryptic Soy Agar plate. In this case, we will be comparing two organisms and their results, so it will be efficient to mark the bottom of the plate with a line down the middle, thus dividing the plate into two sections, one for each organism.

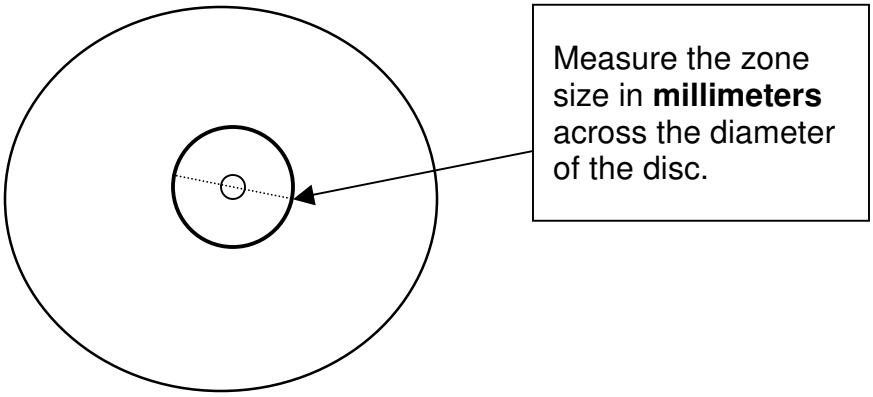


b. On one half write: ***S.saprophyticus*** and on the other ***S. epidermidis***. You will make a **lawn** of each bacterium in the appropriate section. A lawn (also known as **confluent growth**) in microbiology is made by streaking a **<u>small</u>** amount of bacteria onto a plate in one direction with a sterile loop or swab, and then turning the plate a quarter of a turn and streaking in the same direction. In this way we are covering the area completely with bacteria.

Be careful not to overlap your organisms. Keep them separate.

c. In the center of each organism area, place a **Novobiocin disc** using a sterile forceps (tweezers). You can sterilize your forceps by **briefly** passing it through the Bunsen burner flame and wait until they have cooled. You may also flame them using alcohol if available. Grab a disc with your forceps and place it onto the lawn. GENTLY press the disc down to ensure contact. Repeat this procedure with the other organism.

d. Incubate this plate along with all your other plates. It is helpful to use tape to keep all your plates together so that tomorrow you will find them easily. After incubation, measure the zone of inhibition of your discs with a ruler.



Susceptible: A zone of inhibition of >16 millimeters (mm.) indicates the organism is susceptible.

Resistant: A zone of inhibition of<16 mm indicates a resistant result.

Purpose:

Principle:

Date:

Results:

Conclusion:

Further Notes and Discussion (if applicable):

Date:

Title: ►Exercise #2 Optochin Disc.

Lab Procedure:
Label a blood agar plate and divide it into 2 sections. Streak a lawn of ***Streptococcus pneumoniae*** onto one half and **Alpha strep viridans** onto the other. Apply an **Optochin disc** onto the center of each section as previously described. Incubate. Measure zones around the discs.

Susceptible: A zone of inhibition of ≥14 mm.
Resistant: A zone of inhibition of< 14 mm.

Purpose:

Principle:

Date:

Results:

Conclusion:

Further Notes and Discussion (if applicable):

Date:

Title: ►Exercise # 3 Bacitracin Disc.

Lab Procedure:
Label a blood agar plate and divide it into 2 sections. Make a lawn of ***Streptococcus agalactiae*** and ***Streptococcus pyogenes*** with only **ONE COLONY** onto its respective section. **DO NOT OVERINOCULATE!!!** Apply a Bacitracin disc onto the center of each section. Incubate. Read. **Do not confuse hemolysis with inhibition.**

Positive reaction- any zone of inhibition (no growth) around the disc.
Negative reaction-no zone of inhibition, the organism grows up to the disc.

Purpose:

Principle:

Date:

Results:

Conclusion:

Further Notes and Discussion (if applicable):

Date:
Title: ►Exercise #4 CAMP Test.

Lab Procedure:
Label a **Blood Agar Plate (BAP).** Using the loop, make a central stripe of ***Staphylococcus aureus* for CAMP** down the middle of the plate. Flame your loop, and then, at a 90˚ angle and starting 10 millimeters **(1 cm)** away from the Staph stripe, make a stripe of ***Streptococcus agalactiae***. Flame the loop and do the same with **Alpha Strep. viridans**. Incubate. Read plates.

> **Positive reaction-**Arrowhead of enhanced beta hemolysis where both zones of hemolysis meet.
> **Negative reaction-**No arrowhead of enhanced beta hemolysis.

Purpose:

Principle:

Date:

Results:

Conclusion:

Further Notes and Discussion (if applicable):

Date:

Title: ►Exercise #5 Bile Esculin Agar.

Lab Procedure:
Label three **Bile Esculin Agar (BEA)** slants. Inoculate by **fishtailing** one slant with ***Enterococcus*** *species,* another with **Group D non-enterococcus**, and finally one with **Alpha Strep viridans**. Incubate. Read tubes.

Positive reaction- Black color throughout slant
Negative reaction-No black color

Purpose:

Principle:

Date:

Results:

Conclusion:

Further Notes and Discussion (if applicable):

Date:
Title: ► Exercise #6 Salt Broth.

Lab Procedure:
Label three **Salt Broth (NaCl)** tubes. Inoculate one with ***Enterococcus*** *species,* another with **Group D non-enterococcus**, and finally one with **Alpha Strep. viridans**. Incubate 24-48 hours. (If negative at 24 hrs. re-incubate.) Read tubes.

Positive reaction- Turbid (cannot see through liquid).
Negative reaction- No growth, clear.

Purpose:

Principle:

Date:

Results:

Conclusion:

Further Notes and Discussion (if applicable):

THE GRAM POSITIVE COCCI
Part 2

Activities for day 2:

1. Record results of exercises #1-6. Complete the write-ups for day one. Are there any unexpected results?.

2. Perform Exercises # 7 & 8.

Date:
Title: Exercise #7 Catalase Test.

Lab Procedure:
Test a **Staphylococcus** species and a **Streptococcus** species for the enzyme **catalase.** Using a sterile toothpick, apply a small amount of bacteria onto a clean microscope slide. Add a drop of hydrogen peroxide to each. A positive reaction is indicated by the formation of bubbles.

Be careful not to transfer any blood from the Blood Agar Plate onto the slide, since this may cause a False Positive reaction.

Purpose:

Principle:

Results:

Conclusion:

Further Notes and Discussion (if applicable):

Date:
Title: ► Exercise #8 Slide Coagulase

Lab Procedure:
Test two ***Staphylococcus*** species, ***Staphylococcus aureus*** and ***Staphylococcus epidermidis*** for the enzyme **Coagulase** by preparing a heavy, uniform suspension of bacteria in a small drop of distilled water on a slide. Using a sterile transfer pipette, add a drop of rabbit plasma, without the pipette tip touching the slide or bacterial suspension. Mix briefly with a sterile toothpick, and observe for clumping within 10 seconds. Do not over mix, as it will make the clumps smaller.

Purpose:

Principle:

Results:

Conclusion:

Further Notes and Discussion (if applicable):

3. Family Album and Family Tree

a. Instructions:

One of the ways we use at home to remember our ancestors is the family album. Another method is our story telling. In this class, we will use both methods to remember and keep track of our bacteria. We are organizing our album by types of organisms, just as we can organize our pictures by subject or time.

Every time we study a new organism; write its name, defining characteristics – Colony morphology, results of key tests, and gram stain morphology. **Review what we have already learned about colony morphology descriptions and viewing organisms under a microscope**

Remember, you may have this organism later on as your UNKNOWN. The Family Album and Family Trees will help you study and identify your unknown

Begin your Family Album by describing and gram staining the staphylococci and streptococci. You can put 3 different organisms per slide, each in their own wax ring.

Part of a family album consists of a family tree. Using the tests we have performed, construct a flowchart (Family Tree). Show the differentiation between the members (Genera and species).

Cultures: *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus pneumoniae, Streptococcus agalactiae, Streptococcus pyogenes, Enterococcus species,* Alpha strep viridans, and Group D non-enterococcus.

THE GRAM POSITIVE COCCI: STAPHYLOCOCCI FAMILY ALBUM
COLONY MORPHOLOGY AND STAINING

Organism name	
Gram Stain/Morphology	
Colony Morphology	
Key Identifying Tests	

Organism name	
Gram Stain/Morphology	
Colony Morphology	
Key Identifying Tests	

Organism name	
Gram Stain/Morphology	
Colony Morphology	
Key Identifying Tests	

“STAPHYLOCOCCI FAMILY TREE”

THE GRAM POSITIVE COCCI: STREPTOCOCCI FAMILY ALBUM
COLONY MORPHOLOGY AND STAINING

Organism name	
Gram Stain/Morphology	
Colony Morphology	
Key Identifying Tests	

Organism name	
Gram Stain/Morphology	
Colony Morphology	
Key Identifying Tests	

Organism name	
Gram Stain/Morphology	
Colony Morphology	
Key Identifying Tests	

THE GRAM POSITIVE COCCI: STREPTOCOCCI FAMILY ALBUM
COLONY MORPHOLOGY AND STAINING (Continued)

Organism name	
Gram Stain/Morphology	
Colony Morphology	
Key Identifying Tests	

Organism name	
Gram Stain/Morphology	
Colony Morphology	
Key Identifying Tests	

Organism name	
Gram Stain/Morphology	
Colony Morphology	
Key Identifying Tests	

“STREPTOCOCCI FAMILY TREE”