Learning Objectives

The student will

- Use aseptic techniques in the safe inoculation of various forms of media.
- Follow oral and written instructions and manage time in the lab efficiently.
- Apply correct terminology regarding microbiological techniques when making observations.
- Use the bright field light microscope to view microbes under oil immersion, make accurate observations and appropriate interpretations and store the microscope according to lab procedures.
- Properly prepare a bacterial smear for accurate staining and describe the chemical basis for simple staining and negative staining.
- Accurately perform a Gram stain and describe the molecular basis for the technique.
- Correctly perform various inoculation techniques including the quadrant streak and the T streak techniques and describe each technique’s purpose.
- Make accurate observations and appropriate interpretations of biochemical test results and use them in the identification of potentially disease causing microbes.

Background/Theory

In this exercise, you will be sampling your throat and observing the types of colonies produced on a blood agar plate. In another exercise, you will have the opportunity to test a colony from your plate for some of the disease causing organisms in the genus *Streptococcus*.

The genus *Streptococcus* includes important pathogens that are categorized in serological Lancefield groups based on the distinguishing characteristics of their surface carbohydrates. The most clinically important streptococcal species in humans is *S. pyogenes*, also known as Group A Streptococcus (GAS). *S. pyogenes* causes a wide variety of diseases not only in the skin, but in other organ systems as well. Examples of diseases elsewhere in the body include pharyngitis and scarlet fever. *S. pyogenes* produces a variety of extracellular enzymes, including streptolysins O and S, hyaluronidase, and streptokinase. These enzymes can aid in transmission and contribute to the inflammatory response. *S. pyogenes* also produces a capsule and M protein, a streptococcal cell wall protein. These virulence factors help the bacteria to avoid phagocytosis while provoking a substantial immune response that contributes to symptoms associated with streptococcal infections. (OpenStax CNX, 2018)

Your normal throat flora will include a variety of organisms. Normal body flora plays an important role in keeping you healthy. Your resident microbes will compete with potential pathogens for resources keeping pathogen growth in check. For this reason, some students will carry pathogenic strains, but never get sick themselves.

Experiment/Exercise

**Materials per student pair**

- 2 TSA w/ 5% SB (Blood Agar) plates
- 1 pack of cotton swabs (2 swabs/pack)
- Tongue depressors, sterile
Cultures
None

Procedure Lab 1
1. Label one plate with your name (your name only) and the rest of the items required. If you wish, you can draw the T pattern on the bottom of the plate to be used as a guide.
2. Tear open the swab package at the end opposite the cotton tip. Leave swabs in the package until needed.
3. Your partner will remove one of the swabs and sample the pharyngeal region of your throat. Tongue depressors are available if needed. Take care to avoid the tongue while swabbing.
4. Take the swab from your partner and use it to inoculate the first area of the streak. Rotate the swab to create maximum contact between the swab and the plate surface.
5. Place the swab and tongue depressor in the bench top disposal container immediately after use. DO NOT set either on the bench top.
6. Continue the T-streak with a sterile inoculating loop. Be sure to incinerate the loop between areas and after you are finished.
7. Use two small pieces of tape to secure the lid to the bottom of the place.
8. Reverse roles so that your partner can inoculate their plate with a sample from their own throat.
9. Place both plates for incubation at 37°C for 48 hours.

Procedure Lab 2
1. Observe three different colonies from your plate without removing the lid. Record the information in the Colony Observations table.
2. Choose one of the colonies to Gram stain. Make a smear on one side of a slide. Remember you are taking a sample from solid medium, so you will need to place a loopful of water on the slide first. The other side of the slide should have a smear of the mixed culture control broth. You will not need a loopful of water on this side. Allow to air dry, heat fix and then stain it.
3. As always, locate the control side under oil immersion and verify your Gram staining technique first. An instructor must see both rods and cocci and then initial your data table.
4. Once you know your technique is good, make observations of your sample cells in the data table Microscopic Observations. Be sure your drawing conforms to the guidelines in the SSt exercise.
5. If you have some good beta hemolytic colonies, you may wish to use your throat culture to test in the Latex Agglutination test. If not, dispose of your plate properly.
Lab Report: Title

Name ______________________________
Lab Section __________

Data and Observations
Colony Observations

<table>
<thead>
<tr>
<th>colony</th>
<th>Sketches (top and side views)</th>
<th>Size and color</th>
<th>Form, margin and elevation</th>
<th>Appearance of medium</th>
<th>Type of hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony type 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colony type 2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Colony type 3</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Microscopic Observations

<table>
<thead>
<tr>
<th>Source</th>
<th>color</th>
<th>Gram Rxn</th>
<th>Drawing</th>
<th>Morphology and Arrangement</th>
<th>instr initials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed culture control rods</td>
<td></td>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Mixed culture control cocci</td>
<td></td>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Throat Culture sample</td>
<td></td>
<td></td>
<td></td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>
**Post Lab Questions**

1. If your control smear has purple rods and purple cocci, what conclusions can you draw about your throat sample cells if they are also purple cocci?

2. Why are you only testing a beta hemolytic colony with the Agglutination test? (You may need to read the ImLA exercise for this answer.)
References