Laboratory Exercise # 5: Morphological Study and Simple Stain

Purpose:

The student will study macroscopic growth of bacteria and prepare a simple stain of the bacteria for observation.

Introduction:

In order for bacteria to be seen macroscopically it is allowed to grow on media within a petri dish. As the bacteria replicate and divide they eventually produce a colony that can be seen visually with the naked eye. These colonies can be described as to their pigment, elevation, margin, hemolysis, etc. Each colony is approximately several hundred bacterial cells.

To be able to describe the individual bacterial cell a smear must be made of the colony. Since bacteria are almost colorless and show little contrast a stain is applied so they can be seen more easily. Dyes or stains can be categorized as either cationic or anionic. If the colored portion of the stain carries a positive ion it is called a **Cationic dye** (examples: methylene blue, crystal violet, or safranin). If the colored portion of the stain carries a negative ion it is called **Anionic dye** (example: nigrosin). The bacterial cell wall carries a negative charge within it and therefore will attract the positive ion of a cationic dye more readily then it will an anionic dye.

Materials:

- Ubiquitous plates from Lab exercise # 4
- Glass slides
- Safranin stain
- Crystal violet stain
- Inoculating loop
- Bunsen burner
- Wax marker

Procedure:

Examine your ubiquitous plates from exercise # 4. Using the “Colonial Description” sheet provided, describe two colonial morphologies present. This is recorded on the Morphological Study Sheet.

1. Make a smear of the colony using the following technique:

2. Using a wax marker, make a circle of wax on one side of a glass slide. Flip the slide over so the wax circle is on the bottom.

3. Place a small drop of water within the wax circle.
4. Aseptically remove a small portion of the colony with your inoculating loop and mix it in the drop of water. Spread it out well so it will air dry more quickly.

5. Let the smear air dry.

6. Heat fix the smear by running it through the flame of the bunsen burner twice. The back of the slide should be warm to the touch, not hot!

7. Let the smear cool completely.

8. Apply crystal violet or safranin to the smear for one minute.

9. Rinse the slide gently with water from the squirt bottle.

10. Blot the smear dry with bibulous paper.

11. Observe the smear on 10X objective first. Make sure the wax circle is in the down position on the stage. Focus on the wax marking, then move into the circle and focus again.

12. Rotate the 10X objective out of the way and place a drop of oil on the slide. **Don’t move the course adjustment during this step!!**

13. Rotate the 100X objective into the oil. Now focus with the fine adjustment.

14. Record on the data sheet the following:
   - Morphology: cocci, bacilli, spirillum
   - Arrangement:
     - For cocci: single, diplo, tetrads, strepto, or staphylo
     - For bacilli: stacked, chained or irregular
     - For spirillum: single or irregular

15. Save the smear to it can be turned in. **Please blot the slide once with a paper towel to remove the oil. Do not rub as it will remove the smear!!**

16. Label the slide using a stick on label. Include the following on the label: student name and smear #. **Place the label on the same side of the slide as the smear is on!**

17. Turn in the report sheet, your two smears and the ubiquitous plate that the colonies came from for your grade.
Morphological Study

(Worth 10 points)

Using your ubiquitous plates to describe colonial morphology.

**Morphology Colony Type # 1: _______________________

  - Form _______________________
  - Elevation _______________________
  - Surface Appearance _______________________
  - Edge or Margin _______________________
  - Pigment _______________________
  - Simple Stain: _______________________
    - Morphology _______________________
    - Arrangement _______________________

**Morphology Colony Type # 2: _______________________

  - Form _______________________
  - Elevation _______________________
  - Surface Appearance _______________________
  - Edge or Margin _______________________
  - Pigment _______________________
  - Simple Stain: _______________________
    - Morphology _______________________
    - Arrangement _______________________

In order for your grade to be determined, the student should turn in plate with colonies that are being described and the simple stain of each colony.
## Colonial Morphology Chart

<table>
<thead>
<tr>
<th>Form</th>
<th>Punctiform Circular &lt; 1 mm diameter</th>
<th>Circular &gt; 1 mm Diameter</th>
<th>Irregular Not circular or Punctiform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevation</td>
<td>Flat</td>
<td>Raised</td>
<td>Convex</td>
</tr>
<tr>
<td>Surface Appearance</td>
<td>Smooth</td>
<td>Rough</td>
<td>Concentrically Ridged</td>
</tr>
<tr>
<td>Margin</td>
<td>Entire</td>
<td>Undulate</td>
<td>Erose</td>
</tr>
<tr>
<td>Chromogenesis (Examples)</td>
<td>Red</td>
<td>Yellow</td>
<td>Cream</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Green</td>
</tr>
</tbody>
</table>