Lab Exercise 6: The smear and simple staining

OBJECTIVES

1. Prepare bacterial smears for the microscopic visualization of bacteria.
2. Perform a simple staining procedure.
3. Compare the shapes and arrangements of bacterial cells.

INTRODUCTION

Smears
Much of your success in staining microbes will come from preparing a good smear. In doing so, you must keep in mind three key goals. First, you want to adhere the cells to the slide so that they are not washed off in subsequent procedures. Second, in doing so, you do not want to overheat the smear so that the cells shrink and you are left with distortion and artifacts. Third, you need to prepare a thin smear, because the thickness will determine whether or not you can visualize individual cells, their arrangement, or details regarding gram-reaction or internal structure.

Simple staining
The use of a single stain or dye to color a bacterium is called a simple stain. These types of dyes, called basic dyes, are positively charged, containing cationic chromophores. Because the bacterial cells are slightly negatively charged, there is an attraction between the positive dye and the negative cell. Some common basic dyes used in staining are methylene blue, crystal violet, and basic fuchsin.

However, not all dyes are positively charged. Acidic dyes are negatively charged, containing anionic chromophores, and do not stain bacterial cells. They are usually used for negative staining protocols, and you will get experience with this in future labs. Common acidic dyes are nigrosin, India ink, and eosin.

Today, you will stain your environmental sample as well as an avirulent strain of Corynebacterium diphtheriae, which is the species that causes the serious disease, diphtheria. You will want to notice the following features of C. diphtheriae:

Pleomorphism: This refers to the irregular shapes of C. diphtheriae. It is usually a Rod shaped organism, but can appear club-shaped, needle-shaped or spermlike.
**Palisade arrangement:** This refers to the parallel arrangement of rod-shaped cells. It is sometimes referred to as the “picket fence” arrangement and is common to the genus *Corynebacterium*.

**LAB EXERCISES**

**I. The smear**

<table>
<thead>
<tr>
<th>Table supplies</th>
<th>Individual supplies</th>
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<tbody>
<tr>
<td>Solid and liquid cultures of <em>Corynebacterium diptheriae</em></td>
<td>Body broth cultures from Lab 5</td>
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<tr>
<td></td>
<td>Glass microscope slides</td>
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<td>Inoculating loop</td>
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**Protocol:**
1. Prepare lab bench by removing extraneous items and cleaning surface with table disinfectant.
2. Wipe slide clean with paper towel or kimwipe.
3. Draw a circle on the bottom of the slide to indicate area of smear.
4. Transfer culture to top of slide:
   - **From a solid:**
     1. Place one drop of water from your loop on the center of the circle
     2. Transfer a *small* amount of solid inoculum into the drop of water and disperse thoroughly.
     3. Spread both into a thin area about the size of a nickel
     4. Allow smear to air-dry.
   - **From a liquid:**
     1. Place one to two loopfuls of liquid suspension on the center of the circle.
     2. Spread the suspension into a thin area about the size of a nickel.
     3. Allow smear to air-dry.
5. When the smear is *completely* dry, heat-fix by quickly passing the smear over the flame two to three times.
6. You should make *at least* one smear from a solid inoculum and one smear from a liquid inoculum.

**II. Simple stain**

<table>
<thead>
<tr>
<th>Individual supplies</th>
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<tbody>
<tr>
<td>Heat-fixed slides (see Step I above)</td>
</tr>
<tr>
<td>Methylene blue dye</td>
</tr>
<tr>
<td>Staining tray</td>
</tr>
<tr>
<td>Water bottle</td>
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<tr>
<td>Bibulous paper</td>
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</table>
**Protocol:**
1. Place slide on staining tray and cover smear with methylene blue dye for 1 minute.
2. Gently wash the smear with water to remove excess stain.
3. Use bibulous paper, blot dry but do not wipe the slide.
4. Examine all stained slides under 100x oil immersion lens.

**DATA AND OBSERVATIONS**
1. Draw observations of cells from your body broth/environmental sample and of *C. diphtheriae*.

\[
\text{Total magnification} \quad \underline{_______} \quad \text{Total magnification} \quad \underline{_______}
\]

**DISCUSSION**
1. How does smear preparation of cells from a liquid medium differ from preparation of cells from a solid medium?

2. Why is it important to limit the quantity of cells used to prepare a smear?

3. For preparation of a smear on a slide, what is the purpose of heat fixation? What problems can arise when the slide is heated in a flame?

4. What causes a stain to adhere to bacterial cells? Why are all colored dyes not necessarily useful for simple staining?