Staining and Preparing Slides of Archaeal Cells

Archaeal cells are prokaryotes and thus are very small and difficult to see without staining. The staining procedure we will use is a simple stain, not unlike what you used to stain your cheek cells. It is also necessary to use the highest power on the light microscope with what is called an oil immersion lens. This lens is designed to allow an unbroken layer of oil to be between the lens and microscope slide, essentially closing the gap between the two. This improves magnification and resolution by eliminating these two refractory surfaces. First, we will prepare and stain our cells. Then we will examine them using the oil immersion lens. See Figure 2-3 below.

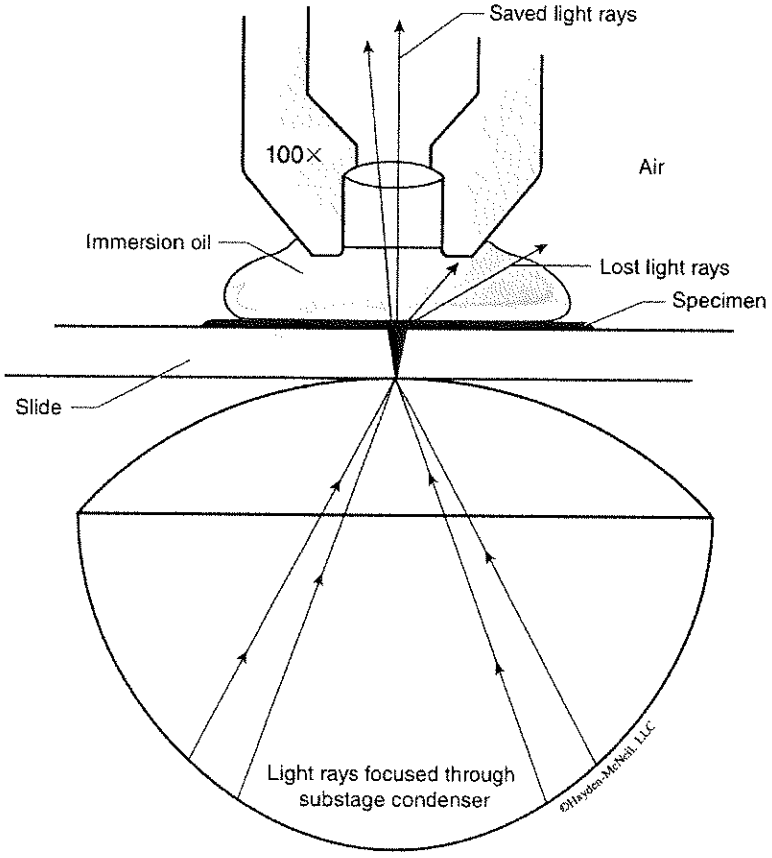


Figure 2-3. How an oil immersion lens works

Procedure C: Simple Staining of Archaeal Cells

1. Obtain a new glass slide from the side counter.
2. Sterilize an inoculating loop by placing it in a flame (from an alcohol burner) until the loop turns bright red. After removing it from the flame, allow a few seconds for the loop to cool.
3. Using the sterile inoculating loop, place a drop of distilled water in the center of the slide.

4. Using the sterile inoculating loop, touch only the tip into the archaeal colonies on the agar surface (if you transfer too many cells, the stained smear will be so dark that you will not be able to see individual cells). Place the tip in the water droplet on the slide; use circular movements to spread the cell suspension over an area equal to that of your thumbnail.
5. Allow the smear to *air-dry*. Do *not* speed up this process by blowing on the slide.
6. When the smear is completely dry, pass the slide 2–3 times over the flame of an alcohol burner to fix the cells to the glass surface. Use the clothespin so that you do not burn yourself.
7. The archaeal cell smear is ready for staining. Go to the staining area and perform the following steps.
8. Holding the slide at one end with a clothespin, completely add a few drops of *either* **crystal violet, methylene blue**, or **carbol fuchsin** to cover the smear. Allow the crystal violet to react 1 min; methylene blue 1 min; and carbol fuchsin 15 sec.
9. Wash excess stain from the smear by holding the slide under running tap water. Keep the slide *parallel to the stream* so that the water will not remove cells.
10. Blot, do not rub, the washed slide dry with bibulous paper.
11. Examine the slide using a compound microscope. After focusing on lower, medium, and high power, have your TA show you how to use the oil immersion lens.
12. Describe the morphology (shape) and arrangement of the archaeal cells.

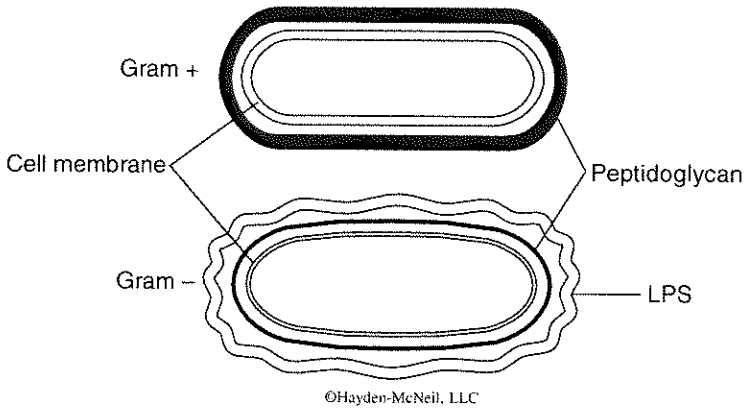


Figure 2-4. *Gram-positive vs. Gram-negative bacteria*

Staining and Preparing Slides of Bacterial Cells

The chemical composition of the cell walls of bacteria can be a major factor in determining both pathogenicity (disease-causing capacity) and the choice of an antibiotic that may be used. The chemical composition of the cell wall also determines reactivity with specific stains.

The capacity of the bacterial cell wall to bind with stain molecules provides a means for separating these organisms into groups. Among a mixture of many species of bacteria, the cell walls typically react differently when stains are applied. This difference is readily observed in the color of the cell wall, either purple or red. With this procedure, we can differentiate among groups of bacteria. We call this procedure **differential staining**. We will perform differential staining by using the reagents of the **Gram stain**. This procedure is one of the fundamental tools employed by the microbiologist in the identification of bacterial species. Cells are classified as **Gram-positive (+)** if they stain **purple**. They are classified as **Gram-negative (−)** if they stain **red**. Gram-positive cells have simpler walls. Among pathogenic (disease-causing) bacteria, Gram-negative species are generally more toxic; this toxicity is, in large part, a function of the complexity of the cell wall (see Figure 2-4).

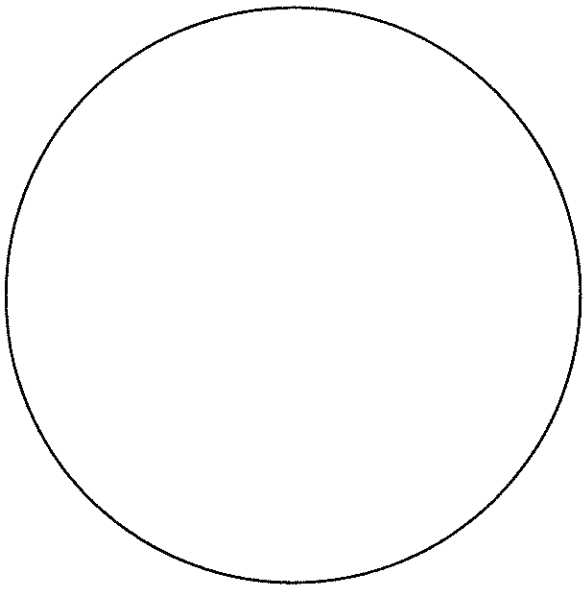
The reagents of the Gram stain are:

- Crystal violet (primary stain that appears purple)
- Gram's iodine (intensifies the color of the crystal violet)
- Ethanol [95%] (decolorizer)
- Safranin (counterstain that appears red)

Procedure D: Gram Staining of Eubacterial Cells

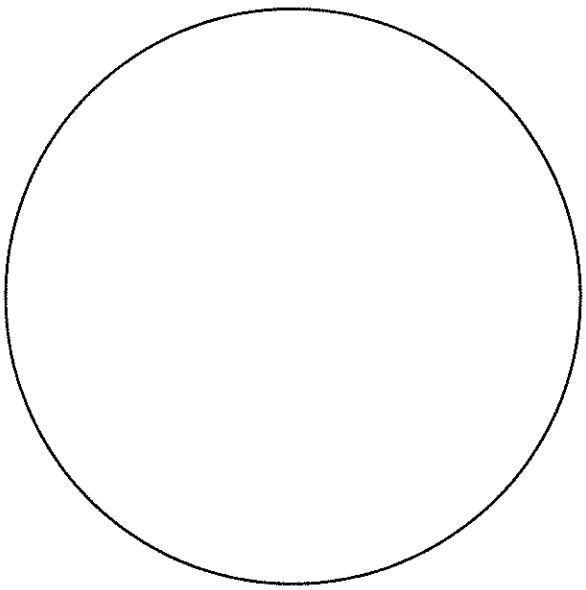
1. Prepare a smear of bacterial cells from one of the available cultures. Use the same techniques as those used in preparing the archaeal cells in Activity 1C steps 1–7.
2. After the smear has been heat fixed, go to the staining area and perform the following steps.
3. Holding the slide at one end with a clothespin, completely cover the smear with crystal violet.
4. Allow the stain to react for 1 minute, and then wash gently with tap water.
5. Now add 2–3 drops of Gram's iodine to the smear. Allow it to react for 1 minute, and then rinse gently with tap water.
6. Carefully apply 95% ethanol drop by drop from a pipette. Stop when the alcohol washing from the smear no longer has any purplish color (one to two drops usually). **Do not remove all of the stain!**
7. Rinse the smear gently with tap water.
8. Add 2–3 drops of safranin to the smear. Allow the counterstain to react for 45 seconds. Gently rinse with tap water and blot dry with bibulous paper. **Do not wipe the slide.**

9. Examine the slide using a compound microscope. Use the oil immersion lens for highest magnification. Illustrate and describe your observations below. *In particular, what differences did you observe between the Gram-positive and Gram-negative bacteria?*



Gram-negative bacteria

Scientific name:_____



Gram-positive bacteria

Scientific name:_____