Preparation of a Bacterial Smear and the Simple Stain Technique

Most bacteria have no color, so they generate little contrast in the microscope field. Therefore, to see bacteria with the microscope, it is necessary to apply color by using a staining reagent. Once stained, the bacteria may be observed and studied with respect to their shape, size, and arrangement.

The preparation of a stained bacterial smear involves several steps. First, bacteria are placed on a glass slide and "fixed" with heat, partly to ensure that they remain attached to the glass. Then, for simple staining, a **basic** stain is applied. Such a stain carries a positive electrical charge. The negatively charged surface and cytoplasm of bacteria attracts the stain, and staining takes place. In this exercise, we shall explore the proper methods for simple staining. The technique is called the **simple stain technique** because only a single stain is used. Other staining techniques are explained in Exercises 5, 6, and 7.

This exercise also will demonstrate the important techniques for handling bacterial cultures. Each is a "pure culture;" it contains only one species of bacterium. The bacteria have been cultivated in a liquid growth medium such as nutrient broth or on a solid growth medium such as nutrient agar. Your instructor will specify the medium used.

A. Preparation of a Bacterial Smear

PURPOSE: to prepare a bacterial sample for staining.

A **bacterial smear** is a thin layer of bacteria placed on a slide for staining. Preparing the smear requires attention to a number of details that help prevent contamination of the culture and ensure safety to the preparer. Each step in this procedure has an important reason, and each should be followed carefully as a prerequisite to successful work.

Special Materials

- Pure cultures of selected bacterial species

Procedure

1. Begin this exercise by disinfecting the laboratory desk with the available disinfectant as directed by the instructor. Then place a clean sheet of paper toweling or other absorbent material over the area to be used. Should a spill occur, the paper will absorb the liquid and can be disposed of as directed by the instructor.
2. Wash a glass slide with soap, rinse it well, and dry it thoroughly, preferably with a lint-free cloth. With a wax marking pencil or felt marker, mark off areas of the slide where smears will be placed. One area should be reserved for a **slide label.** Some laboratory workers prefer to use the underside of the slide for marking to prevent wax from mixing with the smear. Others use the top because the wax barrier holds the dye in that area and prevents spreading.
3. Obtain cultures of the bacteria, and place the tubes in a suitable rack on the bench. Sterilize your inoculating loop. If agar slant cultures are used, place a loopful of water into one area of the slide. If broth cultures are used, no additional liquid is necessary. Light the Bunsen burner. The smear is now ready to be prepared. Review the transfer procedure outlined in Exercise 1.

When lighting the Bunsen burner for use in the lab be sure to face it away from you.

4. Referring to **Figure 1A**, firmly hold the loop as you would hold a pencil, and place it nearly vertically into the blue tip portion of a Bunsen burner flame for a few seconds or until it glows red hot. Allow the loop to cool several seconds.
5. Take the bacterial culture in the opposite hand, remove the cap as shown in Figure 1B. Place the tip of the tube in the flame for a few seconds (Figure 1C). This will burn off any dust or lint and kill any airborne organisms that might happen to fall into the tube.
6. Insert the loop into the tube, and touch it very gently to the main portion of the slant (Figure 1D). Be careful not to dig into the medium or scrape it. If a broth medium is used, dip the loop carefully into the liquid. Remove the loop, reflame the tip of the tube briefly (Figure 1E), then replace the cap (Figure 1F), and return the culture to the rack on the desk.
7. Mix the loopful of bacteria with the loopful of water on the slide (Figure 1G), and swirl the liquid out to the area of a dime. For broth cultures, simply swirl the loopful of bacteria on the marked slide area. Complete the procedure by reflaming the loop (Figure 1H). You now have a bacterial smear.
8. Prepare other smears in the remaining spaces with different bacteria as directed by the instructor. It is important that you become comfortable with the methods for handling cultures and preparing smears since they are essential elements of laboratory microbiology. Your instructor may recommend other sources to be examined, as specified in the next part of this exercise, Part B, step 6. All smears on one slide should be prepared before proceeding with the next step.

Quick Procedure
Smear Preparation

1. Label a clean microscope slide.
2. Sterilize the inoculating loop.
3. Remove a bacterial or broth sample.
4. Smear on slide area.
5. Air dry.
6. Heat fix.

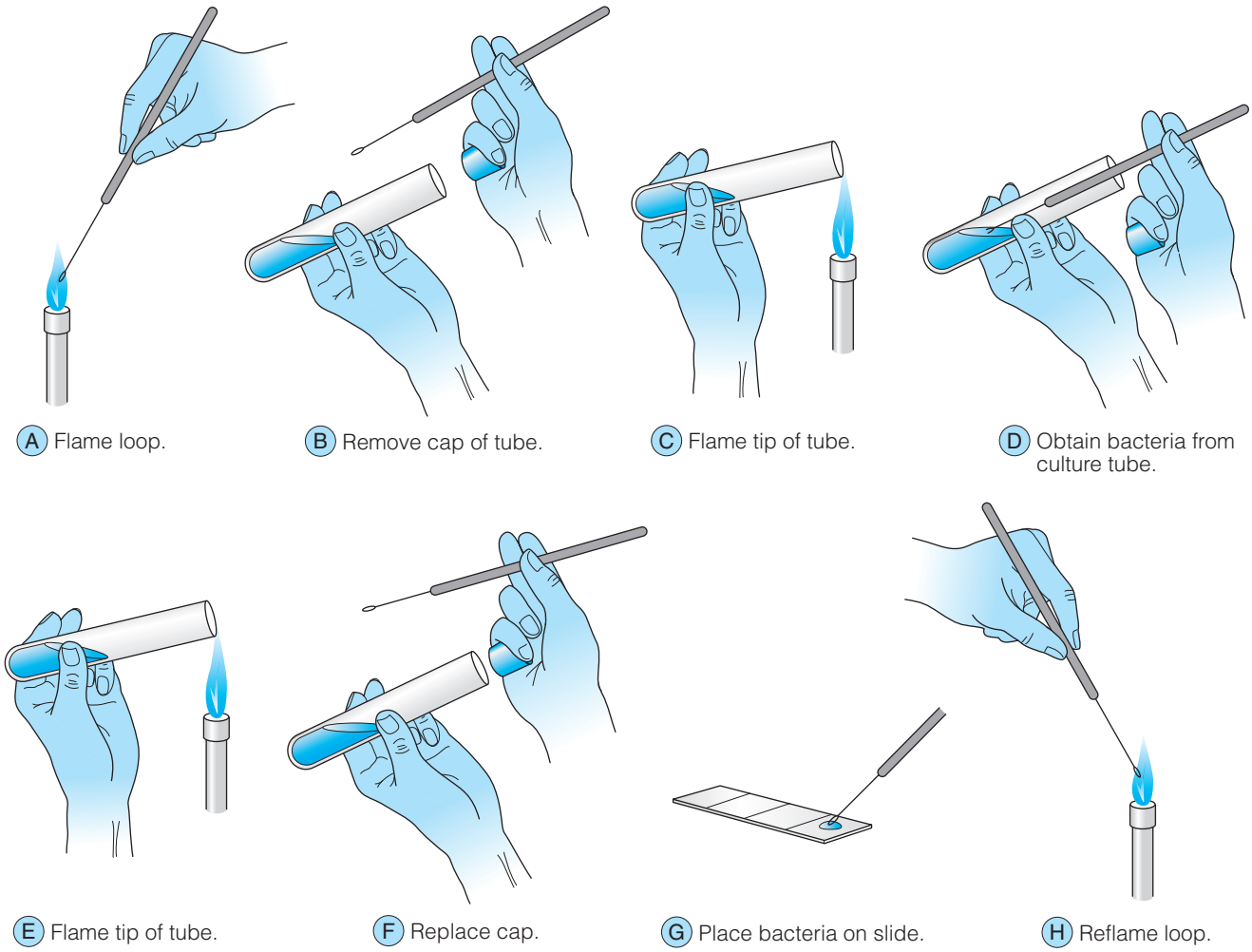


FIGURE 1
Important steps in the preparation of a bacterial smear.

9. **Air-dry** the slide until all the liquid evaporates. An electric warming tray may be used for this purpose. Caution should be taken to avoid extreme heat because cell distortion and splattering may occur.
10. **Heat-fix** the smears by quickly passing the slide through the Bunsen burner flame three times (**Figure 2**). The heat should contact the underside

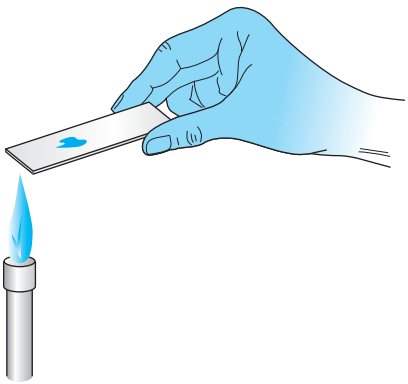


FIGURE 2
A slide is heat-fixed by passing it quickly over a flame several times.

of the slide. This procedure 1) kills any bacteria that may still be alive, 2) facilitates stain penetration, and 3) fixes cells to the slide so that they do not wash off when stained. The slide is now ready to be stained.

B. The Simple Stain Technique

PURPOSE: to determine the size, shape, and arrangement of bacterial cells.

The **simple stain technique** is a rapid and effective way of preparing a bacterial smear for viewing. It is a one-step procedure in which the smear is covered with stain and allowed to sit undisturbed for a minute or so, during which the bacterial cytoplasm chemically unites with the basic stain. The remaining stain is then washed away.

Special Materials

- Basic stains such as crystal violet, methylene blue, and safranin
- Air-dried, heat-fixed bacterial smears
- Wash bottle

Procedure

Quick Procedure
Simple Stain

1. Stain heat-fixed slide for 1 min with basic stain.
2. Wash; dry; observe.

1. Prepare air-dried, heat-fixed smears of bacterial species as outlined in Part A. One or more smears may be prepared on a slide and stained together.
2. At the staining rack or staining tray, cover the smears with the selected basic stain, and let the preparation remain undisturbed for one minute. The instructor may modify these directions for individual situations.
3. Gently wash off the excess stain with a gentle stream of water by holding the slide parallel to the stream. Then blot the slide with absorbent paper or between the pages of a blotting (bibulous) paper booklet (**Figure 3**). Do not wipe the slide. The slide is now ready for examination.
4. To orient yourself, observe the slide with the low power objective and then the high power objective. No drawings should be made yet. Now place a drop of immersion oil directly on the smear and switch to the oil immersion (100x) objective, as described in Exercise 3. After locating the bacterial cells, thoroughly scan the smear, looking for a very thin layer of bacteria apart from other clumps and masses.

   Determine the shape, size, and arrangement of the bacterial cells. When you are confident of your observations, use a pencil with a sharp point to prepare drawings of the bacteria under oil immersion in the Results section of this exercise. Usually about three dozen cells per drawing will suffice. Include with the drawing the full binomial name of the organisms, as well as the total magnification and stain used.
5. When your slide work is completed, the oil may be removed from the slide by gently blotting in the blotting paper booklet. If the slide is to be retained, label with a slide label containing the names of the organisms, the stain, your name, and the date. Avoid placing the slide label in your mouth for moistening purposes.

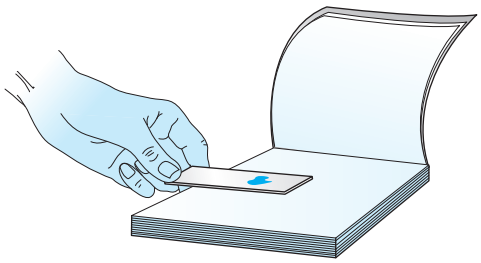


FIGURE 3
A booklet of bibulous (blotting) paper is used to dry the slide after it has been prepared.

6. Additional smears may be prepared of tooth-gum scrapings, a sample of yogurt or sour cream, a hay infusion, a yeast suspension, water from a bird bath, a soil sample, or other specimens supplied in the laboratory. Add your drawings in the Results section.
7. At the conclusion of the laboratory period, lens paper should be used to wipe the microscope objectives; the oil immersion objective should be wiped last. The microscope should be stored with the low power objective in position, the stage lowered, and the cord wound neatly around the base or arm.

Questions

1. Identify the steps taken in the preparation of a bacterial smear to prevent contamination of the culture and the preparer of the smear.
2. Summarize the fundamental theory of simple staining.
3. Judge the result of placing a large drop of water on the slide instead of a loopful.
4. Discuss the possible outcomes if an air-dried smear were not heat-fixed before proceeding with the staining step.
5. When you observe your stained smear at 1000X, you see two different shaped bacteria; some are rod shaped while others are spherical. Explain this result considering the original source culture was pure.

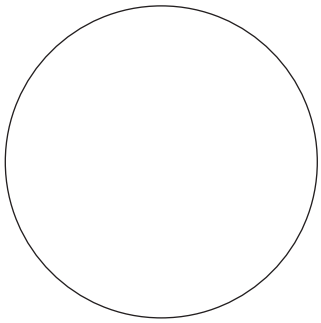
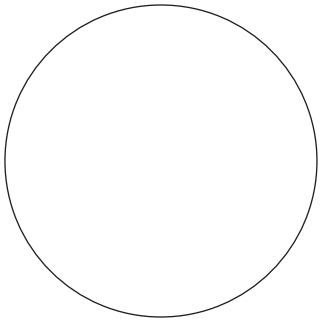
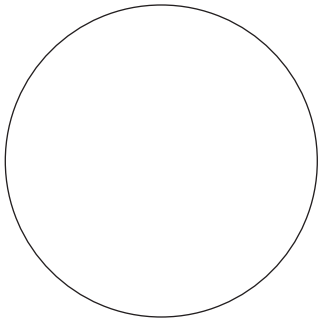
Name _____

Date _____ Section _____

Exercise Results

Preparation of a Bacterial Smear and the Simple Stain Technique

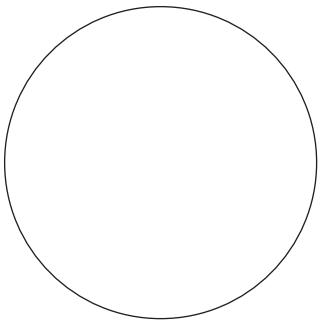
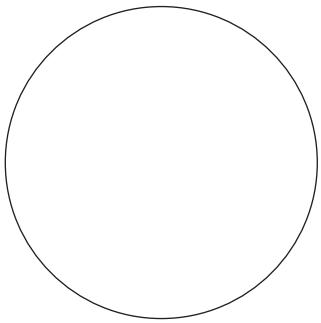
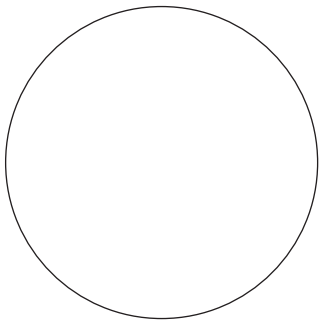
B. The Simple Stain Technique

Organism: _____ _____ _____

Stain: _____ _____ _____

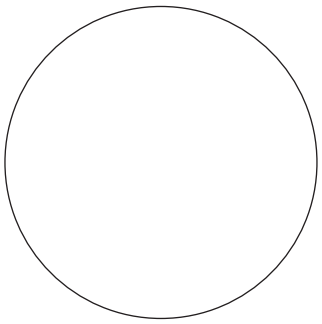
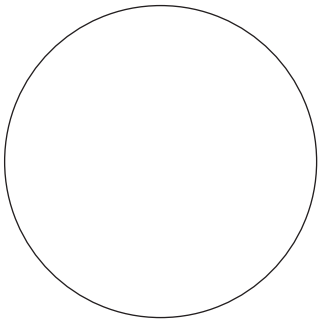
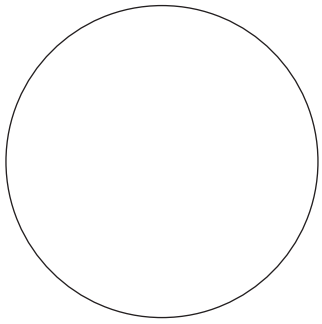
Magnif.: _____ _____ _____

Organism: _____ _____ _____

Stain: _____ _____ _____

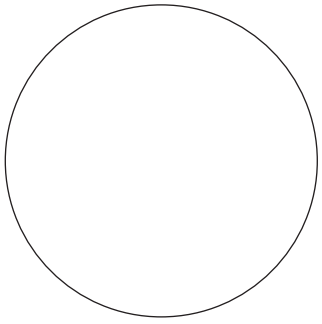
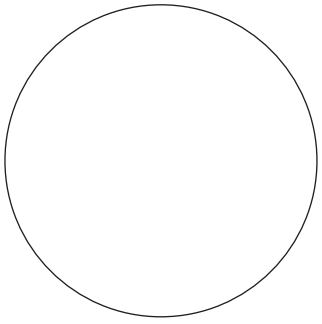
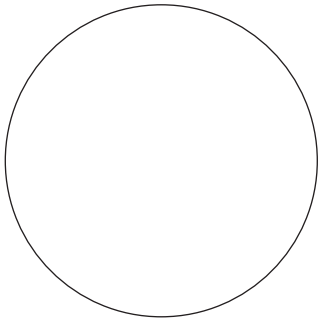
Magnif.: _____ _____ _____

Organism: _____ _____ _____

Stain: _____ _____ _____

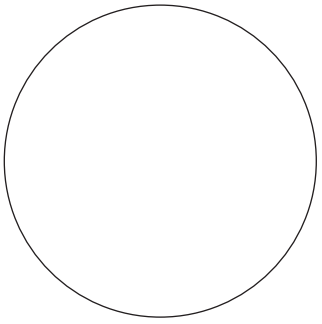
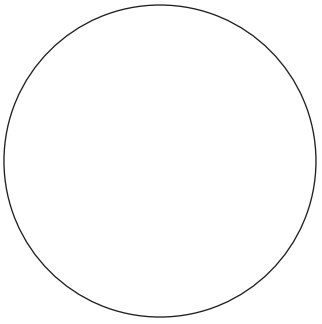
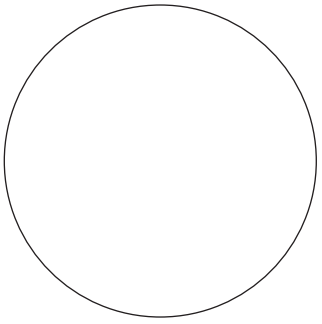
Magnif.: _____ _____ _____

Organism: _____ _____ _____

Stain: _____ _____ _____

Magnif.: _____ _____ _____

Organism: _____ _____ _____

Stain: _____ _____ _____

Magnif.: _____ _____ _____

Observations and Conclusions: _____

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