

E X E R C I S E

7

Smear Preparation

OBJECTIVES

At the conclusion of the exercise, you should...

1. be able to make smears from culture broths and agar.
2. understand the difference between broths and agar.
3. understand the different uses of agar slants and plates.
4. understand the different uses of broth cultures.
5. know why the slide is air dried and heat fixed before staining.

INTRODUCTION

Bacterial cultures are grown in broths (liquid media) and on agar (solid media). Each type of growth has different uses for further studies of bacteria. These uses will be discussed in this exercise and used in later exercises. In this exercise, you will learn how to transfer bacteria from broths and agar to microscope slides to be used for the staining exercises.

MATERIALS

Cultures:

Agar slants, plates, and broths of various bacteria to be used in the staining exercises

Supplies:

Microscope slides with frosted ends

Pencil (lead)

Marking pen (China marker, Sharpie, or Gram Stain pen)

Inoculating loop

Inoculating needle

Clothespins (for holding slides)

Technical Background

Bacteria are grown in broth cultures to yield large numbers of organisms in a small space for further studies. Agar slants are also used to grow bacteria in large numbers for further studies and for transport. Agar slants can be stored for long periods of time. Bacteria are grown on agar plates to observe colony morphology and to check for purity. To make smears, bacteria are air dried on a microscope slide and then heat fixed to kill the bacteria and to adhere the bacteria to the slide so it does not wash off during the staining process.

PROCEDURES

Steps to Make a Smear:

From broth:

1. Label a microscope slide on the frosted end using a pencil.
2. Make a dime-sized circle on the underside of the slide (in the center) with the Gram Stain pen (or grease pencil).
3. Follow the aseptic technique described earlier (Ex. #6) for flaming the loop and transferring bacteria from a test tube.
4. Pick up a loopful of bacteria from the broth tube.
5. Transfer the loopful of bacteria onto the slide, within the marked circled area.
6. Spread the bacteria by moving the loop in a circular pattern. Flame the loop!
7. Allow the slide to completely air dry.
8. Heat fix the slide by holding it with a clothes pin and passing it quickly through the top of the Bunsen burner flame two or three times

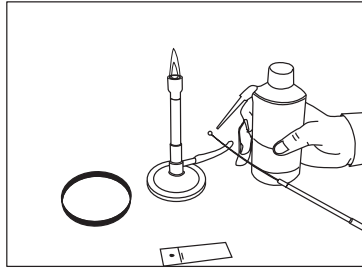
From agar (See Figures on next page):

1. Label another microscope slide on the frosted end with a pencil.
2. Make a circle on the underside of the slide (in the center) with the marking pen, as above.
3. Place a small drop of water on the slide within the circle.
4. Using the needle or the loop, pick up a barely visible amount of bacteria from the agar slant or plate.
5. Mix the bacteria into the drop of water.
6. Spread the bacteria by moving the loop in a circular pattern. Flame the needle or loop!
7. Allow the slide to completely air dry.
8. Heat fix the slide by holding it with a clothes pin and passing it quickly through the top of the Bunsen burner flame two or three times

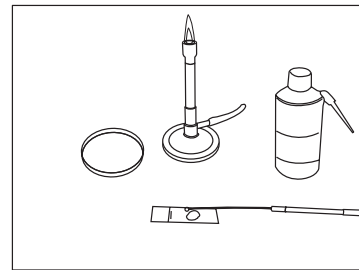
Note: Follow the instructions in the staining exercises (#8-10) once you have air dried and heat fixed your smears.

CAUTION: It is tempting to speed up the drying of the smear by heating the slide over the Bunsen burner flame. Do not do this! Heating bacteria in the presence of water seriously distorts them, and your results will be difficult to interpret. Allow the smears to dry completely (i.e., no sign of moisture) before heat fixing. Once heat fixed, the smears can be stored for later use.

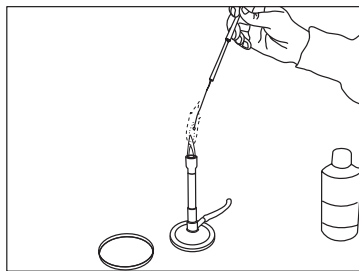
SMEAR PREPARATION PROCEDURE



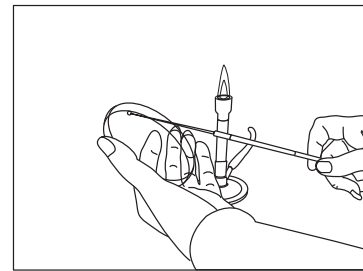
1. After flaming and cooling the loop, add a drop of water to the loop



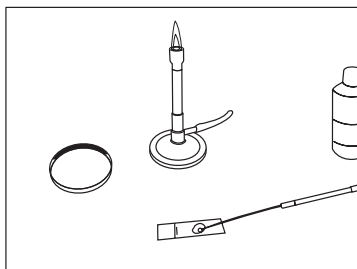
2. Draw a circle on the bottom of the slide, and place the water droplet on the microscope slide in the area of the circle



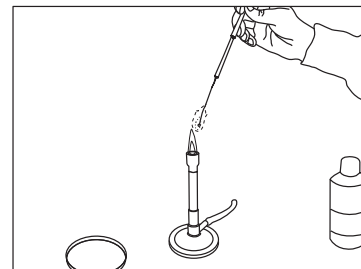
3. Flame and cool the loop



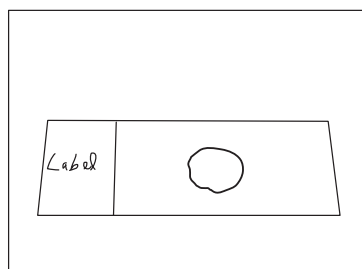
4. Pick up a small amount of culture from an isolated colony on a plate



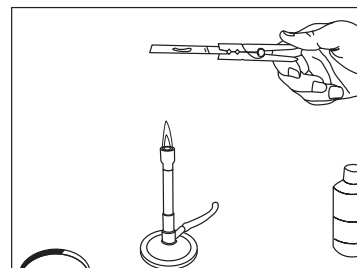
5. Spread the loop of bacteria in the water droplet in the area of the circle



6. Flame and cool the loop



7. Air dry the slide



8. Heat fix the slide by passing it over the top of the flame a few times

EVALUATION OF RESULTS (EXERCISE 7: SMEAR PREPARATION)

Purpose

Data

This exercise demonstrates the first steps for preparing slides to be stained; therefore, data from this exercise will be entered in subsequent staining exercises.

CONCLUSIONS, DISCUSSIONS, AND QUESTIONS

1. List two reasons for heat fixing the bacteria on a smear.
2. Discuss the advantages and disadvantages of using broths and agar for making smears.
3. Why would the inoculating needle be better than the inoculating loop for making a smear from agar?

