# SIMPLE BACTERIOLOGICAL STAINS

Cytoplasm is transparent and, as such, makes viewing cells with the light microscope difficult without the aid of stains. Staining makes cells more visible so that size, shape and arrangement may be determined more easily. In this set of exercises, you will learn how to correctly prepare a bacterial smear for staining and how to perform simple and negative stains.

Exercise 3–3

## Preparing a Bacterial Smear

### MATERIALS

Bacterial culture (as assigned)
Clean glass microscope slides
Inoculating loop or needle

### PROTOCOL

1. A bacterial smear is made prior to most staining procedures. Figures 3-5a through 3-5d illustrate the procedure for preparing a bacterial smear.
2. Follow with the appropriate staining procedure.

### PRECAUTIONS

⚠ Do not use too much water in preparing the slide. This will prolong the air drying step.

⚠ Do not over-inoculate the smear. When dry, it should be barely visible as a film on the glass.

⚠ Although air drying is the most time-consuming step in the procedure, resist the temptation to wave the slide, blow on it, or heat it to speed up the drying process, as contaminating aerosols may result.

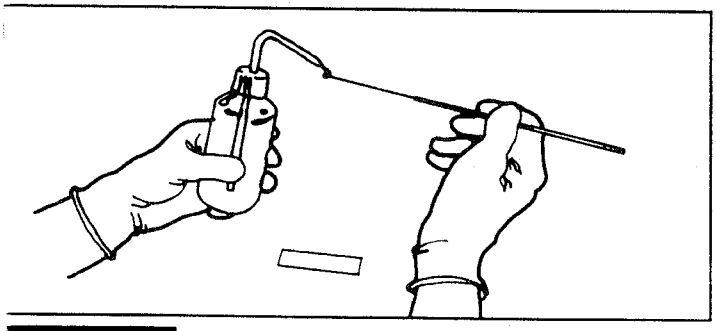

**FIGURE 3-5a Begin With Water**
Capture a drop of water with your inoculating loop. If your specimen is growing in broth, you may omit the water drop and continue with step 3-5c.

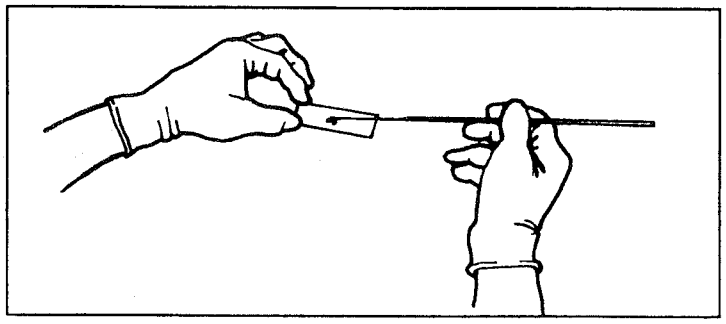

**FIGURE 3-5b Place the Water on the Slide**
Transfer the water drop to the center of a clean slide. Avoid using too much water.

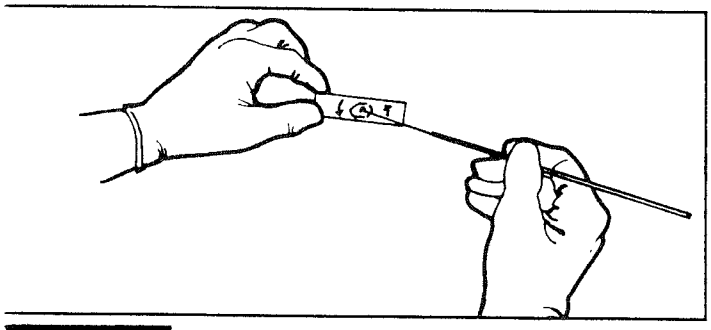

**FIGURE 3-5c Transfer the Organisms to the Slide**
Use your loop to aseptically transfer the cells to the water drop. Avoid excessive inoculation as thick smears are more difficult to stain consistently and individual cells may be difficult to find.) Then, without "springing" your loop, gently *emulsify* (mix) the cells in the drop. As you do so, spread the drop out over the slide's surface so it will air dry more quickly. The slide must be completely dry before continuing.

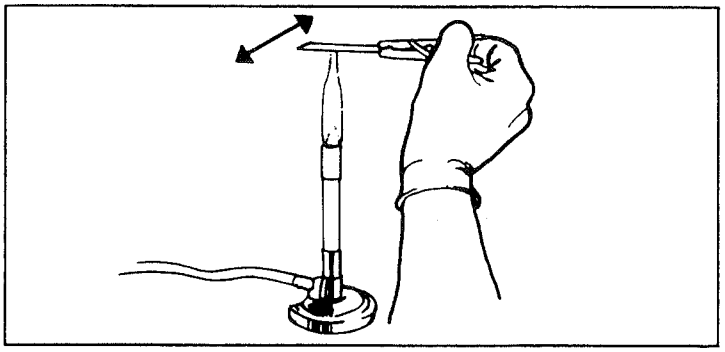

**FIGURE 3-5d Heat-Fix the Slide**
Once the drop has air dried, use a slide holder and pass the slide through the upper part of a flame two or three times to heat-fix the smear. Heat-fixing the dried smear makes the cells adhere to the slide, kills them, and makes them more easily stained as protein becomes coagulated. Do not overheat the slide as aerosols may be produced.

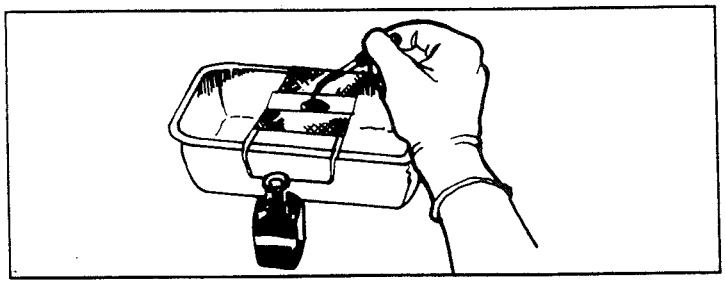

**FIGURE 3-6a Flood the Smear with Stain**
Place your slide with its smear on the staining rack. Flood the smear with stain for the correct length of time. Hold the slide with a slide holder to minimize staining of your hands. Wearing latex gloves is also a good idea.

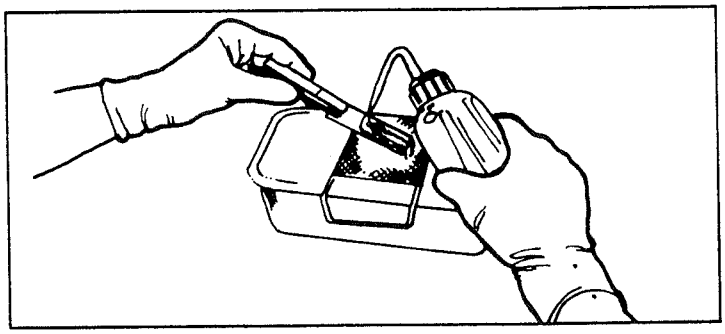

**FIGURE 3-6b Rinse with Water**
Tilt the slide to an angle of 45°. Direct a stream of water towards the top of the slide and allow the water to run down across the smear's surface. Continue washing until the runoff is clear.

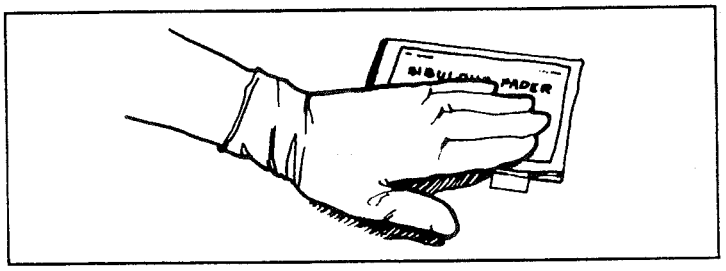

**FIGURE 3-6c Blot Dry**
Blot (do not wipe) the slide dry in a tablet of bibulous paper. Then observe the specimen using the oil immersion lens.

## PRECAUTIONS

⚠ Remember that consistency in preparation produces consistent results. Once you have learned proper smear technique and cell density to achieve optimum staining, stick with it.

⚠ When rinsing the slide, avoid spraying the water directly on the smear as this may dislodge your specimens.

⚠ Dispose of the specimen slides in a jar of disinfectant after use.

## REFERENCES

Murray, R.G.E., Raymond N. Doetsch and C.F. Robinow. 1994. Page 27 in *Methods for General and Molecular Bacteriology*, edited by Philipp Gerhardt, R.G.E. Murray, Willis A. Wood, and Noel R. Krieg. American Society for Microbiology, Washington, D.C.

Norris, J. R. and Helen Swain. 1971. Chapter II in *Methods in Microbiology, Volume 5A*, edited by J. R. Norris and D. W. Ribbons. Academic Press, Ltd., London.