## Exercise 3.1 – Smear Preparation

LEARNING OUTCOMES

1. List the steps of preparing a smear for staining.
2. State the purpose of air-drying and heat-fixing a slide.

Many simple and differential staining techniques begin with a *smear.* A smear is prepared by adding bacteria from solid media to a drop of water on a glass slide or adding a loopful of bacterial broth to a slide directly (Figure 3.3). The smear is allowed to air dry completely, after which it is heat-fixed using an incinerator or Bunsen burner. Heat-fixing adheres cells to the glass, preventing them from washing away during the staining procedure. Because heat-fixing also kills bacteria, smears can be stored and stained later.

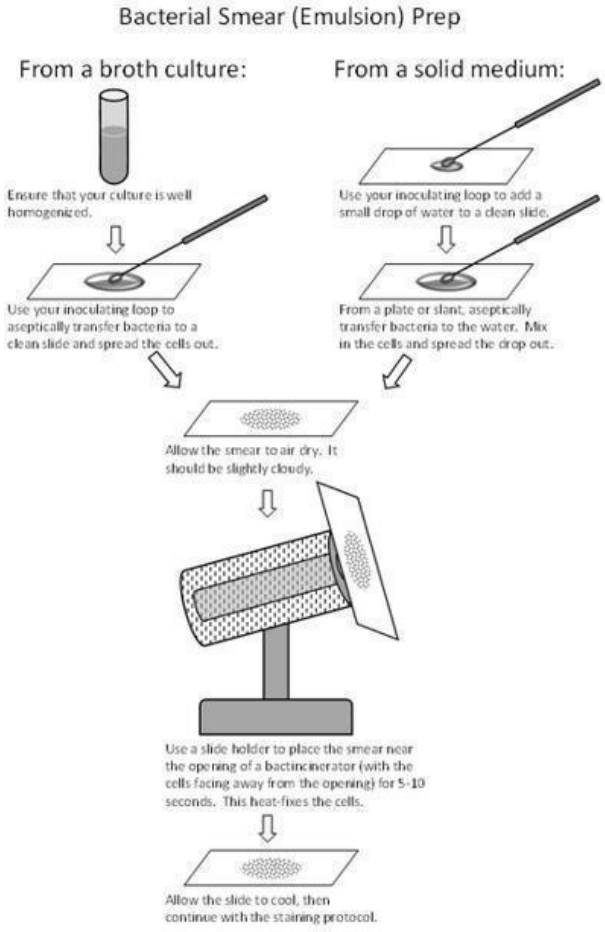


*Figure 3.3. Preparation of a bacterial smear*

## Exercise 3.1 – Smear Preparation

***NOTE: To best manage time, prepare smears first and then follow-up Module 2 exercises while smears are air-drying.***

OBJECTIVE

Prepare heat-fixed smears for simple, Gram, and acid-fast staining in the next lab.

MATERIALS

- CULTURES: Slants of *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Mycobacterium smegmatis,* and an unknown sample (U)
- SOLUTIONS: Deionized water in small dropper bottles
- EQUIPMENT: Inoculating loop, incinerator, glass slides, Sta-Clear paper or Kimwipes, clothespin or slide holder, slide box, pencil

PROCEDURE – STUDENTS WORK IN PAIRS

1. Obtain seven glass slides and wipe both sides with Sta-Clear paper.
2. Place slides directly on the lab bench, not on paper or a paper towel, and use a pencil to label the frosted edge of the glass with your initials, stain, and culture number:

   SS = Simple stain, two slides *B. cereus* and *S. aureus*
   GS = Gram stain, three slides *E. coli, S. aureus,* and an unknown sample (U)
   AF = Acid fast stain, two slides *M. smegmatis* and *S. aureus*
3. Add a small drop of water to the first slide.
4. Using aseptic technique, obtain bacteria on the inoculating loop and close the culture tube.
5. Mix the loop in the drop of water, spreading it out over the slide surface to facilitate drying.
6. Allow the smear to air-dry completely.
7. Repeat steps 4 through 7 for remaining slides.
8. Once slides are completely dry, use a clothespin or slide holder to hold the **back** of the slide (e.g., side of the slide without cells) against the incinerator opening for 10 seconds.

   ***TO AVOID AN AEROSOL, SLIDES MUST BE COMPLETELY DRY BEFORE HEAT FIXING.***
9. Heat-fixed smears are ready to stain or may be stored in a slide box to stain in the future.