SLIDE PREPARATION AND STAINING

Preparing a Wet Mount

This procedure is used to look at live microorganisms.

1. Place a loopful of water onto a microscope slide using an inoculating loop.
2. Flame the loop, cool it, then touch a colony with the loop.
3. Put the edge of the loop that has the bacteria on it into the drop of water and gently mix the bacteria from the loop into the water.
4. Flame the loop to kill the remaining bacteria.
5. Take a cover slip and hold it by the sides at an angle next to the water drop.
6. Gently release the cover slip so that it falls over the drop.
7. The slide is now ready to view.

Preparing a bacterial smear slide for staining

This procedure kills the bacteria and fixes them to the slide so they won't be washed off during the staining procedure.

1. Place a loopful of water onto a microscope slide using an inoculating loop.
2. Flame the loop, cool it, then touch a colony with the loop.
3. Put the loop with the bacteria on it in the drop of water and gently mix the bacteria from the loop into the water. Using circular motions, spread the drop out into a thin layer on the slide.
4. Flame the loop to kill the remaining bacteria.
5. Leave the slide on your bench until all the liquid has evaporated. If you don't wait until the slide is dry, your bacteria will boil in the liquid and explode - sounds fun but there is nothing to see afterwards.
6. Heat fix (to kill and attach the bacteria to the slide) by passing the slide through a flame 3 times, smear side up. Be careful not to burn your fingers.

Simple Stains

The primary purpose of simple staining procedures is to provide contrast between the specimen and the background (slide). Although the bacteria are no longer alive, they are much easier to see than with a wet mount, because they are colored by the dye. Direct stains use dye salts that are positively charged and adhere to the negatively charged molecules in the cells.

1. Prepare a bacterial smear slide.
2. Place the slide smear side up in the staining tray.
3. Cover the smear completely with the dye solution. Methylene Blue and Crystal Violet are always available in the lab.
4. Tilt the slide at a 45 degree angle. Wear gloves or use a clothes pin to hold the slide at this step. Using a squirt bottle of water, direct the stream of water above the smear (not directly on it) and gently rinse the slide.
5. Drain off excess water by touching the edge of the slide to a paper towel. You can gently blot the slide on a paper towel too.
6. The slide is now ready to view.

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Gram stain (a differential stain)

The Gram stain is a classical technique of microbiology used to classify Gram-negative and Gram-positive bacteria. Two stains are used. Crystal violet is applied first to colorize all cells. Gram's iodine acts as a mordant (mordants increase the affinity of the dye for the object to be dyed) and, when added to the specimen, leads to the formation of a crystal violet-iodine complex within the bacterial cell wall. All cells will be colored purple at this point. Alcohol is added next as the decolorizer. The alcohol solubilizes the lipids in the outer membrane of gram negative bacteria, so that the crystal violet diffuses away and the cells are decolorized. In contrast, crystal violet is retained in the Gram-positive bacteria, which lack an outer membrane and have a thick peptidoglycan layer. A counter stain, safranin, is then used to colorize the Gram-negative cells. At the end of the procedure, Gram-negative cells will be red, and Gram-positive cells will be purple.

1. Prepare a bacterial smear slide.
2. Place the slide smear side up in the staining tray.
3. Cover the smear completely with Crystal Violet and let stain for 30 seconds.
4. Tilt the slide at a 45 degree angle. Wear gloves or use a clothes pin to hold the slide at this step. Using a squirt bottle of water, direct the stream of water above the smear (not directly on it) and gently rinse the slide.
5. Drain off excess water by touching the edge of the slide to a paper towel.
6. Put the slide back in the staining tray and cover the smear with Gram's iodine. Leave the iodine on the slide for 30 seconds, then rinse as described in step 4.
7. Put the slide back in the staining tray and cover the smear with alcohol for ~10 seconds. Immediately rinse with water as described above. If you leave the alcohol on for too long, you will decolorize the Gram-positive cells along with the Gram-negative cells, and all the bacteria will be red at the end of the procedure.
8. Put the slide back in the staining tray and cover the smear with safranin. Leave the stain on the slide for 1 minute. Rinse with water as in step 4.
9. Drain off excess water by touching the edge of the slide to a paper towel. You can gently blot the slide on a paper towel too.
10. The slide is now ready to view.

We will provide Gram-negative and Gram-positive bacteria that you can use to practice your staining technique before you test your pet microbe.
If you would like to try other stains, just ask and we will try to provide them.

adapted from http://openwetware.org/wiki/BISC209/S11:_Stains