SMEAR PREPARATION

The preparation of a smear is required for many laboratory procedures, including the Gram-stain. The purpose of making a smear is to fix the bacteria onto the slide and to prevent the sample from being lost during a staining procedure. A smear can be prepared from a solid or broth medium. Below are some guidelines for preparing a smear for a Gram-stain.

1. Place one needle of solid bacterial growth or two loops of liquid bacterial growth in the center of a clean slide.

2. If working from a solid medium, add one drop (and only one drop) of water to your specimen with a water bottle. If using a broth medium, do not add the water.

3. Now, with your inoculating loop, mix the specimen with the water completely and spread the mixture out to cover about half of the total slide area.

4. Place the slide on a slide warmer and wait for it to dry. The smear is now ready for the staining procedure.
Gram-staining Procedure

Gram-staining is a four part procedure which uses certain dyes to make a bacterial cell stand out against against its background. The specimen should be mounted and fixed on a slide before you proceed to stain it. The reagents you will need to successfully perform this operation are:

- Crystal Violet (the Primary Stain)
- Iodine Solution (the Mordant)
- Decolorizer (ethanol is a good choice)
- Safranin (the Counterstain)
- Water (preferably in a squirt bottle)

Before starting, make sure that all reagents, as well as the squirt-bottle of water, are easily accessible because you won't have time to go get them during the staining procedure. Also, make sure you are doing this near a sink because it can get really messy. Wear a lab coat.

STEP 1: Place your slide on a slide holder or a rack. Flood (cover completely) the entire slide with crystal violet. Let the crystal violet stand for about 60 seconds. When the time has elapsed, wash your slide for 5 seconds with water. The specimen should appear blue-violet when observed with the naked eye.

STEP 2: Now, flood your slide with the iodine solution. Let it stand about a minute as well. When time has expired, rinse the slide with water for 5 seconds and immediately proceed to step three. At this point, the specimen should still be blue-violet.
STEP 3: This step involves addition of the decolorizer, ethanol. Step 3 is somewhat subjective because using too much decolorizer could result in a false Gram (-) result. Likewise, not using enough decolorizer may yield a false Gram (+) results. To be safe, add the ethanol dropwise until the blue-violet color is no longer emitted from your specimen. As in the previous steps, rinse with the water for 5 seconds.

STEP 4: The final step involves applying the counterstain, safranin. Flood the slide with the dye as you did in steps 1 and 2. Let this stand for about a minute to allow the bacteria to incorporate the saffranin. Gram positive cells will incorporate little or no counterstain and will remain blue-violet in appearance. Gram negative bacteria, however, take on a pink color and are easily distinguishable from the Gram positives. Again, rinse with water for 5 seconds to remove any excess of dye.

After you have completed steps 1 through 4, you should blot the slide gently with bibulous paper or allow it to air dry before viewing it under the microscope. DO NOT RUB THE SMEAR!