Procedure

PREPARING THE SLIDE

1. Place a single drop of water in the middle of a slide
2. Using a sterile inoculating loop, toothpick or pipette tip, gently touch a single colony on your plate. LESS is MORE!
3. Transfer the collected material to the drop of water on your slide. If you see clumps of bacteria from the colony, you probably have too much.
4. Holding the slide with a pair of forceps pass it through the interface of the yellow and blue flame,-this is the hottest region of the flame- 5 times. The slide should be in this region for no more than 1 second. If it gets too hot the bacteria will rupture (or the slide will break).

GRAM STAINING

1. Flood the slide containing the heat fixed bacteria with crystal violet. Let sit for 30 seconds, then rinse with tap water. Hold slide with forceps while rinsing.
2. Flood slide with Lugol's iodine. Iodine forms a strong complex with bound crystal violet. Let sit for 30 seconds then rinse slide with tap water.
3. Decolorization-the most important step*
    a. Hold the slide at a slant over the sink and count for 3 seconds while squirting top edge of the slide with the decolorizing agent, so it runs down the length of the slide.
    b. Immediately rinse with tap water to remove the remaining decolorizing agent.
4. Counterstain with Saffranin: flood the slide with saffranin and let sit for 30 seconds, then wash with tap water. Saffranin is a red dye & actually stains both Gram positive and Gram negative bacteria. However, the Gram positive bacteria are already a deep purple, they remain this color while the previously colorless Gram negative bacteria take up the red stain and will appear red by microscopy.
5. Interpretation: slides need to be viewed at 100X magnification on a microscope. This requires oil immersion to obtain a clear imaging of material on the slide. Gram positive cells will appear dark purple and Gram negative will appear red or pink.
   ** Acetone/ethanol dissolves the outer membrane of Gram negative bacteria, but not Gram positive bacteria. If done properly, Gram positive baceria remain purple at this stage, while Gram negative bacteria become colorless. However, too much decolorization and all bacteria will appear Gram negative (all crystal violet + iodine will be washed away); too little and all bacteria will appear Gram positive (not enough to remove the outer membrane of Gram negatives).*

Materials required

Bacterial cultures
Lugols iodine
crystal violet
acetone
safranin

glass microscope slide
coverslips

Equipment required

Light microscope (w/ 100X oil immersion objectives)
Sink