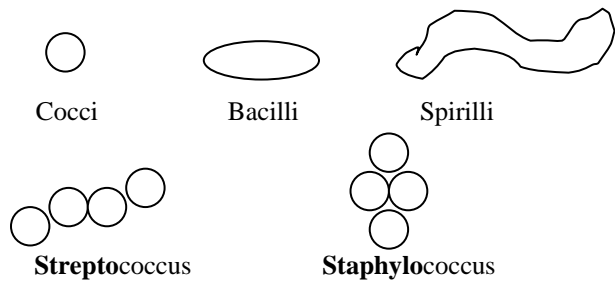
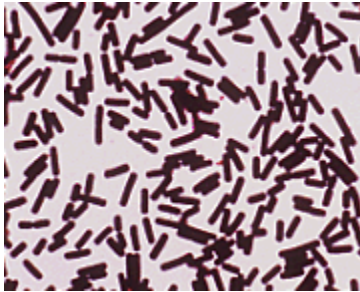


## Gram Staining of Bacteria

The purpose of Gram staining is to determine one characteristic property of bacteria. Some bacteria have different cell wall structure. They either take stain very well (Gram positive) and are purple in color, or they don't take stain very well (Gram negative) and are pink in color. This staining technique will enable the scientist to not only determine if the bacteria are Gram negative or positive, it will also let the scientist determine the structure of the bacteria in the microscope.

### Shape of Bacteria:



### Part I

1. Obtain a prepared slide showing the three types of bacteria.
2. Make sure to start on low power and focus on the color. Look around for a break in the color.
3. Switch to high power. Focus and look for one or two bacteria as shown above.
4. Draw each type on high power. Estimate size.
5. Draw an example of Spiral, Cocci, and Bacilli (All on one slide)
6. After look at a slide that was gram stained previously. In blue box.
7. Draw on high power.

### Part II

#### Gram Staining Procedure:

1. Begin by getting a slide and drawing a circle in the middle of the slide with a wax marker.
2. Get an inoculating loop and flame it. Let it cool and obtain loop full of sterile water. (If staining from a liquid culture no need to add the water)
3. Smear the small loop full of water on the slide in the circle drawn on the slide. Flame loop when finished.
4. Get agar plate that has a colony of bacteria on it. Raise the lid of the petri plate and remove one colony.
5. Smear this into the small amount of water on the slide. Flame loop when finished.

6. Let completely dry.
7. Pass the slide through the flame three times. Do not heat slide very hot. This is to fix the bacteria to the slide, not burn them to it.
8. Cover the slide with Crystal Violet stain. Let stand one minute.
9. Pour off excess stain. Dip slides in water two or three times to remove excess stain.
10. Drain excess water. Cover slide with Gram's Iodine. Let stand one minute.
11. Pour off excess Iodine and Dip in water two or three times to remove excess iodine.
12. Tip the slide and add one or two drops of 95% ethanol to the upper end of the slide and let run over the smear. After 2-3 seconds dip in water once or twice.
13. Cover slide in Safranin O stain and let sit for one minute.
14. Wash as before.
15. Drain excess water and allow to dry completely.
16. This can be examined under high power or oil immersion.