**The Gram Stain**

**Background:** Prior to the mid 1800’s, disease was shrouded in mystery and superstition. If the inhabitants of a town fell ill, they would likely blame the “foul odors” of a nearby sewer or the putrid vapors emanating from a local swamp.

If they were a particularly religious group they might claim that supernatural forces were at work. “God is punishing us for our sins or the Devil is responsible.” The very thought that a delicate unarmed microorganism, so small as to be invisible, could kill a man was absurd. Yet Louis Pasteur, Joseph Lister and Robert Koch, three giants who ushered in the “Golden Age” of microbiology, reached this conclusion.

Since there are many different groups of disease-causing bacteria, it is important to develop methods which enable their differentiation. In 1884 the Danish microbiologist Hans Christian Gram devised a staining procedure which divides bacteria into two large groups. The procedure is based on the ability of bacteria to retain the crystal- violet dye after decolorization with alcohol. **Gram positive** bacteria retain the dye and appear purple after decolorization while **Gram negative** bacteria do not retain the dye. In order to visualize the decolorized Gram negative cells a counterstain (safranin - a red stain) is used. In addition to Gram positive/ negative two other groups have been recognized: **Gram nonreactive**, do not stain or stain poorly by Gram stain (Mycobacteria and Spirochetes) and **Gram variable**, Gram positive organisms that may appear to be Gram negative if an older (>18 hours) culture is used.

It is difficult to overemphasize how fundamental Gram stain

classification has become in medical bacteriology. As a general rule, certain drugs work optimally either on Gram positive or Gram negative organisms; therefore, treatment of a bacterial infection is often based in part on the Gram-staining characteristics of the disease-causing organism. Today you will learn how to use the Gram staining technique to distinguish different groups of bacteria.

**Gram-staining procedure**

1. Place a loopful of a bacterial culture onto a glass slide.
2. Let air dry (5 - 10 minutes)
3. Pass the slide over the flame of a Bunsen burner 3 or 4 times.
4. Cover the smear with **crystal violet** dye - let sit for 30 second and rinse off with water.
5. Cover the smear with **iodine** - let sit for 1 minute and rinse off with water.
6. Decolorize with Gram’s alcohol (10

- 20 seconds). This is the critical step. Slowly add the decolorizer above the specimen, tilt the slide so that the decolorizer runs off the bottom. When the alcohol dripping from the slide is colorless, proceed with the next step.

1. Rinse off with water.
2. Cover the smear with **safranin** - let sit for 30 second and rinse off with water.
3. Blot dry and examine.
4. Sketch your observations in the space provided and record the Gram- staining characteristics in Table 1.

*S. epidermidis*

*B. cereus*

*E. coli*

*S. faecalis*

# Table 1: Gram-staining Characteristics of Different Bacteria.

|  |  |
| --- | --- |
| **Organism** | **Gram Stain** |
| *S. epidermidis* |  |
| *B. cereus* |  |
| *E. coli* |  |
| *S. faecalis* |  |

**NOTES:**