Gram Stain

- Staining is done to increase the contrast between bacterial cells and their environment.
- Differential staining- distinguishes between types of bacteria because of physical and chemical differences.
- The Gram stain is the most useful and widely used differential stain in bacteriology.
- Divides bacteria into Gram positive and Gram negative.
- There are four solutions involved in gram staining:
    1. The primary stain = crystal violet
    2. The mordant = iodine. This increases the interaction between the bacteria and the primary stain. This is arguably the most important step.
    3. The decolorizer = 95 % ethanol *or* 70% ethanol + 30% acetone. This is also a very important step. For Gram positive bacterial cells, this shrinks the pores in the peptidoglycan layer which increases the retention of the crystal violet. For Gram negative bacterial cells, this extracts lipids from the cell wall which increases the porosity. This leads to decolorization of Gram negative cells.
    4. The counterstain = safranin. Stains colorless cells pink.

- The procedure is as follows:
    1. Prepare smear. Make sure the smear is well prepared (air dried and heat fixed)
    2. Flood smear with Gram's Crystal Violet and let sit 30 seconds.
    3. Rinse with deionized water thoroughly.
    4. Flood smear with Gram's iodine and let sit for one minute.
    5. Rinse with deionized water thoroughly.
    6. Decolorize the slide by adding a few drops of decolorizer *.
    7. Rinse with DI water immediately.
    8. Flood smear with Gram's Safranin and let sit for about one minute.
    9. Rinse with deionized water thoroughly.
    10. Blot dry with bibulous paper.
    11. Examine by brightfield microscopy using the 100X oil-immersion objective to discern the colors.

* Different decolorizer solutions may require the addition of more or less volume to obtain proper results. The volume used is something that needs to be adjusted by the individual. It is important to have sufficient decolorization so that Gram-negative bacterial cells do not retain crystal violet, but avoid over-decolorization of Gram-positive bacterial cells. **Stains of Gram-positive and Gram-negative control cellss should be run at the same time as the cells being tested to ensure that all steps of the Gram stain, including the decolorization step, are working properly.**