General Microbiology
Lab 4
Oil Immersion Light Microscopy / Use of the differential Gram stain

All bacteria have cell walls. These semi-rigid structures maintain the cell in a particular shape (Spherical, rodshaped, curved, helical). A major component of bacterial cell walls is **peptidoglycan** a huge, complex molecule that forms a network around the cell membrane. It is composed of both sugars and protein subuinits called amino acids. Most bacteria fall into one of 2 categories with respect to the amount of peptidoglycan in their cell walls. Bacteria may have thick layers of (making up 60-90 % of the cell wall) or thin layers of peptidoglycan (10-20%). This difference allows one to distinguish classes of bacterial using a simple differential staining technique, the Gram Stain. It is often the first test performed in a laboratory to sort out the type of bacteria in a given sample. Bacterial are then initially grouped into **Gram +** (lots of peptidoglycan) or **Gram-** (little peptidoglycan) types.

1. Choose at least one of the bacterial plates from each of groups 1 and 2.

Group 1	A	*Bacillus cereus* (*B. cereus*)
	B	*Bacillus subtilis (B. subtilis)*
	C.	*Micrococcus luteus* (*M. luteus*)
Group 2	A	*Serratia marcescens* (*S. marcescens*)
	B	*Rodospirillum rubrum (R. rubrum)*
	C	*Escherichia coli (E. coli)*

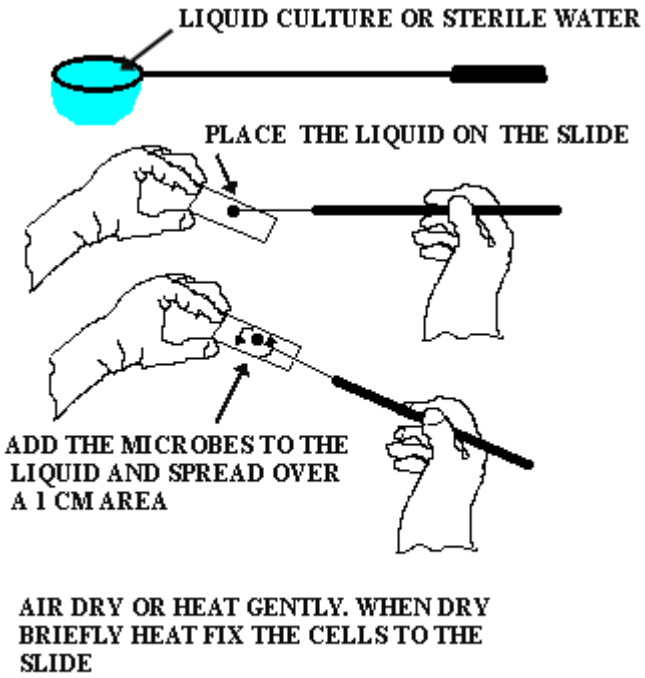
Procedure:

A. Make thorough observations of your bacteria as they appear on the plates. Note any differences between the organisms. Do any of the names give clues as to what the bacteria may look like? (Make use of “simple” tools to differentiate between bacteria.)

B.

Prepare a fixed smear of one organism from Group 1 and one from Group 2 and a mixture smear of the 2 organisms. (3 different slides) **DO NOT CROSS CONTAMINATE THE PLATES----FLAME YOUR LOOP BEFORE <u>EACH</u> SAMPLING OF THE ORGANISMS.**

1. Add a small drop of water to one end of a clean slide.
2. Using a sterile flamed loop (cooled), pick up a VERY small amount of bacteria from an isolated colony.
3. Make a well distributed slurry of your bacteria in the water droplet
4. Air dry
5. Heat fix by passing the bottom of the slide slowly 3 times through a burner flame.



C. **Perform Gram stains on your 3 slides.**

1. Starting with your heat fixed slides, apply crystal violet to the smear area for 30 seconds. (you need not flood the whole slide.)
2. Rinse with water for 5 seconds.
3. Apply Gram's iodine to the smear area for 1 minute
4. Rinse with water for 5 seconds
5. Apply decolorizer to the smear area for 15-30 seconds
6. Rinse with water for 5 seconds
7. Apply safranin to the smear area for 60 seconds.

8. Rinse with water for 5 seconds
9. Gently blot with bibulous paper or paper towel.
10. Observe your smears using oil immersion light microscopy (see last week's handout) and note whether you think each of your samples is gram positive or gram negative. Note the morphology of your organisms (shape). Can you distinguish the 2 organisms on your mixed slide by: morphology (shape)? Size? Gram reaction?