Bio 139 Labs #9 and #10: Gram’s Stain & KOH test

Gram Stain (Lab #9)

- One of the most utilized procedures in all of microbiology
- Differential stain
  - Allows you to distinguish 2 groups of bacteria
  - Used for morphology
Differentiates cells by chemical composition of cell wall
- Gram positive vs. Gram negative
- Based on peptidoglycan content of cell wall
  1. Crystal Violet (primary stain) is retained by G+ cell (large amount of peptidoglycan). Cells will be PURPLE
  2. Crystal Violet is removed from G- cells (small amount of peptidoglycan) by decolorizing agent (acetone)
  3. Safranin (counterstain) dyes G- cells red

Crystal Violet is NOT irreversibly bound to G+ cells: it can be accidentally removed by excessive decolorization with acetone, and then G+ cells will inappropriately stain red. (Include control organism on slide when possible.)

***Only add acetone for a few seconds***
Age of culture can also affect Gram stain results (fresh cultures better)

Iodine is a mordant:
- It complexes with crystal violet to improve stain retention by Gram +’s

Set up your Gram stain slide with known positive controls for each Gram type (Gram – Escherichia coli; Gram + Staphylococcus epidermidis) plus your UNKNOWN and soil sample. ALL samples should be taken from broth cultures.

KOH (potassium hydroxide) test (Lab #10)

- Secondary test for identification of Gram + vs Gram -- cultures
- 3% KOH dissolves cell walls of Gram – (thin layer of peptidoglycan), but does not affect Gram + cell walls. Dissolution of G- cell wall lyses the cell and spills its contents, including the DNA. DNA is very viscous, and with a large enough cell mass, the DNA strands can be seen sticking to / dragging from a loop when touched. G+ cells are not lysed, no free DNA, no viscosity will be observed.
- This test requires a large amount of cells (visible clump). Scrape samples of E. coli, Staphylococcus epidermidis, and your unknown from a solid medium (agar slants).
- Place a drop of 3% KOH on a slide, then mix in cells: do E. coli first so you can see a positive result. Allow a minute for reaction to occur.
- + KOH test = Gram NEGATIVE

KNOW: Text (6th ed.) fig. 3.31 (p. 70) results of each step of the Gram stain