Microbiology Lab Experiment Changes

Experiment #: 3-6

Title: Gram Stain

Live Organisms: *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Micrococcus luteus, Enterococcus fecalis, Serratia marcescens, Klebsiella pneumoniae,*

Changes:

1. Each student will gram stain all seven organisms.
2. Draw in color or take pictures all seven organisms <u>@ **1000x**</u>.
3. Save slides in your slide box. You may put a tissue in the box and allow oil to run off slides.
4. You will pick your best slide and <u>**turn it in for credit with the grade sheet.**</u>

Procedure:

1. Add CRYSTAL VIOLET and cover the smears completely. Stain for 1 minute then rinse with water.
2. Add GRAMS IODINE (mordant). Stain for 1 minute then rinse with water.
3. Decolorize with 95% ETHYL ALCOHOL. For old bacteria, add 1-2 drops then immediately rinse with water. For young cultures, cover smear with ETOH and wait 1 minute, then rinse with water.
3. Add SAFRANIN. Stain for 1 minute then rinse with water.
4. Gently blot dry with paper towel – no wiping!
5. Wash your hands to remove stains, if any, before getting the microscope.
6. Observe your stained bacteria, starting with the lowest magnification. Focus @ 1000X magnification under oil.
7. Your stained bacteria should be of (i) same color and shape (ii) mostly distinct and fairly even distribution (iii) no clumping or aggregates (iv) no uneven staining

Take Home Lesson: Gram stain tells you not only morphology and arrangement but also the cell wall structure [i.e., gram (+) or gram (-)]. You should know how the gram stain relates to the cell wall of the bacterium. You need to be able to recognize Gram positive and Gram negative rods, cocci, spirals and whether they occur in chains, clusters, single etc. by their appearance under the microscope. You should begin to associate particular organisms with their gram stain morphologies. You must know the reagents and the function of each in the staining procedure.