**MCB2010L –Microbiology Lab**

**Week 4: Gram Stain and Acid-Fast Stain**

* Gram Stain
  + Differential stain
    - Tells the difference between gram-positive and gram-negative cells
      * Gram positive – appear purple
        + Have thick layer of peptidoglycan
      * Gram negative – alcohol or acetone removes the crystal violet ; must be counterstained with red dye (safranin) so they appear reddish/pinkish
        + Have outer membrane that covers thin layer of peptidoglycan
    - Look at figure 7-1 – 7-2
  + Drop two loops full of water on blank slide
  + Pick two bacteria (Gram + and Gram –) and make very thin smears on same slide – this is very important (don’t want too much of the sample) – Why?
  + Smear, air dry, and heat fix
  + Procedure – page 48
    - Step 1 – Crystal Violet 1 minute, drain and rinse with water
    - Step 2 – Iodine for 1 minute, drain and rinse with water
    - Step 3 – Decolorization, squirt 95% alcohol for 1-2 seconds and rinse with water. (Decolorization step is the most important step – if done for too long, Gram + will appear Gram –)
    - Step 4 – Safranin for 1 minute, rinse and blot dry. View slide under 100X.
* Acid-Fast Staining
  + Bacteria such as *Mycobacterium phlei* have cell walls that have high lipid content, one of those lipids is a waxy material called mycolic acid – Figure 8-1 and 8-2, page 52
    - Composed of fatty acids and fatty alcohols, affects staining properties and is important diagnostic tool in identifying *Mycobacterium tuberculosis*
  + Procedure – page 53
    - Prepare smear containing both acid-fast (*Mycobacterium*) and non-acid-fast (*S. aureus*) on same slide
      * Drop two loops full of water on blank slide
      * Place *S. aureus* on slide, flame loop, and then small amount of *Mycobacterium* (be sure to break up and spread out very well on slide), air dry and heat fix.
      * Step 1 – Carbolfuchsin for 5 minutes, drain and rinse with water
      * Step 2 – Decolorize with acid-alcohol for 1 minute, rinse with water
      * Step 3 – Methylene blue for 30 seconds, rinse with water and blot dry
      * Using 100X oil immersion to view slide without covers slip