** Introductory Medical Microbiology**

**Laboratory Notes**

**BIOL 2161L**

# ACID FAST STAINING

Certain bacterial species have unusual lipids (**mycolic acid**) in their cell walls.  This substance renders the cell wall very waxy and impenetrable by aqueous stain solutions.

In the late 19th century, Koch and Ehrlich simultaneously introduced a method for staining the previously undetectable Mycobacterium tuberculosis.  Modifications by Ziehl and Neelsen produced the commonly used carbol-fuchsin solution, which requires steaming to drive the stain in.  (The melting point for mycolic acid is 56oC).  Muller and Chermock modified carbol-fuchsin for use at room temperature by addition of a surfactant (wetting agent).  A solution of acid-alcohol removes stain from most cellular and tissue elements.  The mycolic acid, however, resists penetration by this differentiating agent, leaving acid-fast bacteria reddish pink against a colorless background.  Light green or methylene blue is used as a counterstain to aid in the localization of cellular material on the specimen. The acid fast stain is used routinely on sputum samples for preliminary diagnosis of active tuberculosis.

Students will use cultures of *Mycobacterium smegmatis, Micrococcus luteus* (now know as *Kocuria rhizophilia*), and *Serratia marcescens* D1.

Start by making a smear (**its OK if the smear is thick for acid fast staining**) of each organism and then stain them using the procedure below and the instructions provided from the instructor. You should also perform Gram stains on these organisms as well. You may want to also do acid fast stains on the organisms from the previous labs (E.coli, B.sub, S. epi) as well to make sure you are familiar with their characteristics before the skills test.

Below is a diagram that gives a representation of what the organisms would look like through each step of the staining process. Note that AF(+) organisms will appear pinkish red at the end of the process and the AF(-) organisms will be pale blue. These colors must be observed through the microscope and should be determined by looking at the cells, not the slide background which can be misleading.

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| **Acid Fast** |  | **Non-Acid Fast** |
| **Pink** | ***Primary Stain***  **Carbol Fuchsin** | **Pink** |
| **Pink** | ***Decolorizer***  **Acid Alcohol** | **Colorless** |
| **Pink** | ***Counter Stain***  **Methylene Blue** | **Blue** |

Read the following steps and explanations, then try the procedure with each of your cultures.  Remember:  **Do not** cross contaminate your cultures.  Use aseptic techniques to keep them pure.

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| --- | --- |
| **Step** | **Explanation** |
| Make a thin bacterial smear on a separate slide for each organism provided.  You will especially have to work the *M. smegmatis* with the loop.  It will be helpful to add a loopful of water.  Make one slide a mixed sample by adding a loop from both.  **DON'T FORGET TO HEAT-FIX!** | If you can see substance on the loop or slide surface, your smear will be too thick.  The stains cannot penetrate or rinse out of thick samples *M. smegmatis* is branched "higher" bacteria; the cells are difficult to separate. |
| Flood the slide with **carbol-fuchsin** (3-4 drops) and place over a steaming water bath for 5 minutes, adding stain as the edges begin to evaporate.  Try not to drip stain into the boiling water.  A dime-sized piece of paper towel placed directly on the smear can help hold the dye on the smear. | It takes time for the **primary stain** to penetrate the waxy cell wall at room temperature.  Heat hastens the process. |
| Remove from bath and cool.  Rinse with distilled water and blot gently with bibulous paper.  Allow the slide to cool. | Place paper towel into the trashcan at the end of the bench. DO NOT rinse the paper towel into the stain rack as this will cause them to clog!! |
| Remove the excess satin by tilting the slide and  dripping **acid-alcohol** over it for **30 seconds.** | This pre-colorizing step stops the action of carbol-fuchsin and removes excess stain. |
| Rinse with distilled water and blot gently. | Rinsing stops the dye action, blotting keeps the next step from being diluted.  Too much pressure in blotting will break the slide. |
| Differentiate organisms by flooding the slide with **acid-alcohol** and allowing to stand for **two minutes**. | Acid-alcohol is the **decolorizer** and thus differentiates between the acid-fast positive organisms and acid-fast negative organisms.  If the alcohol acts too long, it will eventually decolorize even the acid-fast cells. |
| Rinse with distilled water and blot gently. | Rinsing stops the action of the acid-alcohol. |
| Flood the slide with **methylene blue** for at least **one minute**. | This is the **secondary** or **counterstain**.  It will stain the transparent non-AF cells so they may be seen.  The color will be blue, much paler than the red AF+ cells. |
| Rinse and blot dry. Observe. |  |

# Record the stain results for each of the following organisms below:

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| --- | --- | --- |
| Organism | Color Observed | Acid Fast Result (+/-) |
| *E. coli* |  |  |
| *B. subtilis* |  |  |
| *S. epidermidis* |  |  |
| *M. luteus* |  |  |
| *M. smegmatis* |  |  |
| *S. marcescens* |  |  |