Ziehl-Neelson Acid Fast Stain

Introduction

The Acid Fast stain is very useful method used to identify bacteria. It consists of a primary dye (carbol-fuchsin), a decolorizer (acid:alcohol) and a counter stain (methylene blue). The decolorizer is used to decolorize the bacteria that "do not like" the primary stain. The counter stain is used to stain those bacteria which were decolorized by the acid:alcohol.

Bacteria which retain the carbol-fuchsin are fuchsia-colored and are called acid fast bacteria (AFB). Non-AFB are decolorized by the acid:alcohol and are stained by the methylene blue and appear blue.

The special property that differentiates between AFB and non-AFB is that AFB are covered by a "hard", waxy outer layer around the bacterial cell. It is because of this "wax" that heat and carbol (phenol) are required to get the fuchsin dye inside the layer – sort of like how a hammer works on a chisel. It is also because of this waxy layer that the acid:alcohol doesn't remove the fuchsin from the bacteria, leaving them brightly stained (refer to the Introduction to Stains monograph prior to this experiment).

Experimental

Materials

The table, below, summarizes the supplies you will need to complete this experiment successfully:

Carbol-fuchsin	Methylene blue	Decolorizer
Disinfectant	Loop	Bunsen burner
Burner tubing	Striker	2 - Microscope slides
Microscope	Bibulous paper	Lens paper
Immersion oil	Staining tray	China marking pencil -- RED
Bacteria as directed by your professor.		

CAUTION: Bacteria in this lab are pathogenic. Use care when working with them. The decolorizer is flammable. Use no flames in its proximity. Immersion oil loosens glue on the microscope. When you are done with the microscope, wipe the oil off the oil immersion objective and check the others for possible accidental immersion in oil.

Methods

All staining techniques begin with preparing the sample. This involves one of two methods: 1) Mark a target circle (1 in graphic, below) on a microscope slide with a China marking pencil about the size of your thumbnail. Flame a bacteriological loop from the loop to where it joins the

handle. You want to get each area red-hot. Let it cool to room temperature WITHOUT touching anything. Dip it into a sample of water and place the loopful of water in the target circle (2 in the graphic, below). Reflame the loop and let cool. Carefully remove a single colony of bacteria (certainly no more than the amount of pus you'd get out of a medium sized zit when it pops) from your solid media sample and place it in the water in your target circle. Mix the water and bacteria together inside the target circle to make a thin smear. Let air dry.

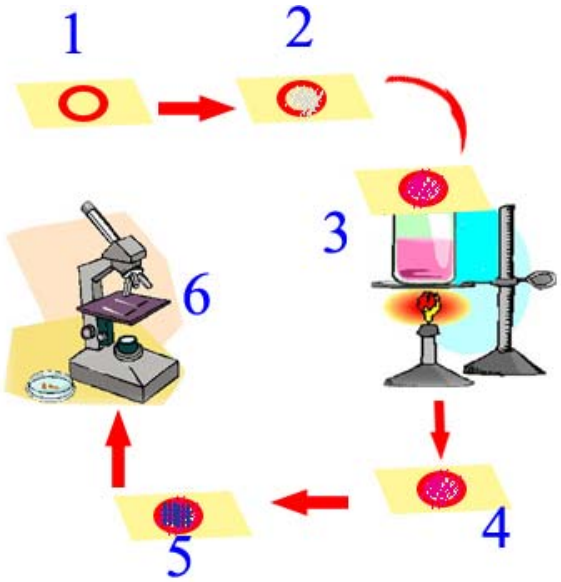
Method two (#2) has the following variation: instead of using water and bacterial colonies, you use a drop of liquid media that has bacteria growing in it. To remove the bacteria-containing sample, you remove the cap with the pinky finger of your strong hand and pass the open neck of the tube through the flame 2-4 times in your weak hand. Dip your flamed loop into the broth with the other fingers of your strong hand, remove the sample without touching the sides or neck of the tube and reflame the neck of the tube. Replace the cap on the tube and smear the sample inside your target circle. Flame your loop to re-sterilize it. Allow the sample to air dry.

Regardless of the staining technique, all slides are prepared as above.

With the exception of the capsule stain, the next step in the majority of microbiological staining is to heat fix the slide. This process kills the bacteria and fixes them to the slide so they won't wash off during staining or rinsing. To do this, you pass the slide through the flame of a Bunsen burner 2-5 times or until it feels like baby's milk on your wrist -- touch it carefully to your wrist so you don't get burned!

Once you've heat fixed the slide, you may stain it as directed. Below is the technique for the Acid-fast stain. All of these techniques work best in our little part of the world -- techniques vary by location, altitude and quality of chemicals.

The graphic, below, summarizes, very succinctly, the steps in the acid-fast staining procedure:



Organisms of the genus Mycobacterium don't stain well by Gram's method, but take up hot carbol fuchsin and hold it so well that they resist decolorization with strong mineral acids (nitric, hydrochloric or sulfuric acid with ethyl alcohol). Point of caution: ethyl alcohol (EtOH) is lipophilic. Each of the three acids is caustic to skin. Since they are mixed with EtOH, the potential burn that this can cause will be worse than just getting burned by the acids alone. That's because the EtOH "drags" the acids deeper into the skin faster than by themselves.

Place your heat-fixed slide on the top of a beaker with boiling water in it (3 in the graphic, above). Cover the target circle with carbol fuchsin and keep it moist as it heats. Continue boiling the water beneath the slide for 10 minutes. After the 10 minutes have passed, remove the slide and allow it to cool to room temperature (4 in the graphic, above). Rinse it, then, with acid-alcohol for about 30 seconds or until no more fuchsia color comes from the target circle. Counter-stain with methylene blue for one minute (5 in the graphic, above), then rinse with water and blot dry with bibulous paper and the sample is ready for examination under the microscope on oil immersion (6 in the graphic, above). What you'll see, here, are a blue background and Mycobacterium tuberculosis and other acid-fast bacteria (AFB) show up as bright red/fuchsia rods that show up against this blue background, i.e., the acid alcohol does not remove the fuchsia from these microorganisms. This method may be modified by using a fluorescent dye in place of the carbol fuchsin. This may then be illuminated with UV light. This method is much simpler to use for the detection of AFB.

Draw what you see in the space provided, below:

Bacterial Slide #1	Bacterial Slide #2

When you have completed your work, wipe the oil off the objectives, discard your slides and clean up your bench.

References

1. Beishir, L.: Microbiology in Practice: A Self-Instructional Laboratory Course, Fifth Edition. (Harper Collins: New York) © 1991.

2. Jawetz, Melnick and Adelberg: Medical Microbiology, Nineteenth Edition. (Appleton and Lange: Norwalk, CT) © 1991.

3. Tortora, Case and Funke: Microbiology: An Introduction, Fourth Edition. (Benjamin Cummings: Redwood City, CA) © 1992.

4. Zubay: Biochemistry. (Addison Wesley: Reading, MA) © 1983.