

Acid-Fast Stain

Acid Fast bacteria, such as *Mycobacterium tuberculosis* and *Mycobacterium leprae*, contain long chain fatty acids (mycolic acids) in their cell walls. These cells are impervious to methylene blue and other basic dyes. The carbolfuchsin dye penetrates the cell wall and stains the bacteria. You must either heat the slides to melt the mycolic acids or add a detergent to the dye so that it will penetrate the cell wall. The mycolic acid does not allow the acid alcohol to penetrate, so the cell resists de-colorization and remains a bright pink. The *Staphylococcus* culture is added as a control, non-acid fast, organism to indicate if you have decolorized sufficiently. The methylene blue is added to stain the *Staph* organism so you can see them as well. The presence of acid-fast organisms in direct clinical specimens is clinically significant. They can be hard to detect if there are only a few of them in the sample.

Materials

- Carbol-fuchsin stain with Tergitol
- Methylene Blue stain
- Acid-alcohol
- *Staphylococcus aureus* broth culture
- *Mycobacterium smegmatis* plate culture

Acid Fast Stain Procedure

1. Prepare a combined smear of both organisms on a glass slide.
 - Add several loop-fulls of *Staph. aureus* on the slide first.
 - Because of its waxy cell wall, the *Mycobacterium* likes to clump together. It also rinses off the slide easily, so add a lot of it to your slide.
 - After adding the *Mycobacterium* to your slide with the inoculating loop, use the inoculating needle to tease the bacteria and really disperse the cells.
 - You need to have some very small clumps on your slide so that you can be sure the acid alcohol can penetrate.
2. Allow the slides to air dry.
3. Methanol-fix the organisms.
4. Apply enough carbolfuchsin with Tergitol to cover the bacteria.
 - Allow this to set for 5 minutes.
5. Rinse the slide with acid-alcohol, drop by drop, just until the alcohol runs clear.
6. Gently rinse the slide with water.
7. Apply enough methylene blue to cover the bacteria.
 - Allow this to set for one minute.
8. Gently rinse the slide with water and allow the slide to air dry.
9. Examine the slide under oil immersion.
 - Positive (acid-fast) organisms will appear pink/red
 - Negative (non acid-fast) organisms will appear blue

