

Acid-Fast Staining

- The acid-fast stain is a differential stain used to identify cells capable of retaining a primary stain when treated with acid alcohol.
- Very useful for identifying bacteria in the genus *Mycobacterium*, some of which are pathogens (i.e. *Mycobacterium leprae*, *Mycobacterium tuberculosis*).
- Also useful for identifying other organisms which could be pathogenic such as members of the *Nocardia* genus and parasites in the genus *Cryptosporidium* and the genus *Isospora*.
- Few organisms are acid-fast, so this stain is run only when there is suspicion of an infection by an acid-fast organism.
- Acid-fast positive cells contain mycolic acids in their cell wall.
- Mycolic acid is a waxy substance which does not allow the cells to be stained by simple stains, but when stained by carbolfuchsin can retain this stain even acid alcohol decolorizer is used.
- There are two methods:
 1. Ziehl-Neelsen (ZN), which uses heat to drive the carbolfuchsin in the cells.
 2. Kinyoun (K), which uses a more concentrated, more lipid soluble form of carbolfuchsin.
- There are three components in the acid-fast procedure. They are:
 1. Carbolfuchsin= A primary stain that is a phenolic compound that is lipid soluble. Stains cells reddish purple.
 2. Acid-alcohol= A decolorizer that decolorizes non acid-fast cells.
 3. Methylene blue= A secondary stain that stains non acid-fast cells blue.

PROCEDURE (Kinyoun Procedure)

1. Prepare a smear, air dry and heat fix.
2. Flood slide for 5-10 minutes with carbolfuchsin prepared with Tergitol No. 7 (heat is not required)
3. Decolorize with acid-alcohol (30 sec) and rinse with tap water. Repeat this step until no more color runs off slide.
4. Counterstain with alkaline methylene blue for 2 min. Rinse with water and blot dry.
5. Examine by brightfield microscopy using the 100X oil-immersion objective. Acid-fast organisms stain red; the background and other organisms (non-acid fast organisms) stain blue.