

Differential Stains

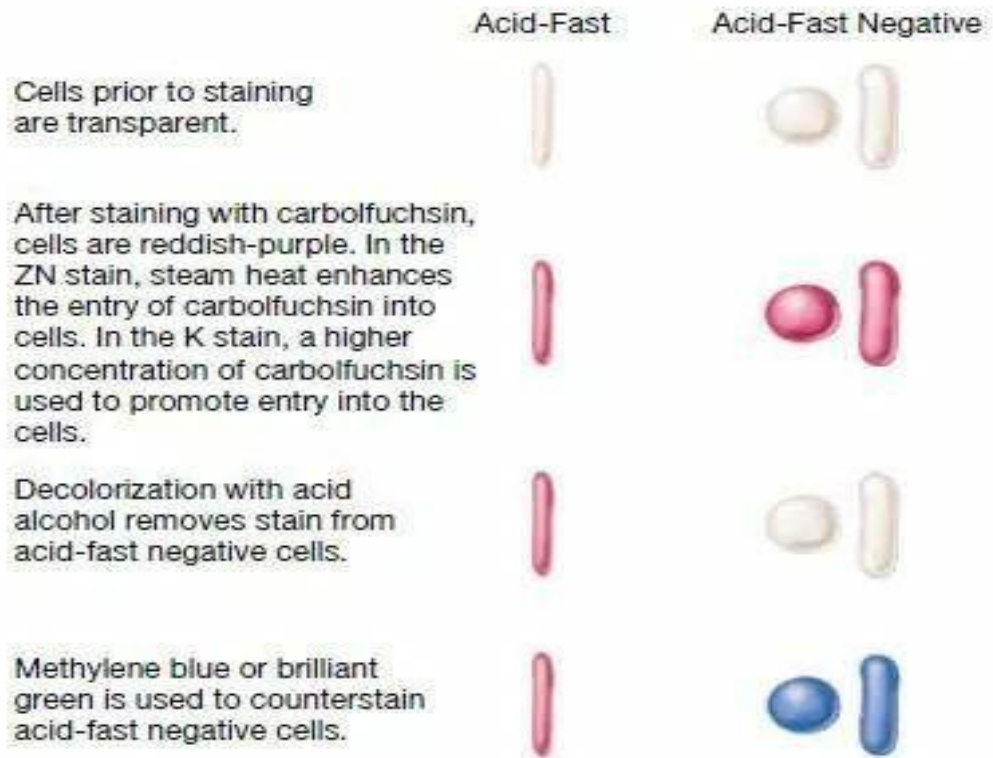
Acid-Fast Stain

Purpose

The acid-fast stain is a differential stain used to detect cells capable of retaining a primary stain when treated with an acid alcohol. It is an important differential stain used to identify bacteria in the genus *Mycobacterium*, some of which are pathogens (e.g., *M. leprae* and *M. tuberculosis*, causative agents of leprosy and tuberculosis, respectively). Acid-fast stains are useful in identification of **acid-fast bacilli (AFB)** and rapid, preliminary diagnosis of tuberculosis (with greater than 90% predictive value from sputum samples).

Principle

The presence of mycolic acids in the cell walls of acid-fast organisms is the cytological basis for this differential stain. Mycolic acid is a waxy substance that gives acid-fast cells a higher affinity for the primary stain and resistance to decolorization by an acid alcohol solution. A variety of acid-fast staining procedures are employed, one of which is the Ziehl-Neelsen (ZN) method that uses heat as part of the staining process and the phenolic compound carbolfuchsin is used as the primary stain because it is lipid soluble and penetrates the waxy cell wall. Staining by carbolfuchsin is further enhanced by steam heating the preparation to melt the wax and allow the stain to move into the cell. Acid alcohol is used to decolorize nonacid-fast cells; acid-fast cells resist this decolorization. A counterstain, such as methylene blue, is then applied. Acid-fast cells are reddish-purple; nonacid-fast cells are blue .



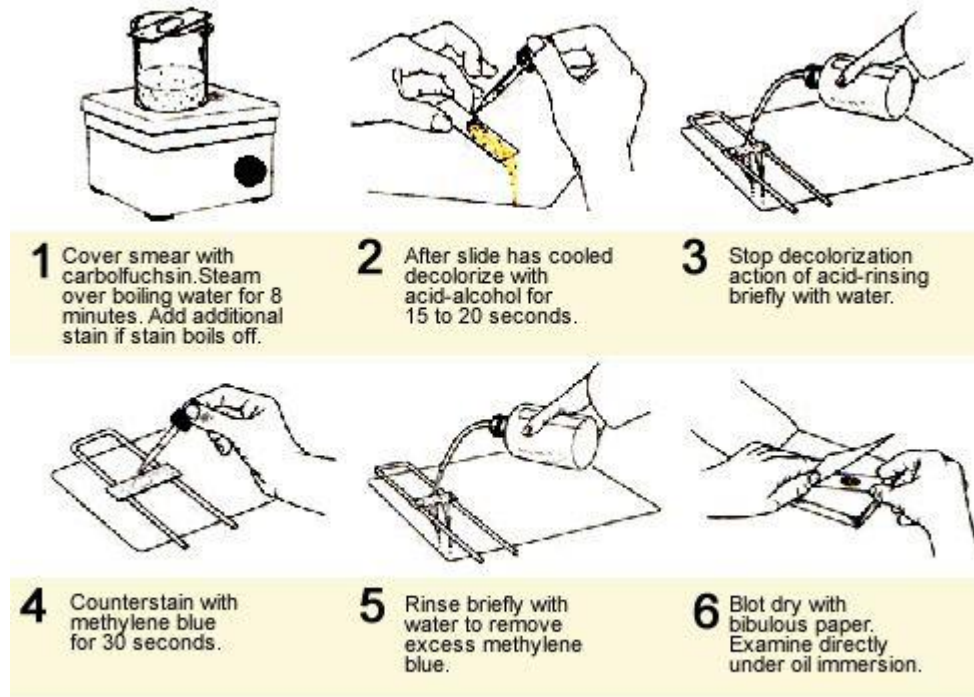
So as a result, there will be two types of bacteria that appear on the slide when examined under microscope:

1. Acid fast bacteria stained red.
2. Non- acid fast bacteria stained blue.

Procedure:

1. Prepare the bacteria fixed smear.
2. Place the slide in the staining rack and cover the smear with carbol fuchsin dye, Maintain steaming for 5 min; add more dye as needed to prevent the smear from boiling and dry on the slide, and if necessary add more carbol fuchsin to cover the smear.
3. Rinse the smear gently with tap water.
4. Apply the acid- alcohol decolorizer drop wise and slowly, continue add this reagent until the dye does not run off from the smear and rinse with tap water.

5. Cover the smear with methylene blue dye for 30 min., rinse with tap water .
6. Blot dry the smear, and examine with oil lens .



Ziehl-Neelsen acid-fast staining procedure

