

Bacterial taxonomy

Lab: Two

Staining & Differential stains

**Acid fast Stain (Ziehl-Neelsen stain)**

**Acid-fast staining** is a crucial differential laboratory technique used to identify waxy-walled bacteria, primarily *Mycobacterium tuberculosis*, by staining them with red carbol-fuchsin that resists acid-alcohol decolorization. This method separates organisms into acid-fast (**red/pink**) and non-acid-fast (**blue**) groups, aiding in the diagnosis of TB, leprosy, and Nocardia infections.

# Acid fast Staining (Ziehl-Neelsen staining)

## Technique

### Procedure Steps

1. Prepare Smear: Air-dry and heat-fix a thin smear of the specimen onto a clean slide.

### 2. Primary Stain (Carbolfuchsin):

- Cover the smear with carbolfuchsin.
- Place a piece of filter paper over the stain and steam the slide over a water bath (or using a Bunsen burner) for 3-5 minutes, ensuring it doesn't boil dry and adding more stain if needed.
- Rinse thoroughly with water.

### 3. Decolorization (Acid-Alcohol):

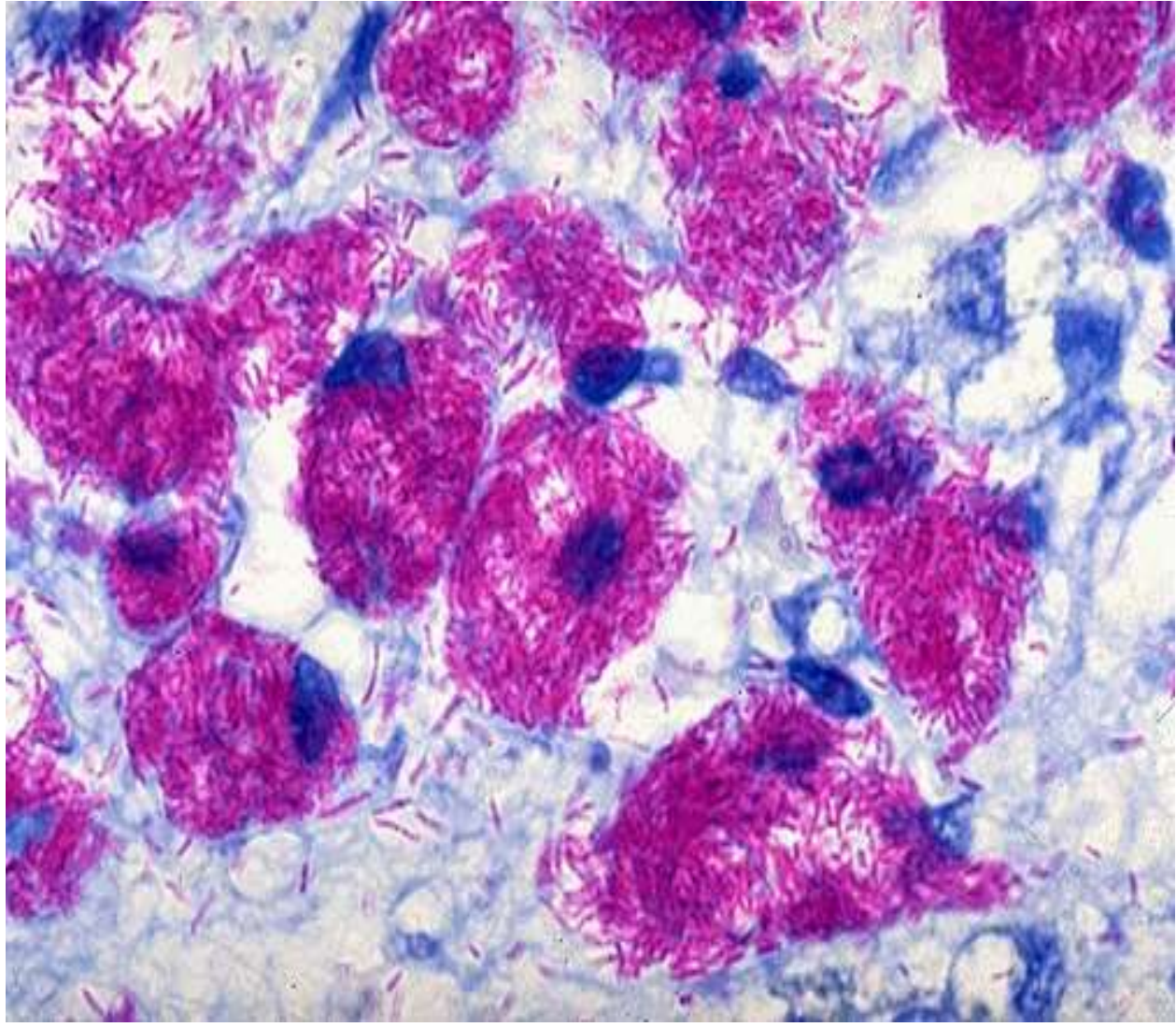
- Apply acid-alcohol for about 15-30 seconds, or until the red color runs pale pink. (Careful: it's flammable).
- Rinse well with water.

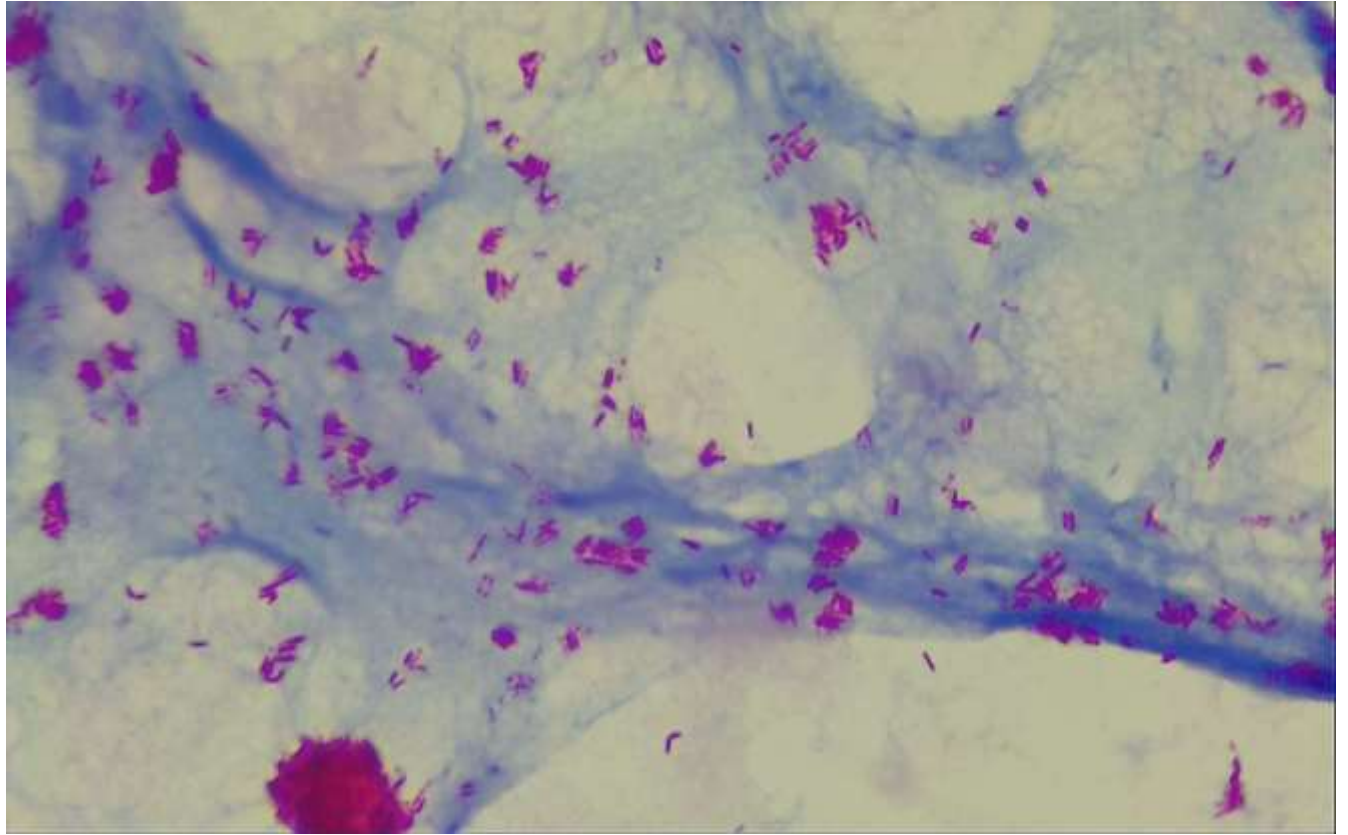
#### **4. Counterstain (Methylene Blue):**

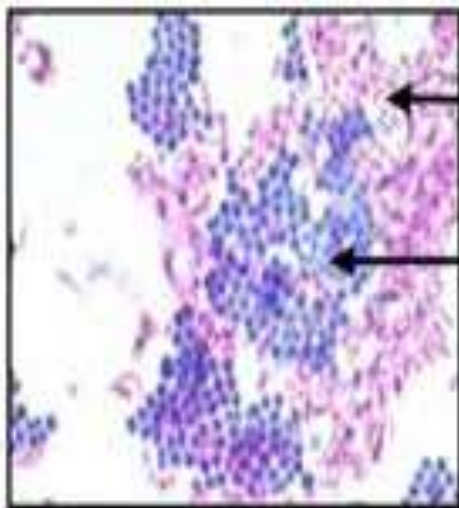
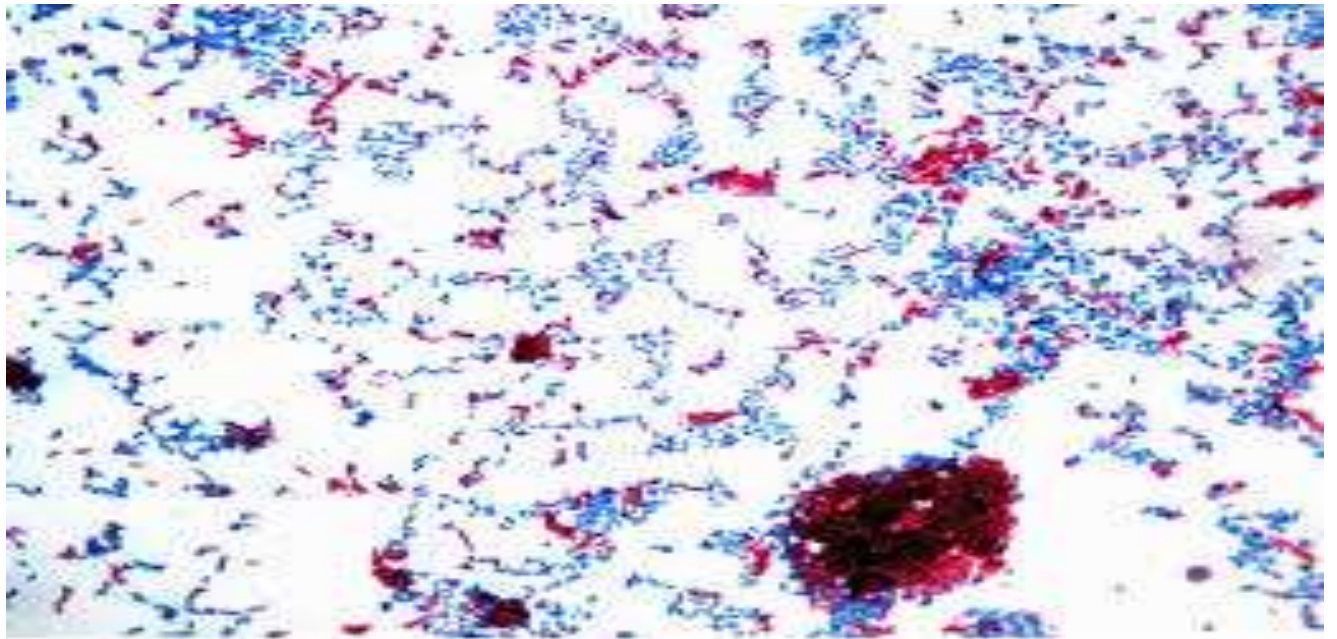
- Flood the slide with methylene blue for 1-2 minutes.
  - Rinse with water.
5. **Dry & Observe:** Gently blot dry with bibulous paper and examine under an oil immersion objective.

#### **Expected Results**

- **Acid-Fast Bacteria:** Retain the red carbolfuchsin and appear bright pink/red.
- **Non-Acid-Fast Bacteria & Background:** Lose the red stain and take up the blue counterstain, appearing blue.

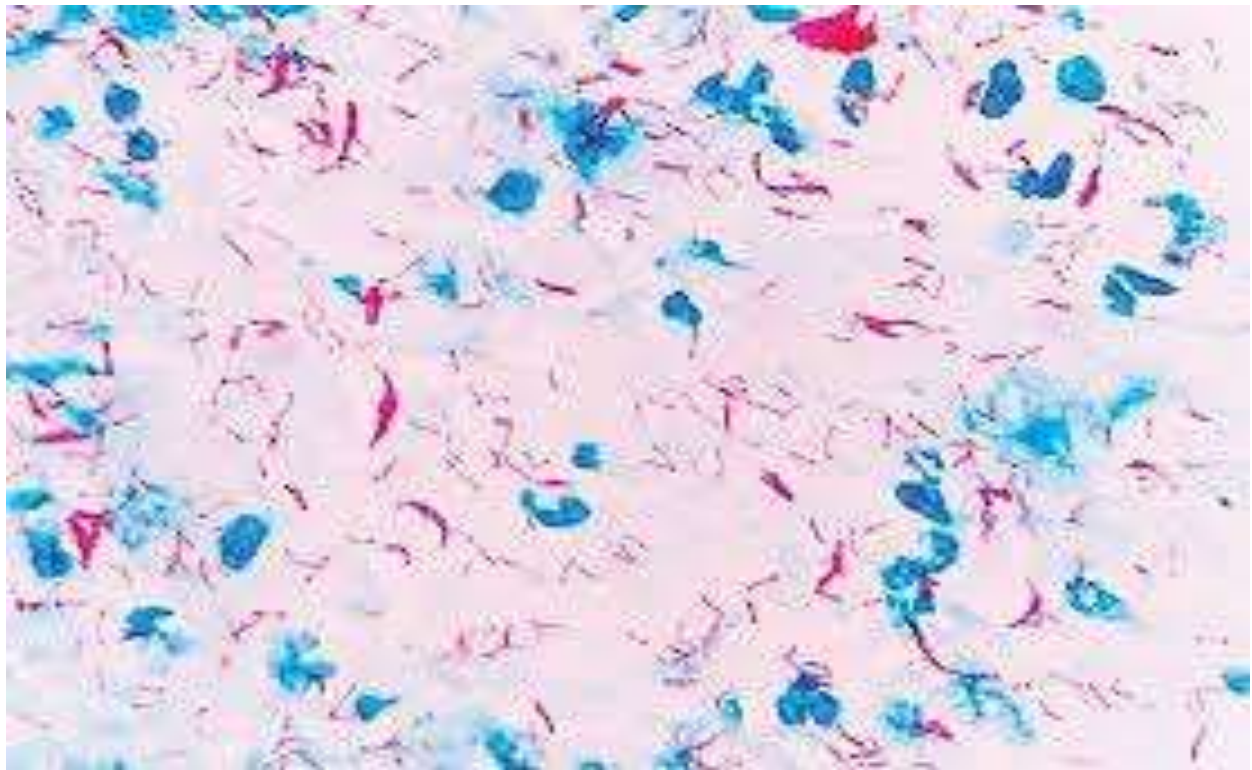






acid fast positive (pink)

acid fast negative (dark blue/purple)



**Acid-Fast**

**Non-AF**



**Heat + Carbol Fuchsin**



**Acid alcohol**



**Methylene blue**



