

## **Exercise No. (9):**

### **Mycobacterium species (Ziehl Neelsen stain)**

**- Type of sample:** Sputum smear

**- Stain:** Ziehl Neelsen Staining Technique (Acid fast stain)

#### **- Procedures**

1- Cover the film with strong carbol fuchsin, heat under the slide till the steam rises, avoid boiling and avoid dryness, leave the hot stain to act for 5m.

**N.B.** the evaporated stain is replaced by addition of new carbol fuchsin.

2- Allow the slide to cool then wash with water.

3- Cover the slide with 20% sulfuric acid in water or 3% HCl in alcohol for 1m then wash with water and pour on fresh acid. Repeat this procedure several times (10m). The film is only very faintly pink, wash with water.

4- Add counter stain, Loeffler's MB or crystal violet for 3-5m then wash with water, dry and examine with oil immersion lens.

❖ Acid fast bacilli appear red (retain the original stain) while other non-acid fast appear blue or violet (colour of the counter stain).

## Microscopic Appearance

