

## Practical No.13

### Mycobacterium

The genus includes pathogens known to cause serious diseases in mammals, including tuberculosis and leprosy

Mycobacteria are aerobic and nonmotile bacteria they are characteristically acid-alcohol fast. Mycobacteria do not contain endospores or capsules. They are not classified as either Gram-positive or Gram-negative they are acid fast bacilli referring to their resistance to decolorization by acids during staining procedures.

#### *Mycobacterium tuberculosis*

*Mycobacterium tuberculosis* (MTB) is the etiologic agent of tuberculosis in humans. The bacterium is a facultative intracellular parasite, usually of macrophages, and has a slow generation time, 15-20 hours.

The cell wall structure of *Mycobacterium tuberculosis* contains peptidoglycan and complex lipids. Over 60% of the mycobacterial cell wall is lipid. The lipid fraction of MTB's cell wall consists of three major components, mycolic acids, cord factor, and wax-D.

#### Laboratory diagnostic tests;

Specimen; sputum, CSF, joint fluids, urine gastric washings, biopsy material.

Direct smear; Ziehl-Neelsen staining for examining the AFB (acid fast bacilli). In order to detect *Mycobacterium tuberculosis* in a sputum sample. One acid-fast bacillus/slide is regarded as "positive" of an MTB infection.

#### Procedure;

- 1- Strong carbol fuchsin is added to a fixed smear of sputum. Flood the slide with stain, heat until steaming.
- 2- Decolorize with 20% H<sub>2</sub>SO<sub>4</sub>.
- 3- Wash with tap water.
- 4- Add methylene blue for 1 minute (counter stain).
- 5- Dry and examine under microscope. Acid-fast bacilli appear pink in a contrasting blue background.

Another method is the Kinyoun method. The procedure for Kinyoun staining is similar to the Ziehl-Neelsen stain, but does not involve heating the slides being stained. The Kinyoun staining method uses carbol fuchsin as a primary stain, followed by decolorization with an acid-alcohol solution and methylene blue as a counterstain. Kinyoun carbol fuchsin has a greater concentration of phenol and basic fuchsin and does not require heating in order to stain properly. When viewed under a microscope; a Kinyoun stained slide will show acid-fast organisms as red and nonacid-fast organisms as blue.

**Culture:** Lowenstein-Jensen medium which is an egg based medium prepared as slants contain eggs, potato, serum, glycerol, malachite green and antibiotics. Colonies appear dry, rough, wrinkled; these bacteria are very slow growers it takes 4-6 weeks until visible growth appears.

### *Mycobacterium leprae,*

*Mycobacterium leprae*, also known as Hansen's bacillus, mostly found in warm tropical countries, is a bacterium that causes leprosy (Hansen's disease). It is an intracellular, pleomorphic, acid-fast bacterium. *M. leprae* is an aerobic rod-shaped (bacillus) surrounded by the characteristic waxy coating unique to mycobacteria. In size and shape, it closely resembles *Mycobacterium tuberculosis*. Due to its thick waxy coating, *M. leprae* stains with a carbol fuchsin rather than with the traditional Gram stain. These bacilli cannot be cultivated on laboratory media. Diagnosis is based only on direct microscopic examination of acid fast smears of material from the lesions. No serologic tests are of value.