**Morphology**

*M. tuberculosis* is a slender, straight or slightly curved rod with rounded ends, about 3

μm × 0.3 μm, in pairs or as small clumps. The bacilli are non-motile, non-sporing, non

capsulated and acid-fast. They are gram-positive but are difficult to stain.

When stained with carbol fuchsin by the Ziehl-Neelsen method, they resist de

colorization by 20 percent sulfuric acid and absolute alcohol for 10 minutes **(acid and** **alcohol fast).** With this stain, the *Tubercle bacilli* stain **bright red,** while the tissue cells

and other organisms are stained blue (Fig. 1). Organisms in **tissue** and **sputum smears**

often stain irregularly and have a beaded or barred appearance, presumably because of

their **vacuoles** and **polyphosphate** content.

Acid fastness has been ascribed to the presence in the bacillus of **mycoloic acid.** It is

related to the **integrity of the cell** and appears to be a property of the lipid-rich waxy cell

wall. Staining may be uniform or granular. In *M. tuberculosis* beaded or barred forms are

frequently seen*,* but *M. bovis* stains more uniformly. *M. bovis* appear straighter, bolder

and shorter with uniform staining.

**Figure-1: *Mycobacterium tuberculosis* in Ziehl-Neelsen stained smear**

**Cultural Characteristics**

*M. tuberculosis* is an **obligate aerobe** while *M. bovis* is **microaerophilic** on primary

isolation, becoming aerobic on subculture. The optimal growth temperature of tubercle

bacilli is 35 to 37°C but they fail to grow at 25°C or 41°C. Most other mycobacteria grow

at one or other, or both, of these temperatures. Optimum pH is 6.4 to 7.0. The bacilli grow

slowly, the **generation time** *in vitro* being **14** to **15** hours. Colonies appear in about **two**

**weeks** and may sometimes take up to **eight weeks**.

The solid medium most widely employed for routine culture is **Lowenstein-Jensen (LJ)**

**medium** without starch.

Human tubercle bacilli produce visible growth on LJ medium in about 2 weeks, although

on primary isolation from clinical material colonies may take up to 8 weeks to appear. On

solid media, *M. tuberculosis* forms **dry**, **rough**, **raised**, **irregular** colonies with a

**wrinkled** surface. They are **creamy white**, **becoming yellowish or buff colored on**

**further incubation**. They are tenacious and not easily emulsified. *Mycobacterium*

*tuberculosis* has a luxuriant growth **(eugenic growth***)* as compared to *Mycobacterium*

*bovis* which grows poorly on LJ glycerol medium **(dysgenic growth)** and colonies, in

comparison are **flat** , **smooth**, **moist**, **white** and **break up** easily when touched. The

growth of *M. bovis* is much better on LJ pyruvate medium (media containing sodium

pyruvate in place of glycerol).

**Antigenic Structure**

Mycobactria contain many unique immune-reactive substances, most of which are

components of the cell wall. Mycobacteria possess two types of antigens, **cell wall**

(insoluble) and **cytoplasmic** (soluble) antigens.