Mycobacteria

- More than 100 spp.: Medically important mycobacteria include:

1. Those that cause tuberculosis (referred as Mycobacterium tuberculosis complex –consisting of *M tuberculosis*, *M bovis*, *M africanum*, *M microti*, *M canettii*).

2. *M leprae* which cause leprosy or Hansen disease.

3. nontuberculous mycobacteria (NTM) or mycobacteria other than tuberculosis (MOTT) cause disease called Mycobacteriosis (those organism live in soil and water).

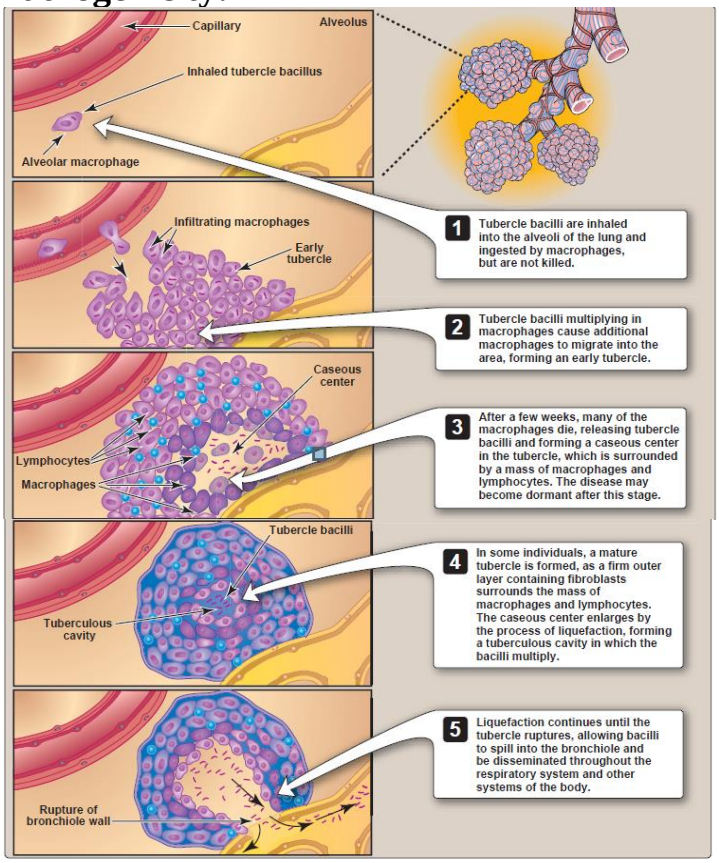
- long, slender rods that are nonmotile and do not form spores.
- cell walls are composed of 60 percent lipid, including a unique class of very long–chain (75 to 90 carbons), β-hydroxylated fatty acids (mycolic acids). These complex with a variety of polysaccharides and peptides, creating a waxy cell surface that makes mycobacteria strongly hydrophobic and accounts for their acid-fast staining characteristic. Mycobacteria are also resistant to drying but not to heat or ultraviolet irradiation.
- Some mycobacterium spp. grows on simple media, some are fastidious, and some (Leprae) do not grow at all.
- Mycobacteria are strictly aerobic. Most species grow slowly with generation times of 8 to 24 hours.

Mycobacteria survive and replicate intracellularly. Mycobacterial infections generally result in the formation of slow-growing granulomatous lesions that are responsible for major tissue destruction.

A. **Mycobacterium tuberculosis**

- **Tuberculosis (Pulmonary, Tuberculous meningitis, Miliary tuberculosis, Renal and urogenital tuberculosis, Bone and joint tuberculosis)**
- It is currently estimated that about one third of the world's population is infected with M. tuberculosis (tubercle bacillus), with 30 million people having active disease.
- Patients with active pulmonary tuberculosis shed large numbers of organisms by coughing, creating aerosol droplet nuclei. Because of resistance to desiccation, the organisms can remain viable as droplet nuclei suspended in room air for at least 30 minutes.
- The principal mode of transmission from person-to-person is by inhalation of the aerosol. A single infected person can pass the organism to numerous people in an exposed group, such as a family, classroom, or hospital ward without proper isolation.

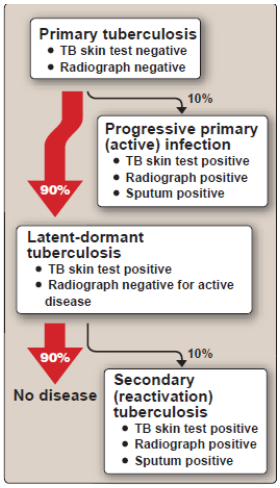
Pathogenicity:

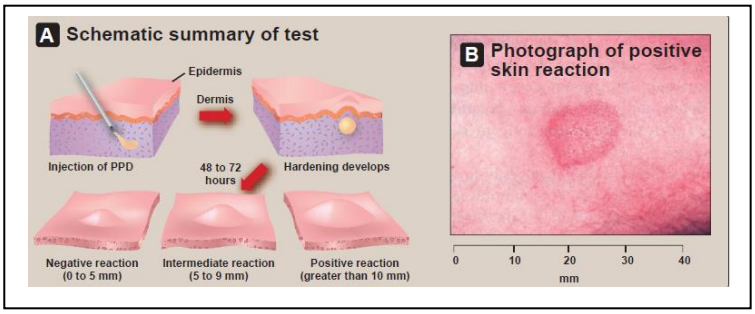


The virulence of M. tuberculosis rests with its ability to survive and grow within host cells. Although the organism produces no demonstrable toxins, when engulfed by macrophages, bacterial sulfolipids inhibit the fusion of phagocytic vesicles with lysosomes.

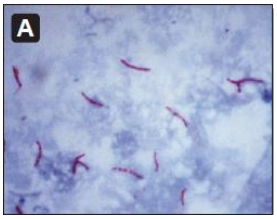
Immunity: M. tuberculosis stimulates both a humoral and a cell mediated immune response. Although circulating antibodies appear, they do not convey resistance to the organism. Instead, cellular immunity (CD4+ T cells) and the accompanying delayed hypersensitivity directed against a number of bacterial protein antigens, develop in the course of infection and contribute to both the pathology of and immunity to the disease.

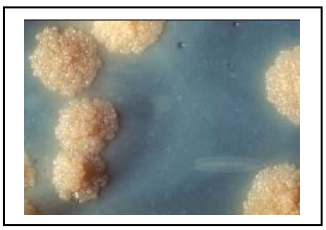
Clinical significance: Primary tuberculosis occurs in a person who has had no previous contact with the organism. For the majority of cases (90-95 percent), the infection becomes arrested, and most people are unaware of this initial encounter. The only evidence of tuberculosis may be a positive tuberculin test. Approximately 10 percent of those with an arrested primary infection develop clinical tuberculosis at some later time in their lives.





Laboratory identification: Diagnosis of active pulmonary tuberculosis includes demonstration of clinical symptoms and abnormal chest radiographs and confirmation by isolation of M. tuberculosis from relevant clinical material.





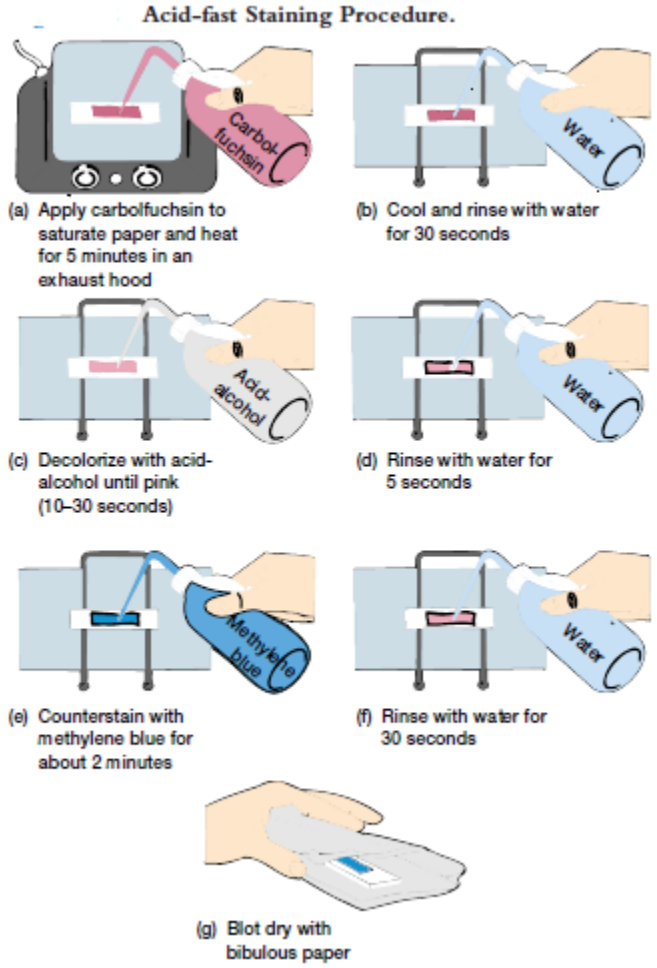
Specimens:
Sputum, not saliva is required to detect AFB. Examination of up to three specimens (at least one as an early morning specimen) may be required to detect the organisms.
In AIDS patients, it is sometimes possible to detect AFB in buffy coat smears prepared from EDTA anticoagulated blood. Cerebrospinal fluid is required to investigate tuberculous meningitis.

Caution: M. tuberculosis is a highly infectious pathogen, therefore handle specimens with care. It is particularly important to minimize the creation of aerosols and to ensure the laboratory is well ventilated. The use of personal respirators should be considered to protect staff working with M. tuberculosis.
Use a hooded Bunsen burner when flaming wire loops. Smears however, can be more easily made using a wooden stick which can be easily discarded and incinerated after use.

AFS:
Concentrating AFB by bleach treating sputum followed by centrifugation, significantly increases the sensitivity of direct microscopy in detecting

AFB in sputum. Bleach treated sputum cannot be used for culture. To be detected microscopically, sputum smears need to contain 5000–10 000 AFB/ml which may be found in samples from patients with cavity lesions.

The cell wall lipids make the cell surface hydrophobic, rendering mycobacteria resistant to staining with basic aniline dyes unless they are applied with heat or detergents, or for prolonged periods of time. Once stained, however, mycobacteria resist decolorization with a mixture of 3% hydrochloric acid and 95% ethanol. These properties are described as **acid fastness** or, more properly, acid–alcohol fastness



Growth requirements

Specimens such as sputum, urine, and pus which contain commensals, require decontamination before being cultured for *M. tuberculosis*. For routine purposes, sodium hydroxide, 40 g/l has been found to be an effective decontaminant.

M. tuberculosis will grow aerobically on a protein enriched medium, e.g. Lowenstein Jensen egg medium. The optimal temperature for growth is 35–37 ºC. The organism is slow-growing.

When cultured on Lowenstein Jensen medium at 35–37 ºC, *M. tuberculosis* produces raised, dry, cream (buff) coloured colonies. Visible colonies are usually produced 2–3 weeks after incubation, but
cultures should be incubated for up to 6 weeks before being discarded.

WHO recommendations for chemotherapy of tuberculosis
Short course antituberculosis therapy recommended by WHO consists of an initial 2 month intensive treatment with rifampicin, isoniazid and pyrazinamide. Ethambutol or streptomycin is added if resistance to one of the former drugs is common in a given region or if twice or thrice weekly therapy is indicated. This phase is followed by a 4 month continuation
phase of rifampicin and isoniazid. WHO recommends the use of directly observed therapy short course (DOTS) to ensure complete cure and prevent multi-drug resistance.

Prevention:
At present, the bacillus Calmette-Guérin (BCG) vaccine (named for its originators, Calmette and Guérin) is the only available vaccine. It has been used for prophylaxis of tuberculosis in various countries since 1923; administration is usually intradermal. It is a **live** vaccine derived originally from a strain of ***M. bovis*** that was **attenuated by repeated subculture**.
This vaccine, according to most studies, **decreased the highly lethal miliary and meningeal forms of tuberculosis among young children.**

Tuberculin reaction: The tuberculin reaction test is a manifestation of delayed hypersensitivity to protein antigens of M. tuberculosis.
Although such tests can be used to document contact with the tubercle bacillus, they do not confirm that the patient currently has active disease. In the Mantoux test, purified protein derivative (PPD) is prepared from culture filtrates of the organism.
A positive reaction usually develops 4 to 6 weeks after initial contact with the organism. It remains positive for life, although it may wane after some years or in the presence of immunosuppression by medications or disease.

An induration of >5 mm is interpreted as positive in the following populations:

- Persons who have had contact with infectious individuals
- Persons with an abnormal chest radiograph
- HIV-infected and other immuno-suppressed persons

An induration of >10 mm is interpreted as positive in the following populations:

- Foreign-born persons from high-prevalence countries
- Residents of prisons, nursing homes, and other institutions
- Healthcare workers
- Persons with other medical risk factors

An induration of >15 mm is interpreted as positive in the following populations:

- Persons with no risk factors