Mycobacterium species

Represented by:-
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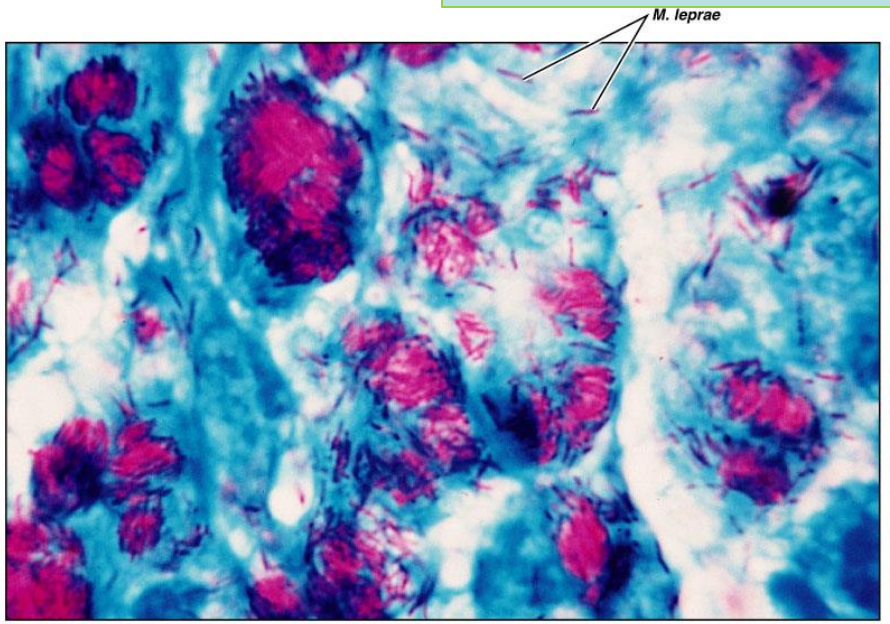
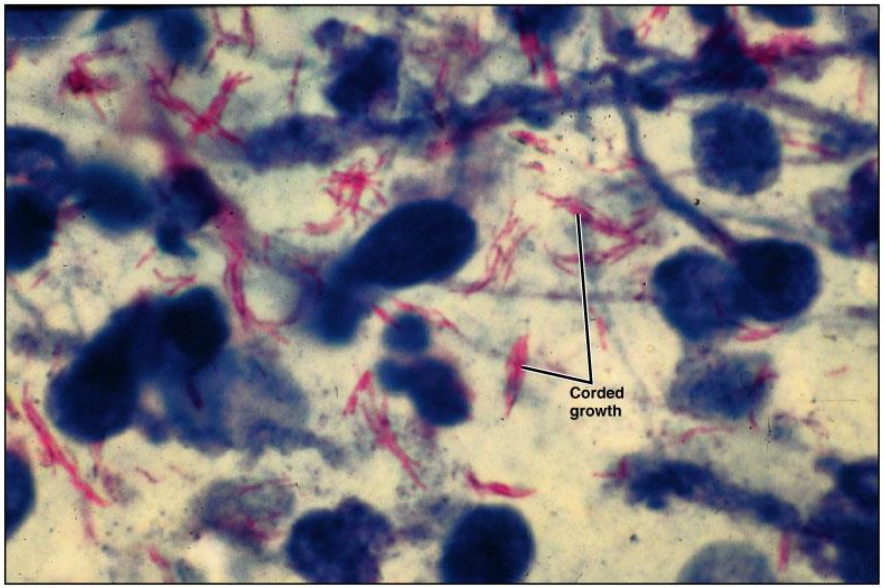




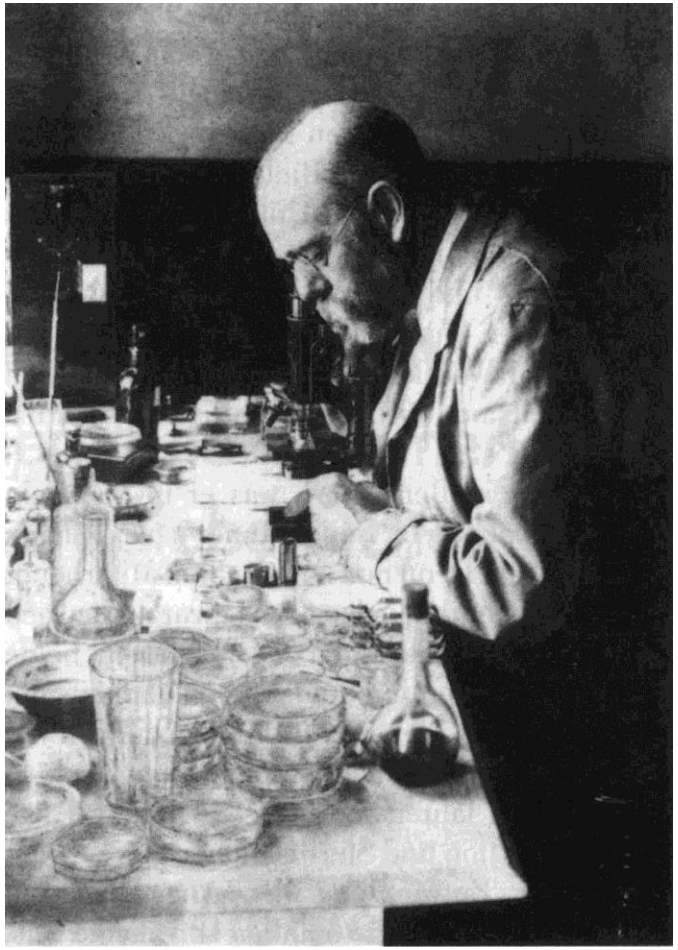
Important Human Pathogens

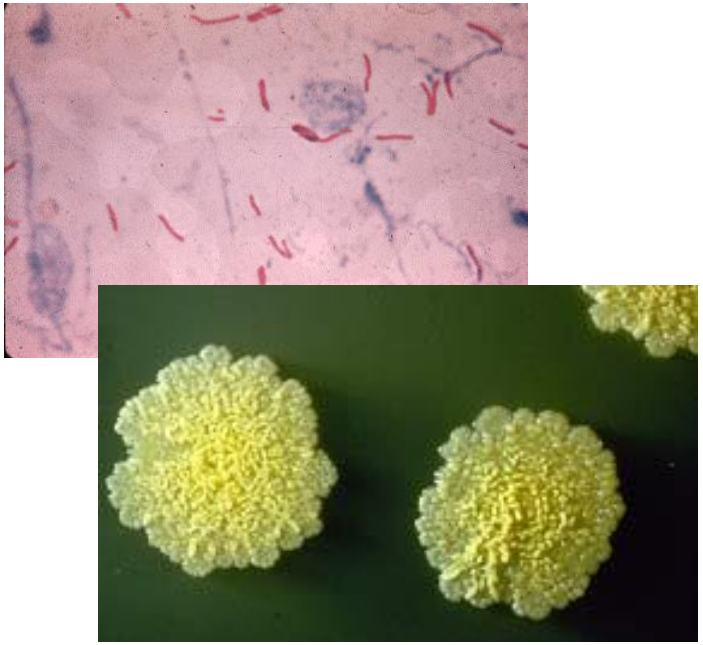
Mycobacterium tuberculosis

Mycobacterium leprae (uncommon)

Mycobacterium avium-intracellulaire Complex (MAC) or (M. avium)

M tuberculosis as causative agent for tuberculosis





Robert Koch

The pathogens include the organisms responsible for human and bovine **tuberculosis** and for **leprosy**.

It is convenient to divide mycobacteria of clinical interest into:

1-*Mycobacterium tuberculosis* complex (MTC) which includes *M. tuberculosis*, *M bovis*, BCG, *M. africanum*, and ***M. microti***. These mycobacteria associated with tuberculosis and they are always pathogenic for man and animal

2-Mycobacteria other than tuberculosis bacilli (MOTT)

which associated with human disease also these mycobacterial spp. Called **atypical**, **anonymous**, **non tuberculosis, tuberculoid bacilli.**

Diseases caused by *Mycobacterium* species

M. tuberculosis **– tuberculosis**

M. leprae **– leprosy**

M. avium **- lung and skin infections in immunocompromised hosts**

General Characteristics

- Slender, slightly curved or straight rod-shaped organisms
- Non-motile
- Do not form spores
- Cell wall with extremely high lipid content
  - Staining requires longer time or application of heat
  - Once stained, resist decolorization with acid-alcohol (acid-fast)

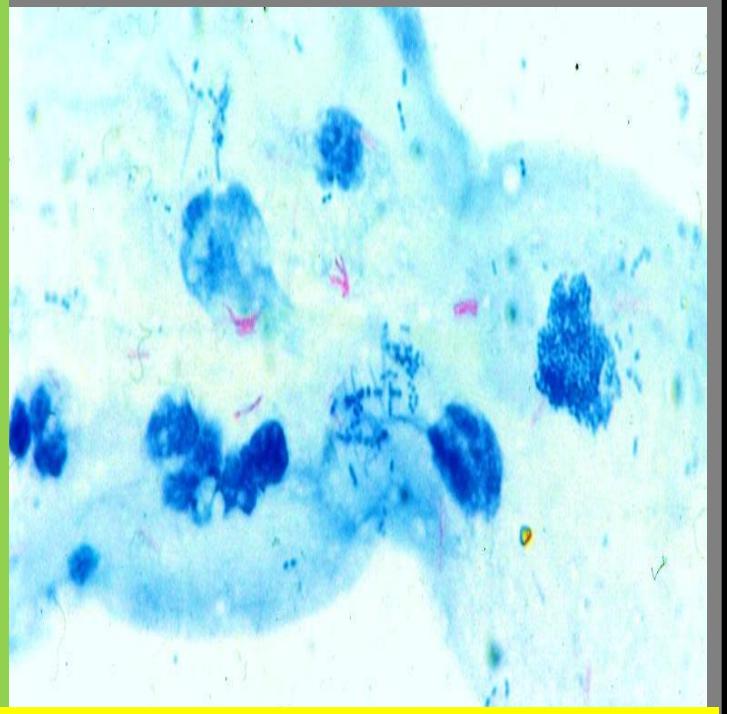
Laboratory Diagnosis

- ***Specimens: include sputum,***

- ***Early morning sputum specimens collected on 3 consecutive days, from a deep productive cough, give the best results***

Laboratory Diagnosis

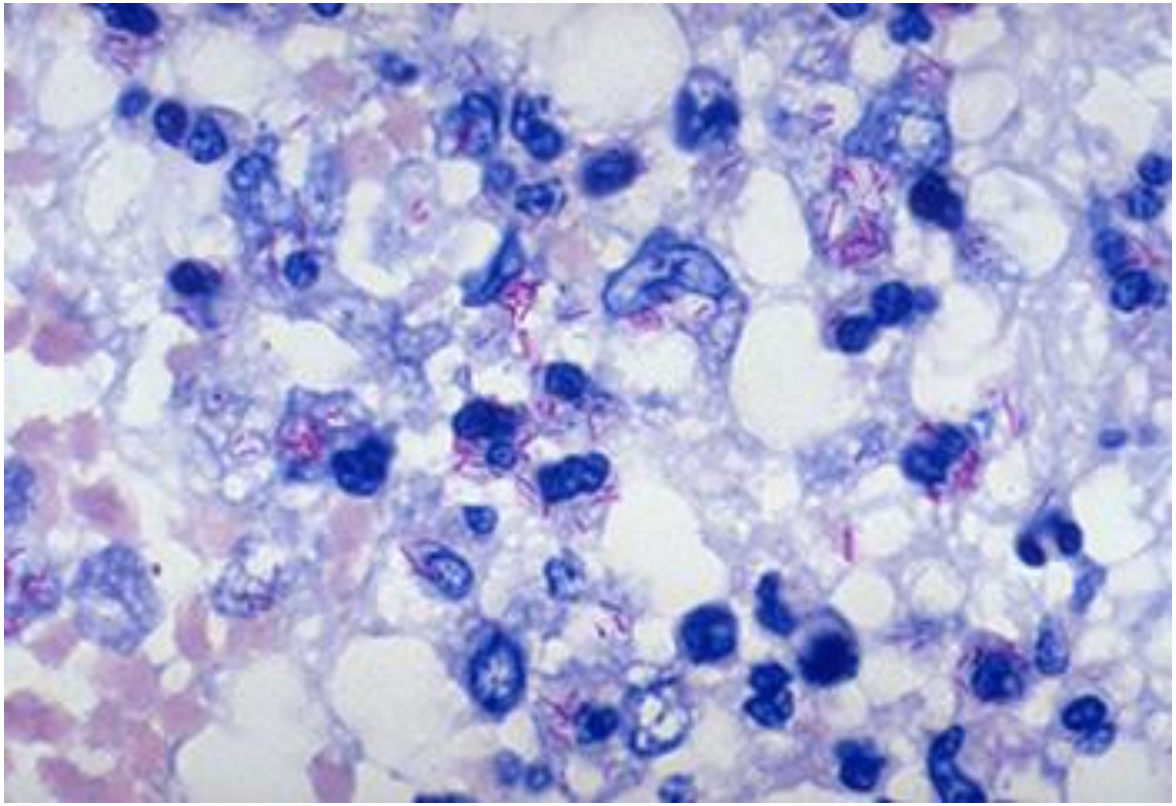
- ***Direct Detection:***
  1. *Smears: Ziehl-Neelsen (Z-N). Under the ordinary light microscope, AFB appear pink in a blue background*



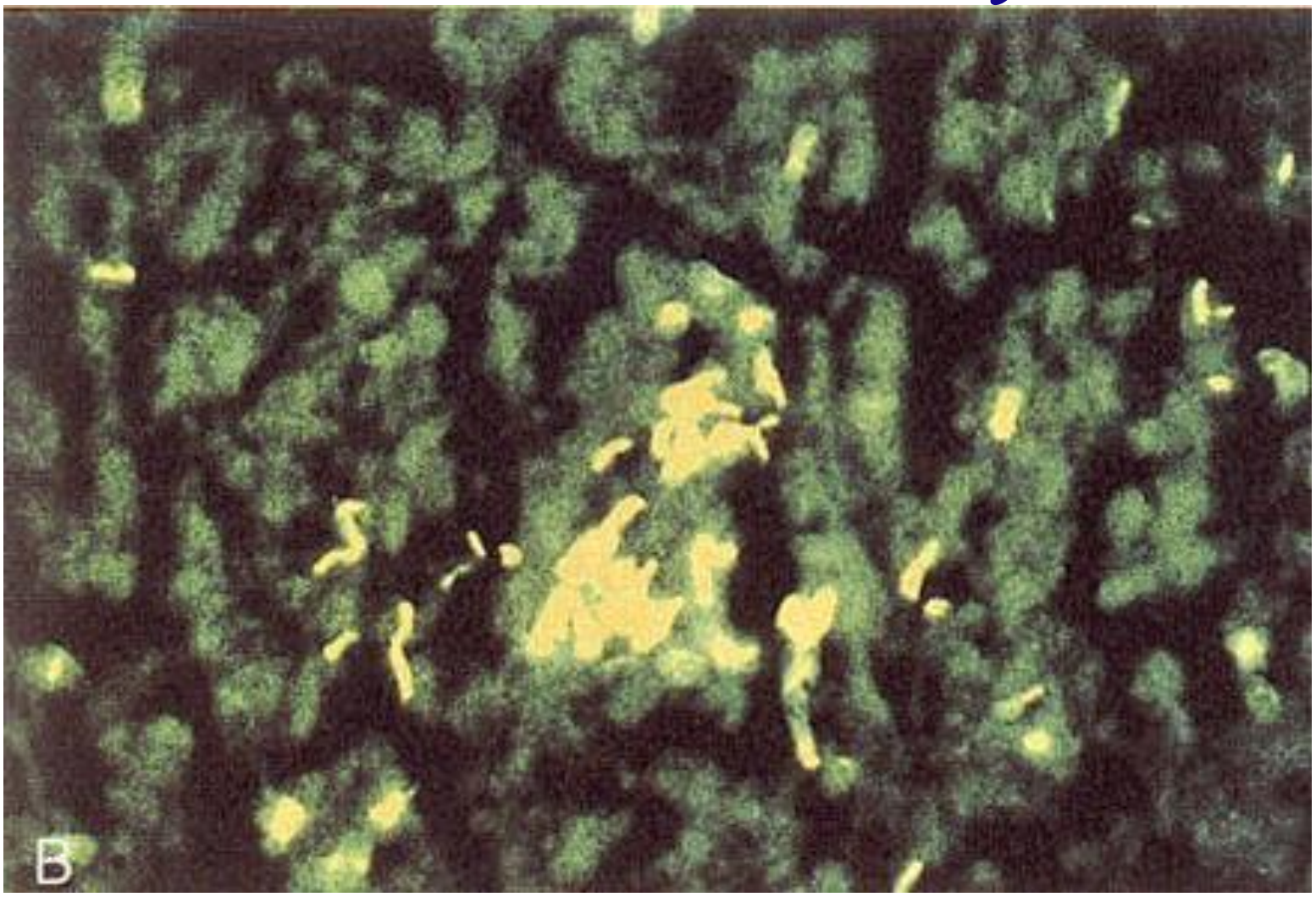
2. Molecular tests: • For rapid detection.

- M.TB. is not classified **as either Gram + or Gram-** because it does not have the chemical characteristics of either, although the bacteria do contain peptidoglycan (murein) in their cell wall. If a Gram stain is performed on M.TB., it stains very weakly Gram-positive or not at all (referred to as "ghosts").

- The bacilli will appear red in sharp contrast to the blue colour stained background .



Mycobacterium Tuberculosis Stained with Fluorescent Dye



Laboratory Diagnosis

- ***Cultivation:***

- *Media include: - Lowenstein-Jensen (L-J) medium – Middlebrook's medium*

Incubation is at 35-37 °C in 5-10% CO2 for up to 8 weeks.

- *If culture negative and acid-fast Positive , a set of inoculated media should be incubated at 24-33°C for 12 weeks.*

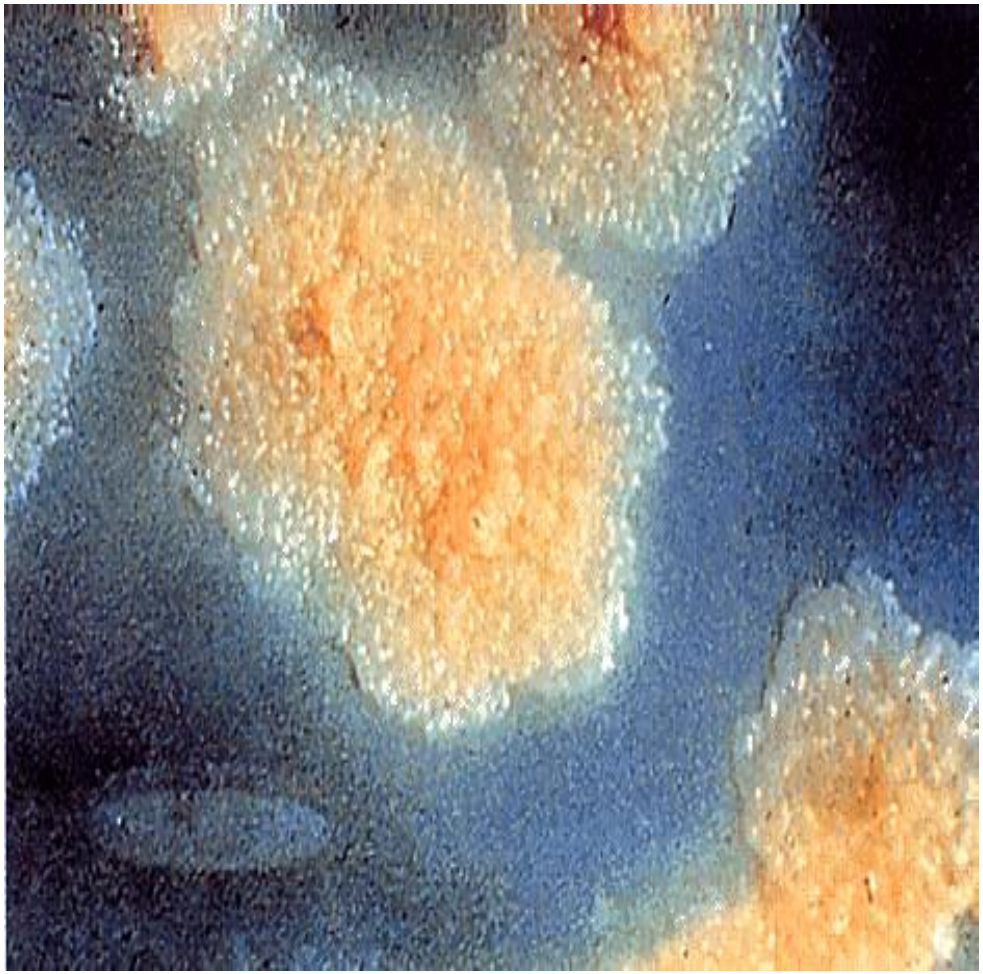
- **Medically important mycobacterial species grow slowly. Their generation (doubling) time ranges from a few hours to >2 days, compared to 40–60 minutes for most other bacteria.**

- **Colonies may take 2–3 weeks to develop from the time of inoculation of culture media.**

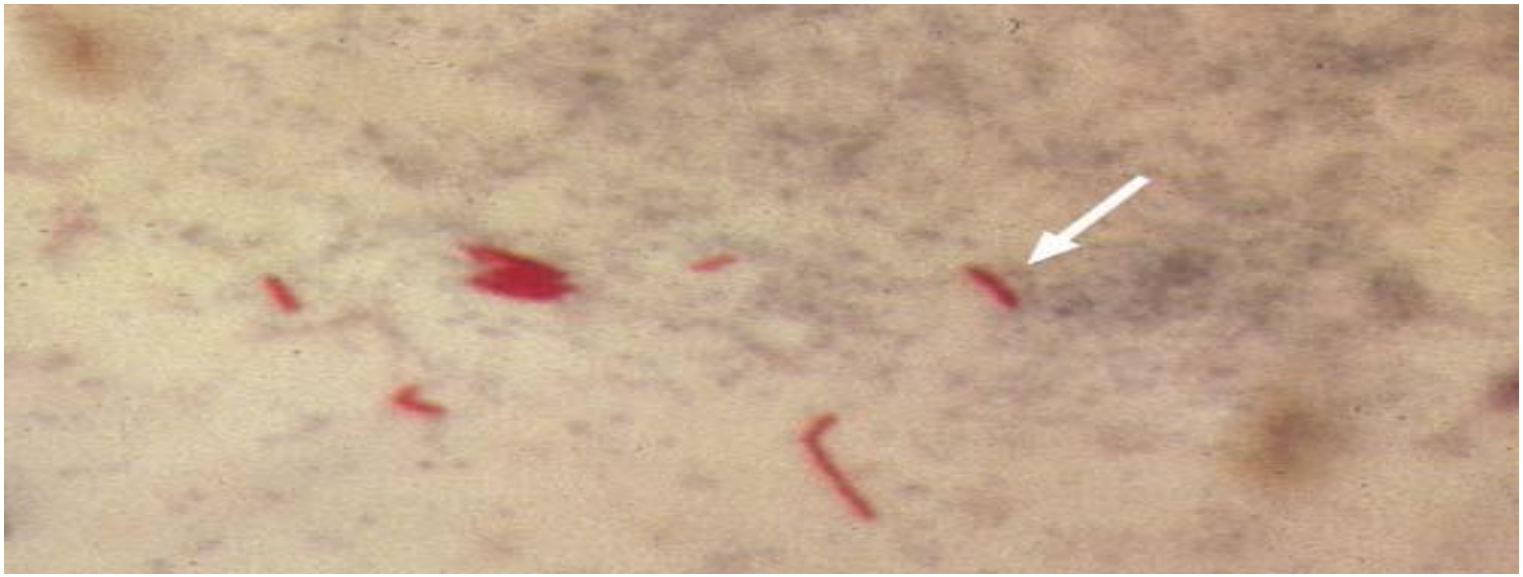
- **Mycobacteria do not grow on ordinary laboratory media but grow well on Löwenstein-Jensen (LJ) medium, containing egg, asparagine, glycerol and malachite green. The latter inhibits contaminants. Two media are used to grow M.TB. Middlebrook's medium**

	Colonial morphology on	
	Lowenstein-Jensen medium	**Stonebrink's medium**
M. tuberculosis	**Rough**=dry and irregular surface **Tough**=Hard and difficult to emulsify **Buff**=in colour (creamy to light yellow)	The same characteristics are shown on this medium

Eight Week Growth of Mycobacterium tuberculosis on Lowenstein-Jensen Agar



In order to detect Mycobacterium tuberculosis in a sputum sample, in excess of 10,000 organisms per ml of sputum are needed to visualize the bacilli with a 100X microscope objective. One acid-fast bacillus/slide is regarded as "suspicious" of an M.TB. infection.



Mycobacterium tuberculosis. **Acid-fast stain.**

Cell Wall Structure

Over 60% of the mycobacterial cell wall is lipid. The lipid fraction of M.TB's cell wall consists of three major components.

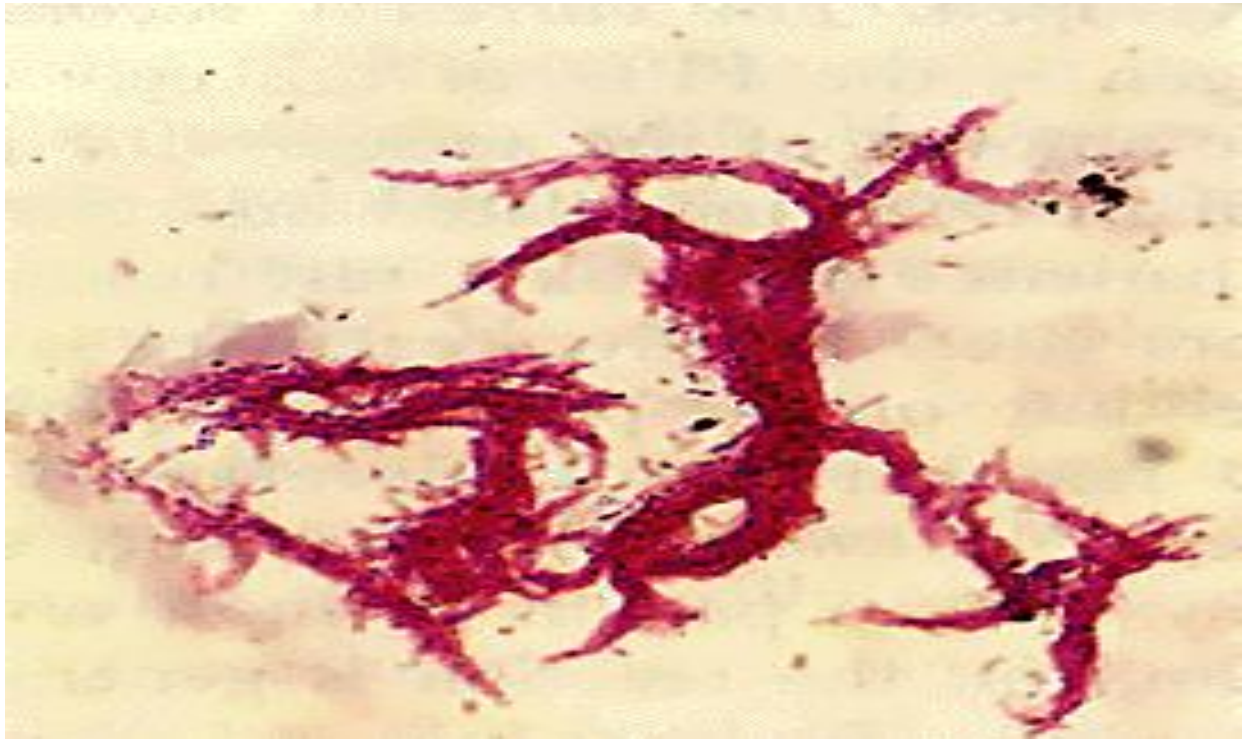
Mycolic acids are unique alpha-branched lipids found in cell walls of Mycobacterium and Corynebacterium.

Mycolic Acids are thought to be a significant determinant of virulence in M.TB. Probably, they prevent attack of the mycobacteria by cationic proteins, lysozyme and oxygen radicals in the phagocytic granule. They also protect extracellular mycobacteria from complement deposition in serum.

Cord Factor is responsible for the serpentine cording (chains of cells form distinctive serpentine cord). Cord factor is toxic to mammalian cells and is also an inhibitor of PMN migration. Cord factor is most abundantly produced in virulent strains of M.TB.

Wax-D in the cell envelope

❑When smear is prepared from **culture**, the bacilli appear short, thick and tend to arrange in a sort of bundles (a phenomenon referred to as a **cord formation** due to the cord factor.



NOTE: cord growth (serpentine arrangement) of virulent strains

In summary, the high concentration of lipids in the cell wall of Mycobacterium tuberculosis has been associated with these properties of the bacterium:

- Impermeability to stains and dyes
- Resistance to many antibiotics
- Resistance to killing by acidic and alkaline compounds
- Resistance to osmotic lysis via complement deposition
- **Resistance to lethal oxidations and survival inside of macrophages**

Predisposing factors for TB infection include:

- Close contact with large populations of people, i.e., schools, nursing homes, dormitories, prisons, etc.
- Poor nutrition
- IV drug use
- Alcoholism

HIV infection is the predisposing factor for M.TB. infection.

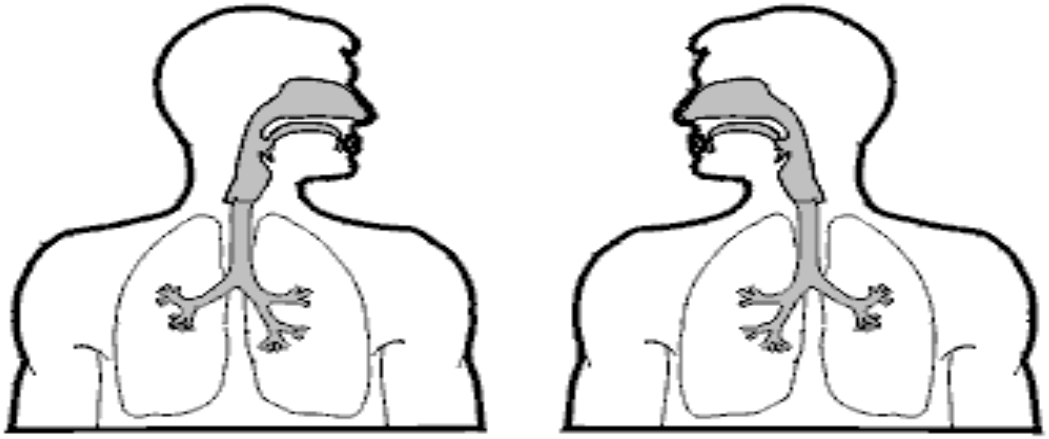
Tuberculosis: Infection vs Disease

TB disease in lungs	TB Infection
M.TB. present	M.TB. present
Tuberculin skin test positive	Tuberculin skin test positive
Chest X-ray usually reveals lesion	Chest X-ray normal
Sputum smears and cultures positive	Sputum smears and cultures negative
Symptoms such as cough, fever, weight loss	No symptoms
Often infectious before treatment	Not infectious
Defined as a case of TB	Not defined as a case of TB

Stages of the Disease:-

Stage 1

Droplet nucleiare inhaled



Spread of droplet nuclei from one individual to another. After droplet nuclei are inhaled, the bacteria are nonspecifically taken up by alveolar macrophages. However, the macrophages are not activated and are unable to destroy the intracellular organisms

Stage 2

Begins 7-21 days after initial infection. M.TB. multiplies virtually unrestricted within inactivated macrophages until the macrophages burst.

Stage 3

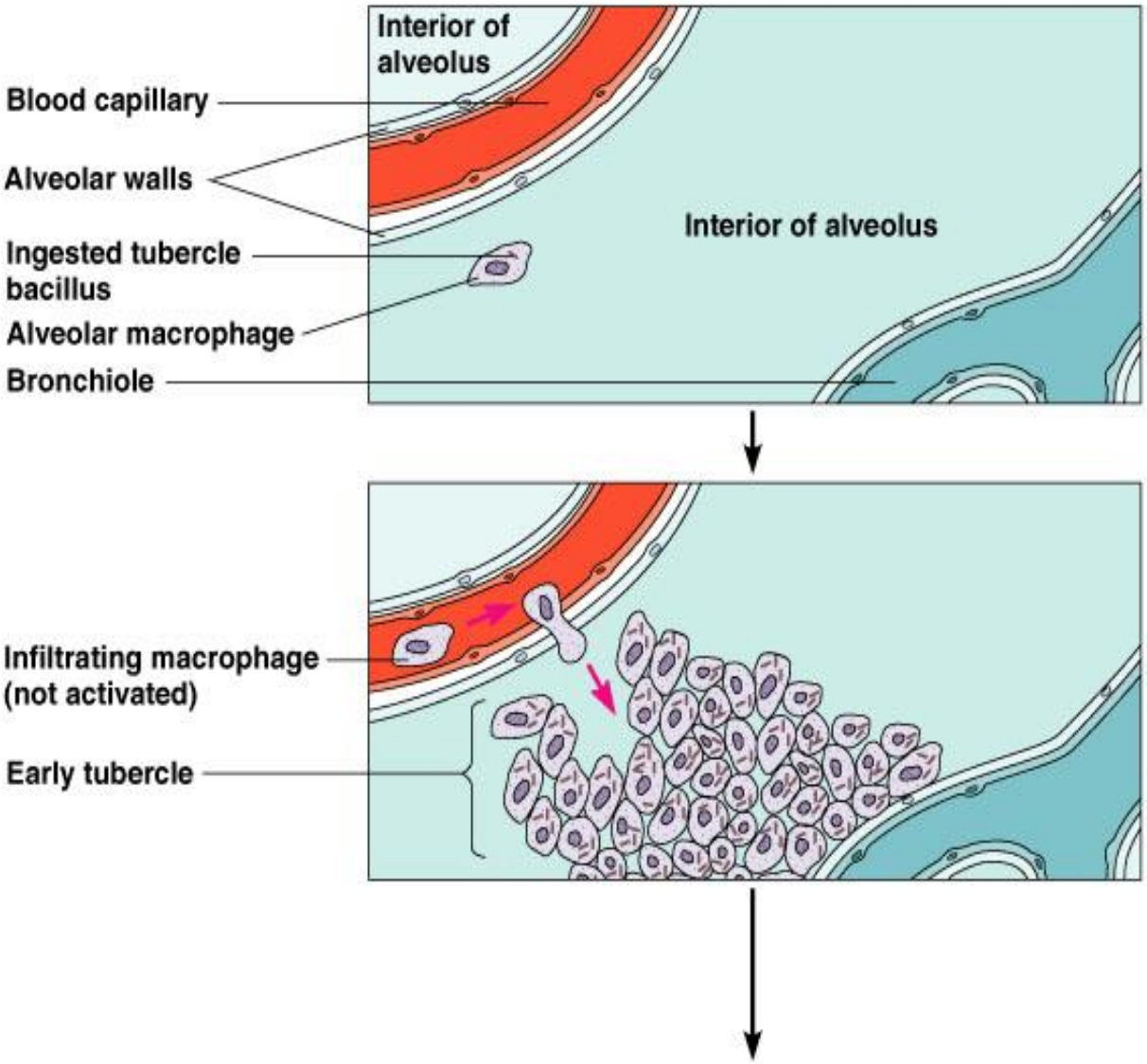
lymphocytes begin to infiltrate.

The lymphocytes, specifically T-cells, recognize processed and presented M.TB

T-cell activation and the liberation of cytokines including gamma interferon (IFN).

tubercle formation begins. The center of the tubercle is characterized by "caseation necrosis" meaning semi-solid or "cheesy" consistency

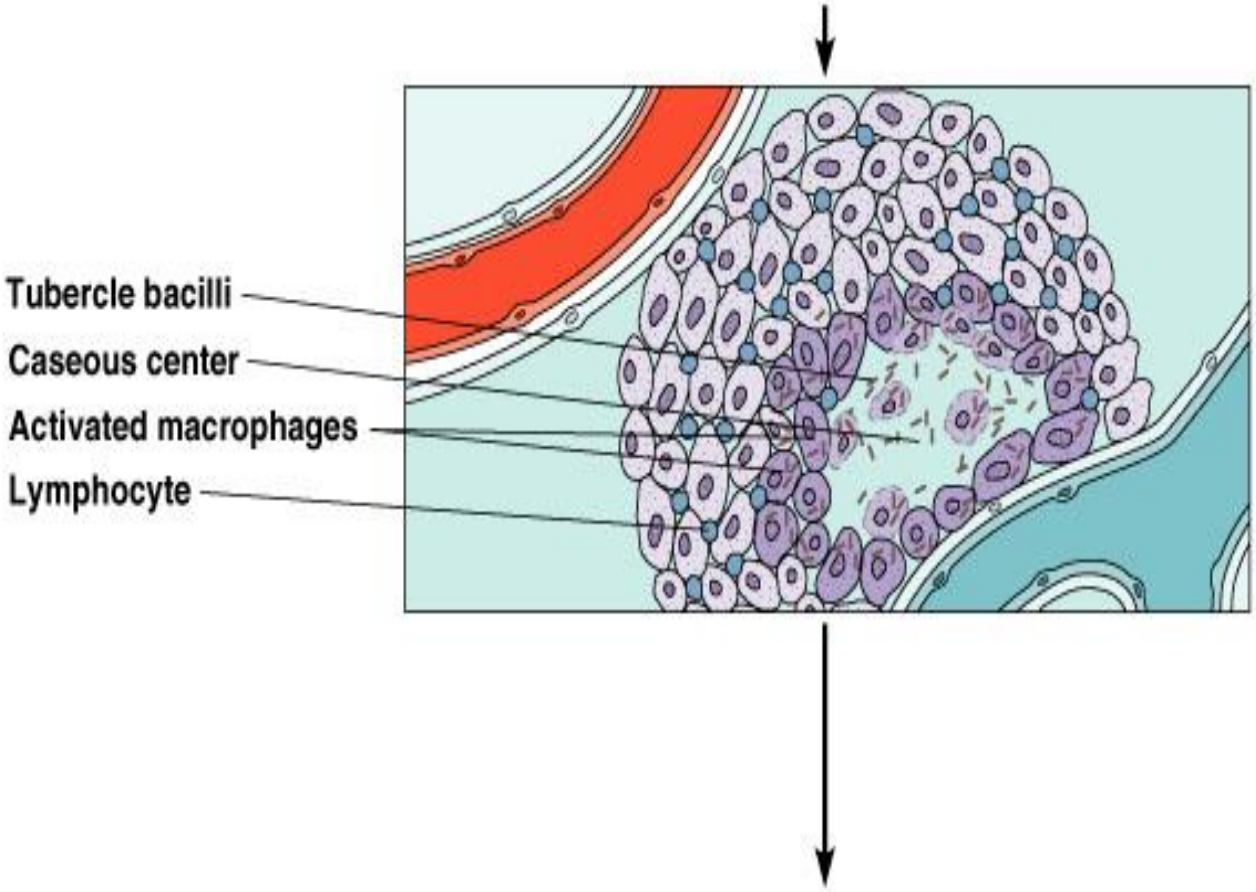
Progression of TB



1. Tubercle bacilli that reach the alveoli of the lung (Figure 24.2) are ingested by macrophages, but some often survive. Infection is present, but no symptoms of disease.

2. Tubercle bacilli multiplying in macrophages cause a chemotactic response that brings additional macrophages and other defensive cells to the area. These form a surrounding layer and, in turn, an early tubercle. Most of the surrounding macrophages are not successful in destroying bacteria but release enzymes and cytokines that cause a lung-damaging inflammation.

Progression of TB



3 After a few weeks, disease symptoms appear as many of the macrophages die, releasing tubercle bacilli and forming a *caseous center* in the tubercle. The aerobic tubercle bacilli do not grow well in this location. However, many remain dormant (latent TB) and serve as a basis for later reactivation of the disease. The disease may be arrested at this stage, and the lesions become calcified.

Stage 4

The growing tubercle may invade a bronchus. If this happens, M.TB. infection can spread to other parts of the lung.

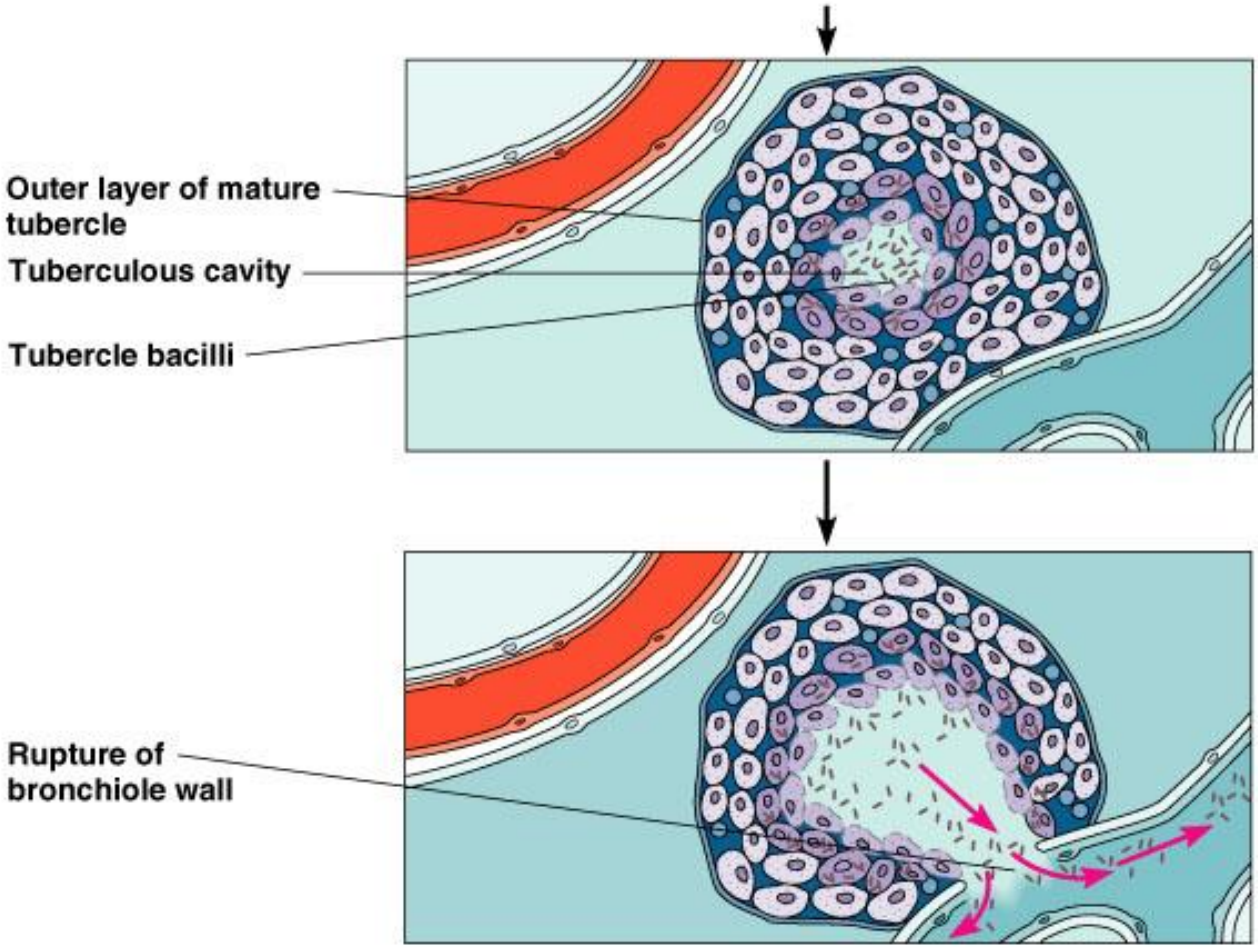
milliary tuberculosis. The name "milliary" is derived from the fact that metastasizing tubercles are about the same size as a millet seed,

Stage 5

The caseous centers of the tubercles liquify.

the walls of nearby bronchi to become necrotic and rupture.

Progression of TB



4 In some individuals, disease symptoms appear, as a mature tubercle is formed. The disease progresses as the caseous center enlarges in the process termed *liquefaction*. The caseous center now enlarges and forms an air-filled *tuberculous cavity* in which the aerobic bacilli multiply outside macrophages.

5 Liquefaction continues until the tubercle ruptures, allowing bacilli to spill into a bronchiole (see Figure 24.2) and thus be disseminated throughout the lungs and then to the circulatory and lymphatic systems.

Symptoms include : fever

coughing (often with blood)

weight loss

malaise (loss of energy)

→ progressive lung damage

Systemic TB

Can infect any area of the body including:

- **Bones and joints**
- **Internal organs**
- **Brain**

Progression of TB

Bacteria in tubercles may survive for decades (latency)

Suppression of immune system may allow bacteria to break out of lesions and multiply (reactivation)

Old age, cancer, immunosuppressive drugs and HIV infection can lead to reactivation

Diagnosis of TB

typical chest X-ray

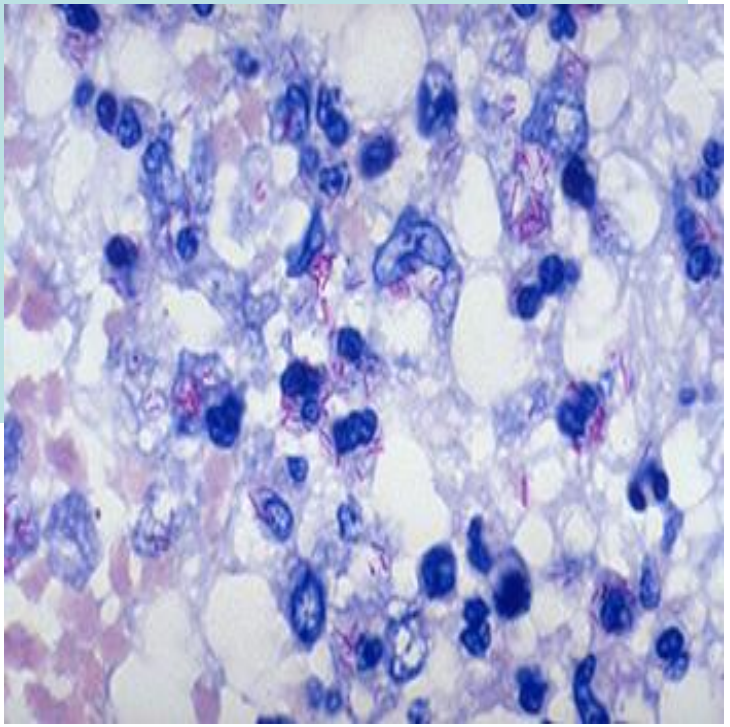
#Acid-fast staining of sputum samples via the Ziehl-Neelsen method

fluorescent microscope is a sensitive but not very specific method of detection

#cultured on LJ slopes

#molecular techniques (e.g. PCR)

#Biopsies

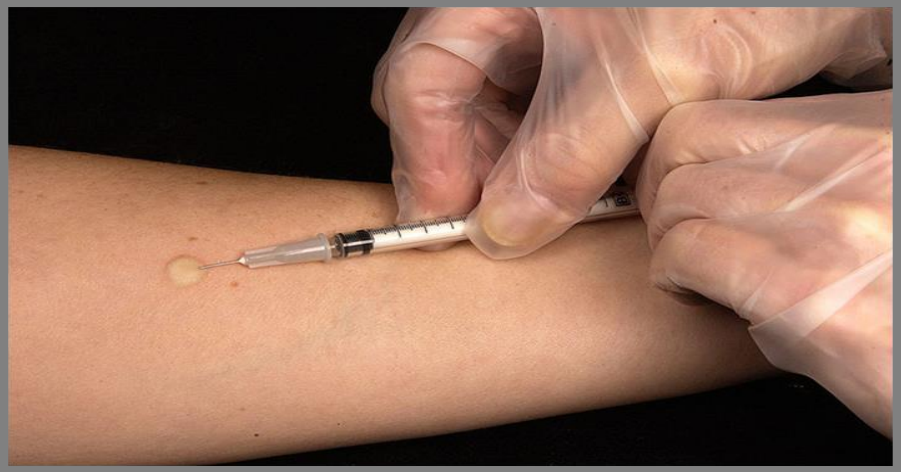


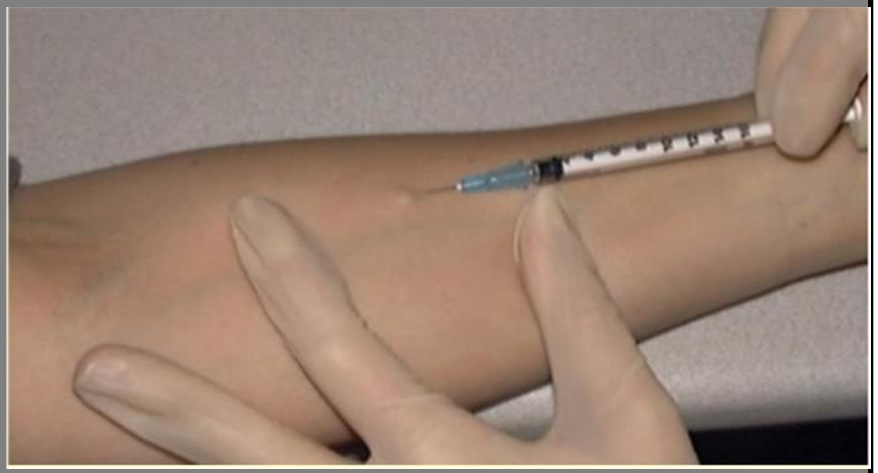
The Mantoux test

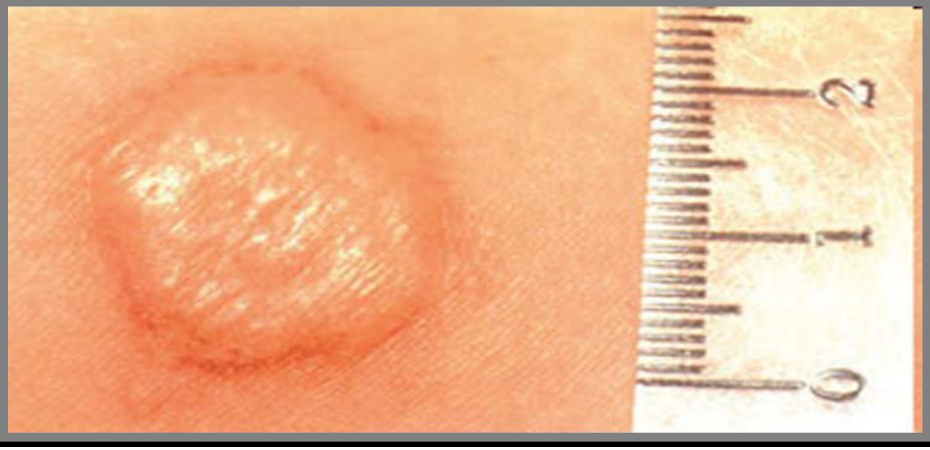
- *also known as the Tuberculin Sensitivity Test, is a diagnostic tool for tuberculosis.*
- *Tuberculin is a glycerol extract of the tubercle bacillus.*
- *A standard dose of 5 Tuberculin units (0.1mL) is injected intradermally and read 48 to 72 hours later.*
- *The reaction is read by measuring the diameter of induration across the forearm in millimeters.*

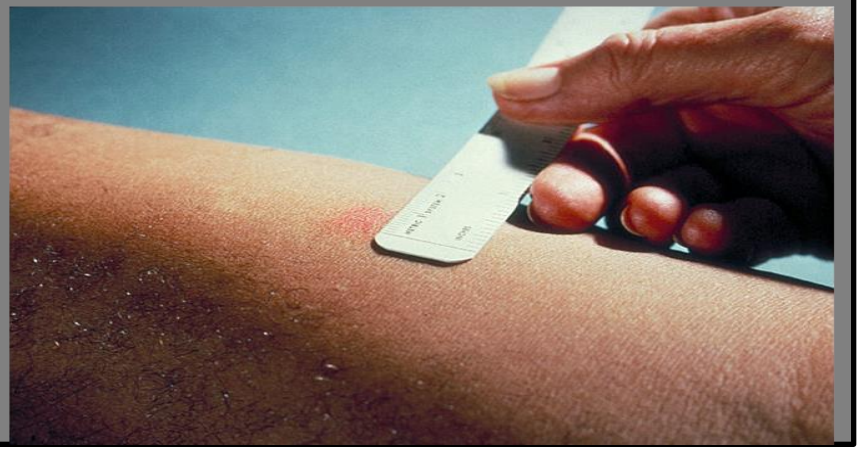
The Mantoux test

- *It is considered positive if the induration 10mm or more in daimeter.*









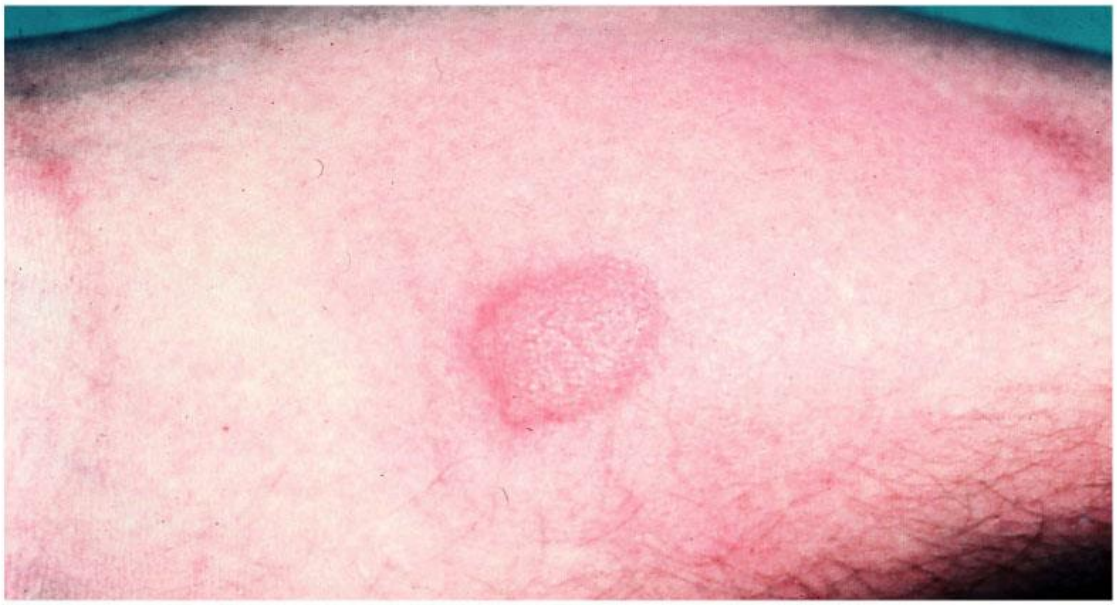
The Mantoux test

- *Apositive test indicates that an individual has been infected in the past.*
- *It does not imply that active disease or immunity to disease is present.*
- *Tuberculin positive person are of risk of developing disease from reactivation of the primary infection.*

Diagnosis of TB

Skin Testing is performed as the tuberulin or Mantoux test. skin test - injection of *M. tuberculosis* proteins **(tuberculin)**

- **positive test** leads to red area at injection site



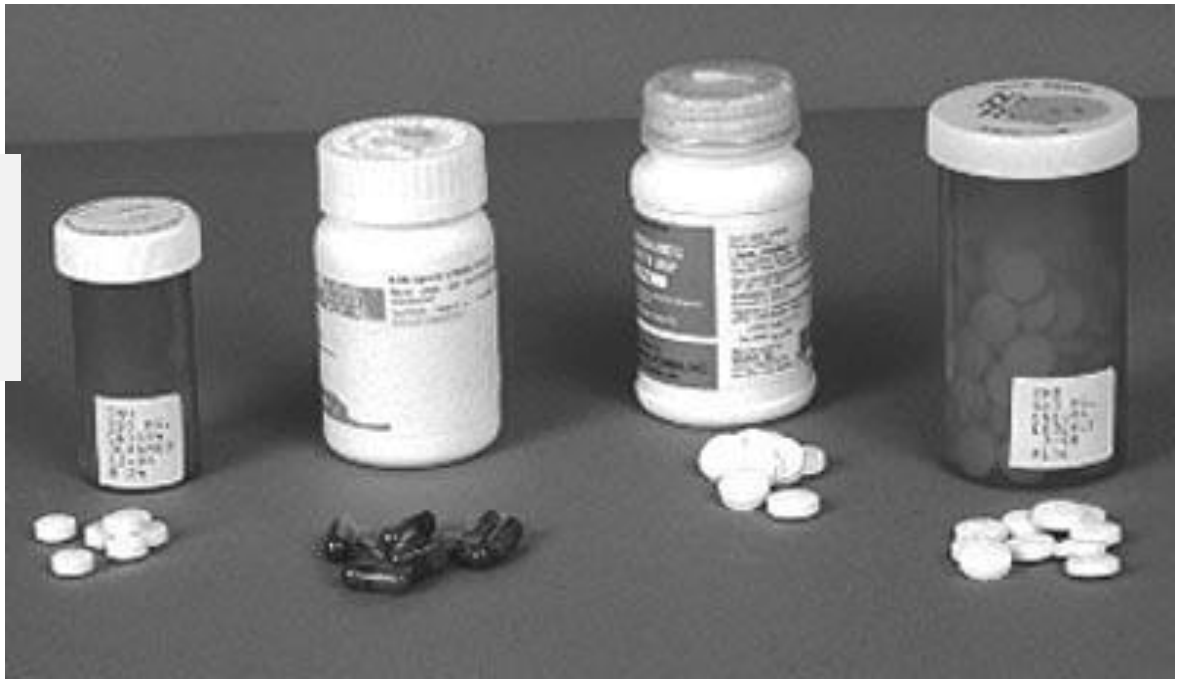
PPD = Purified Protein Derivative from *M. tuberculosis*

BCG (bacille Calmette-Guerin) = attenuated *M. bovis*

Treatment of TB

Treatment requires the use of multiple drugs for long periods of time (6–9 months). Commonly used regimens include 2 months of isoniazid, rifampicin, pyrazinamide and ethambutol followed by 4–6 months of the first two drugs alone.

Multi-drug resistant M. tuberculosis is a major public health problem worldwide.



Drugs used to treat TB disease. From left to right isoniazid, rifampin, pyrazinamide, and ethambutol

Prevention

A vaccine against M.TB. is available.

It is called BCG (Bacillus of Calmette and Guerin).

BCG consists of a live attenuated strain derived from Mycobacterium bovis.

The vaccine is not 100% effective.

Mycobacterium avium

Also known as M avium intracellulare MAI.

These organism grow optimally at 41^{0}C and produce smooth, soft, nonpigmented colonies.

Cultured from water, soil, food, and animals, including birds.

Cause disease in immunocomptent human.

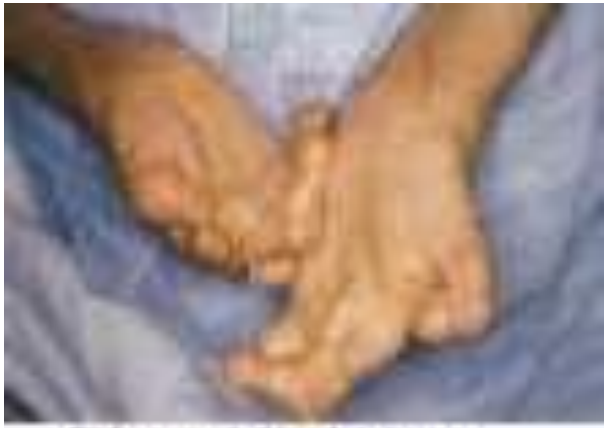
Mycobacterium leprae

- *Typical acid-fast bacilli, singly, in parallel bundles, or in globular masses,*
- *It cause leprosy.*
- *Daignosis: by scraping from skin or nasal mucosa are smeared a slide and stained by the Ziel-Neelsen technique.*
- *Biopsy of skin for histological study.*

Mycobacterium leprae

- Causes leprosy or Hansen's Disease
- Infection of the skin, mucous membranes and peripheral nerves
- Most cases are from warm climates
- Bacteria infect the cooler areas of the body (ears, nose, eyebrows, fingers, toes)
- Diagnosis made from finding acid-fast bacilli in scrapings from lesions
- Not culturable, except in mouse foot pads

Mycobacterium leprae



Disfigurement of hands in leprosy.
M.G.Lebwohl, Atlas of the Skin
and Systemic Diseases, 1995

Mycobacterium leprae Infections

Physiology and Structure

Weakly gram-positive, strongly acid-fast bacilli.

Lipid-rich cell wall.

Unable to be cultured on artificial media.

Diagnosis made with specific skin test (tuberculoid form of disease) or acid-fast stain (lepromatous form).

Virulence

Capable of intracellular growth.

Disease primarily from host response to infection.

Epidemiology

Rare in United States but common in other countries (e.g., Asia, Africa).

Armadillos are naturally infected and represent an indigenous reservoir.

Lepromatous form of disease, but not the tuberculoid form, is highly infectious.

Person-to-person spread by direct contact or inhalation of infectious aerosols.

People in close contact with patients who have lepromatous disease are at greatest risk.

Mycobacterium leprae Infections (cont.)

Diseases

Tuberculoid form of leprosy.

Lepromatous form of leprosy.

Intermediate forms of leprosy.

Diagnosis

Microscopy is sensitive for the lepromatous form but not the tuberculoid form.

Skin testing required to confirm tuberculoid leprosy. Culture cannot be used.

Treatment, Prevention, and Control

Dapsone with or without rifampin is used to treat the tuberculoid form of disease; clofazimine is added for the treatment of the lepromatous form. Therapy is prolonged.

Dapsone is recommended for long-term prophylaxis in treated patients.

Disease is controlled through the prompt recognition and treatment of infected people.

Thanks for your Attention!