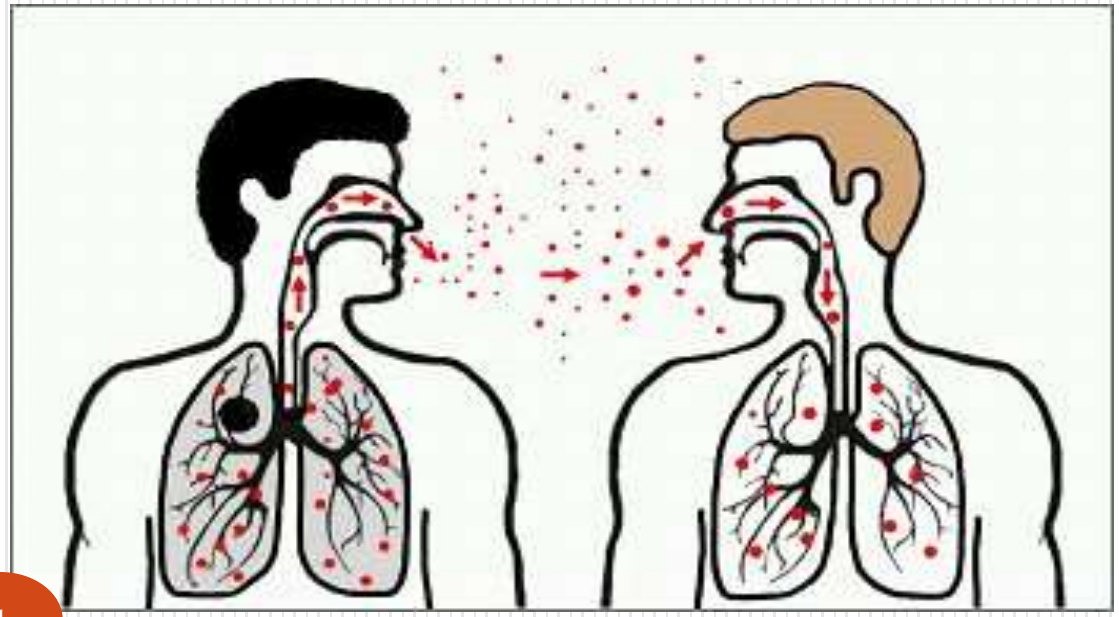
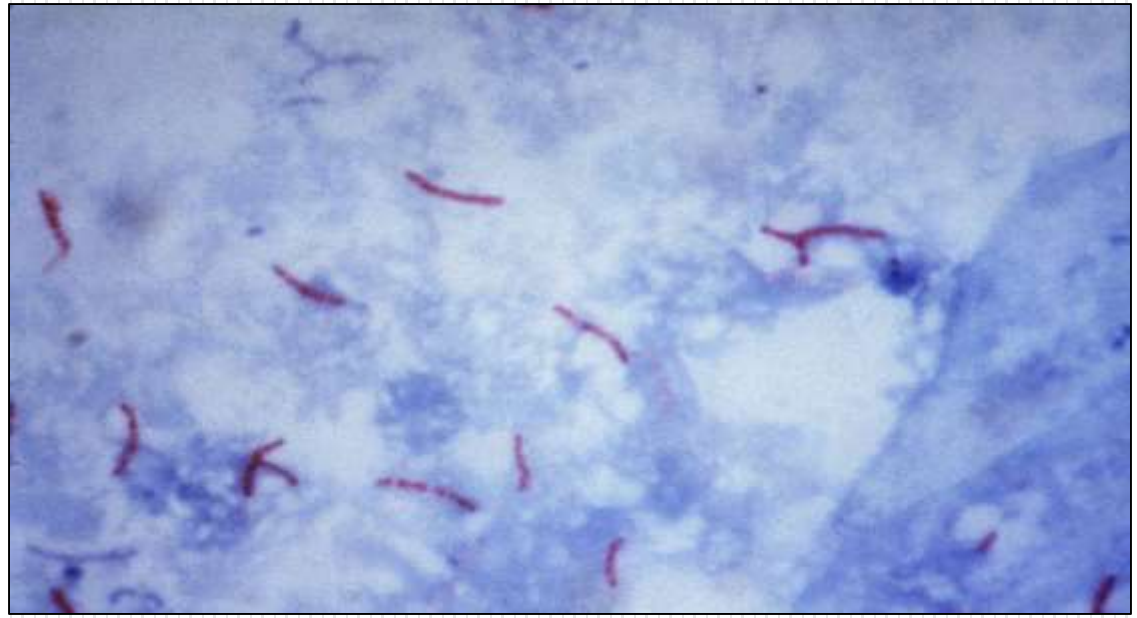
PULMONARY TUBERCULOSIS

MYCOBACTERIUM TUBERCULOSIS





HISTORY of Tuberculosis

- **Tuberculosis is an Ancient Disease**
- **Tuberculosis in Egyptian Mummies**

- **Tuberculosis (TB)** is the leading cause of death in the world from a bacterial infectious disease. The disease **affects 1.8 billion people/year** which is equal to one-third of the entire world population.

Robert Koch discovered Mycobacterium tuberculosis in 1882

Classification of Mycobacteria

1. **Tubercle bacilli**
   a) **Human – MTB**
   b) Bovine – *M. bovis*
   c) Murine – *M. microti*
   d) Avian – *M. avium*
   e) Cold blooded – *M. marinum*

MTB Complex (*M. africanum* also included)

2. **Lepra bacilli**
   a) Human – *M. leprae*
   b) Rat – *M. leprae murium*
3. **Mycobacteria causing skin ulcers**
   a) *M. ulcerans*
   b) *M. belnei*
4. **Atypical Mycobacteria (Runyon Groups)**
   a) Photochromogens
   b) Scotochromogens
   c) Nonphotochromogens
   d) Rapid growers
5. **Johne's bacillus**
   M. paratuberculosis
6. **Saprophytic mycobacteria**
   a) *M. butyricum*
   b) *M. phlei*
   c) *M. stercoralis*
   d) *M. smegmatis*
   e) Others

What are Mycobacteria?

- Obligate **aerobes** growing most successfully in tissues with a high oxygen content, such as the lungs.
- Facultative **intracellular pathogens** usually infecting **mononuclear phagocytes** (e.g. macrophages).

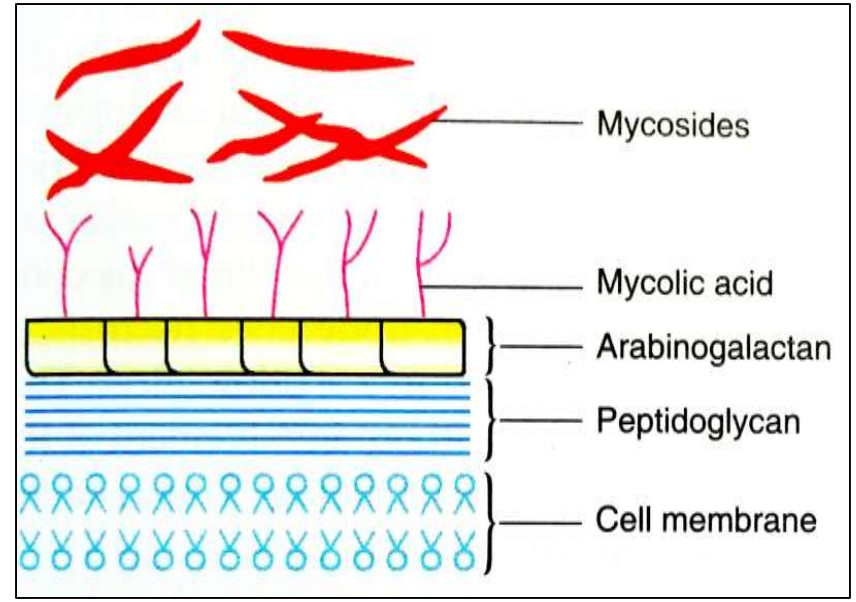
Mycobacterium differ from other routinely isolated Bacteria

- **Slow-growing** with a generation time of 14 to 15 hours (20-30 minutes for *Escherichia coli*).
- **Hydrophobic** with a high lipid content in the cell wall. As they are hydrophobic and tend to clump together, they are impermeable to the usual stains,

  e.g. **Gram's stain**

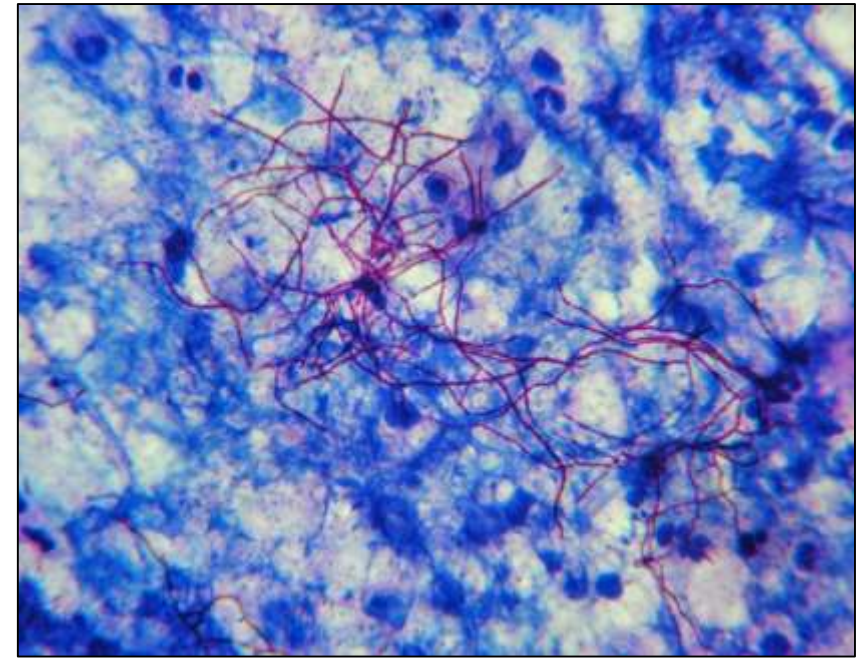
Acid fast bacilli

- Known as **“Acid-fast bacilli"** because of their lipid-rich cell walls, which are relatively impermeable to various basic dyes unless the dyes are combined with phenol.



How they are Acid fast

- Once stained, the cells resist decolourization with acidified organic solvents and are therefore called "acid-fast". (Other bacteria which also contain mycolic acids, such as *Nocardia*, can also exhibit this feature.)



Mycobacterium tuberculosis

MORPHOLOGY:-

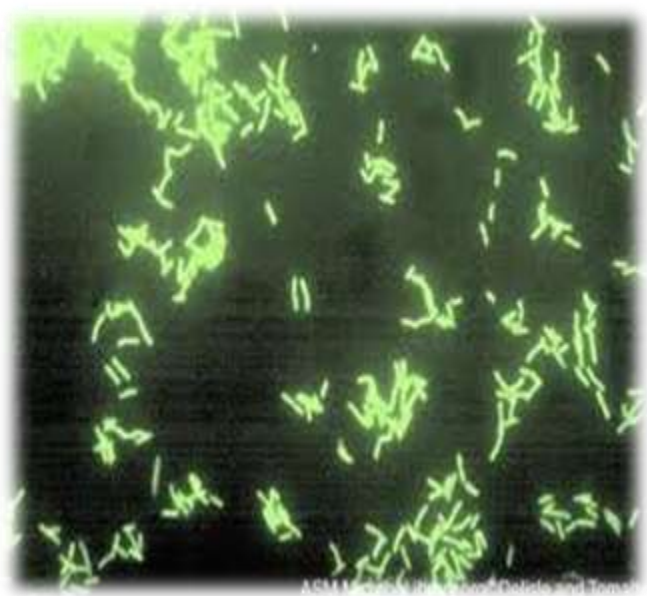
- Slender, straight or slightly curved bacilli with rounded ends, occurring singly or in pairs or in clumps.
- Non-sporing, non-capsulated and non-motile.

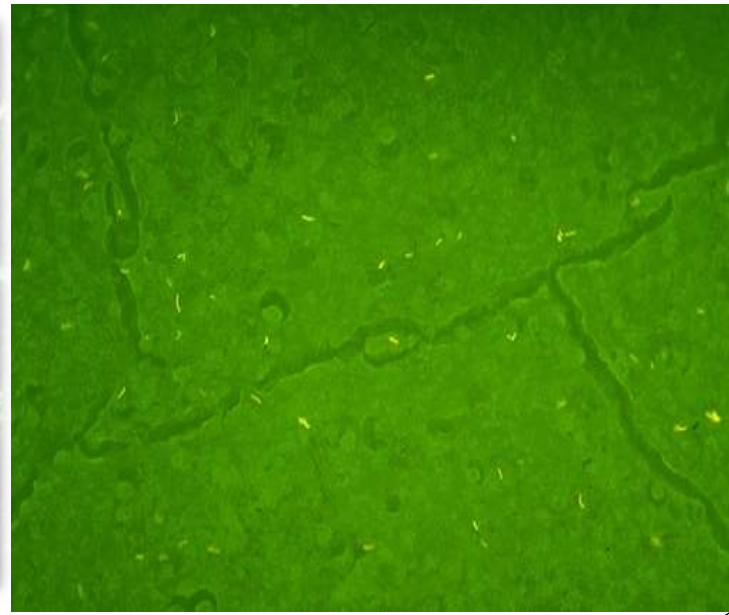
1. **Ziehl Neelsen stain** – stained by carbol fuschin; heat melts wax; resist decolourisation by 20% sulphuric acid . Resist decolourization by absolute alcohol.

   (Acid fast and alcohol fast)

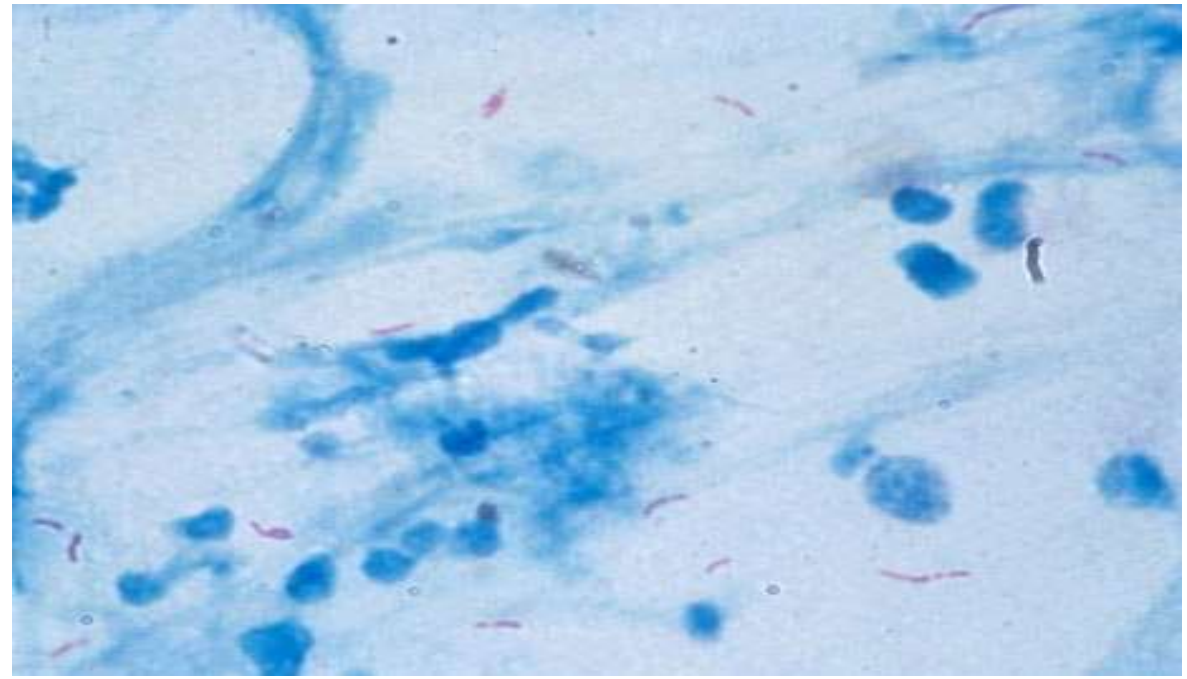
2. **Auramine rhodamine stain**

   (fluorescent stain)



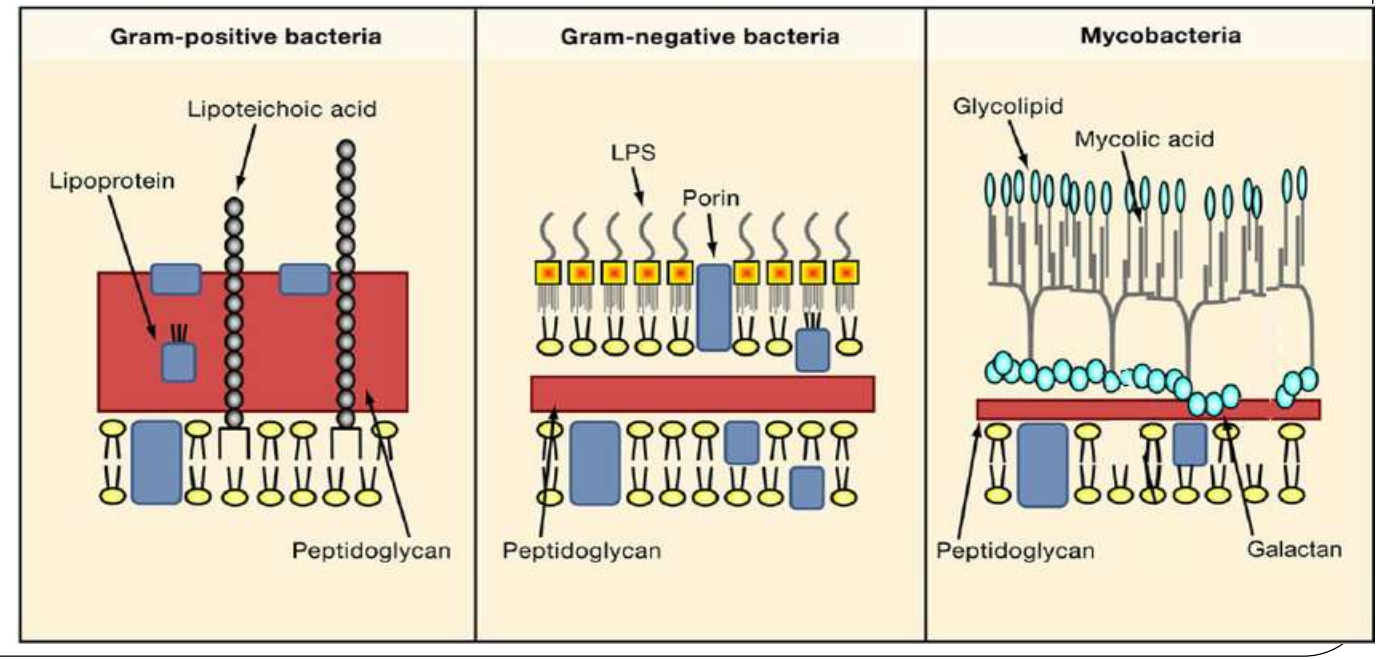


- **Acid fast bacilli**
- Straight or slightly curved.
- 1- 4 x 0.2-0.8 µm.
- Single, small clumps, pairs, long filamentous forms may be seen.
- Other (bacteria, cells – stained blue by)
- Counter stain (methylene blue)



COUNTER STAINS USED:-

- Methylene blue – Blue background
- Malachite green – Green ”



CULTURAL CHARACTERS:-

- Aerobe.
- Growth stimulation by 5-10% CO_2
- Bacilli grow slowly, generation time 14- 15 hrs.
- Colonies appear in about two weeks or delayed upto 6-8 weeks.
- Optimum temp. 37^{o}c
- Optimum pH 6.4-7.0
- Colonies - rough, tough and buff
- *M. tuberculosis* – obligate aerobe
- *M. bovis* – Microaerophilic

1. Solid media:-

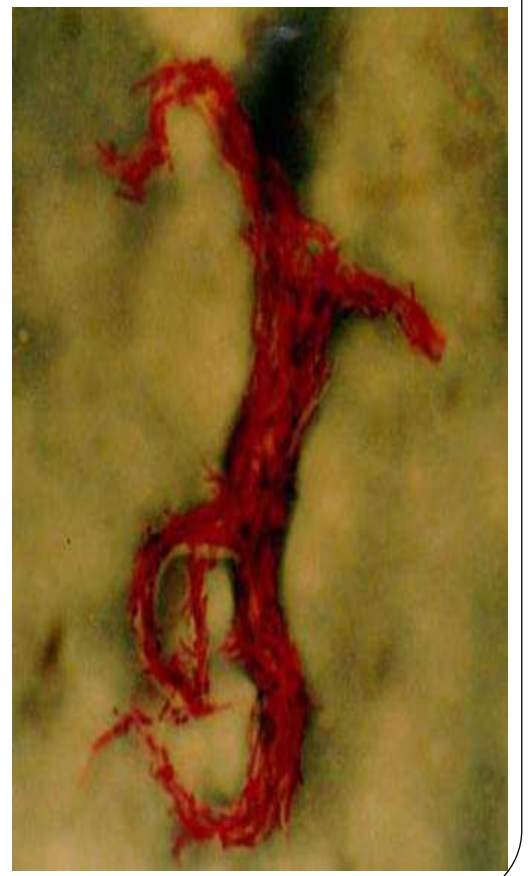
i. Containing egg – **Lowenstein Jensen**, Petragnin, Dorset's egg.
ii. Containing blood – Tarshis medium.
iii. Containing potato – Pawlowsky's medium.

- Medium most commonly used is Lowenstein Jensen medium contain:-
  i. Coagulated hen's eggs (neutralise fatty acid)
  ii. Glycerol (C source)
  iii. Mineral salt solution
  iv. Asparagines (nitrogen source)
  v. Malachite green (inhibits growth of other bacteria)

2. Liquid media:-

- Dubo's, Middlebrooke's, Prouskeur & Beck's, Sula's & Sauton's.

- Liquid media useful for – sensitivity tests, for extraction of Ag & vaccines.
  i. Growth in liquid media- pellicle at surface.
  ii. Dubo's medium with tween 80 – diffuse growth



- **Virulent strain – Serpentine cords**
- **Avirulent strain – Dispersed growth.**

- Tubercle bacilli also grow in chick embryo & tissue culture.

RESISTANCE :

- Not heat resistant
- Resistant to chemical disinfectants like phenol
- Destroyed by tincture iodine -5 min
- 80% ethanol – 2-10 minutes
- Sensitive to formaldehyde and glutaraldehyde

VIABILITY :

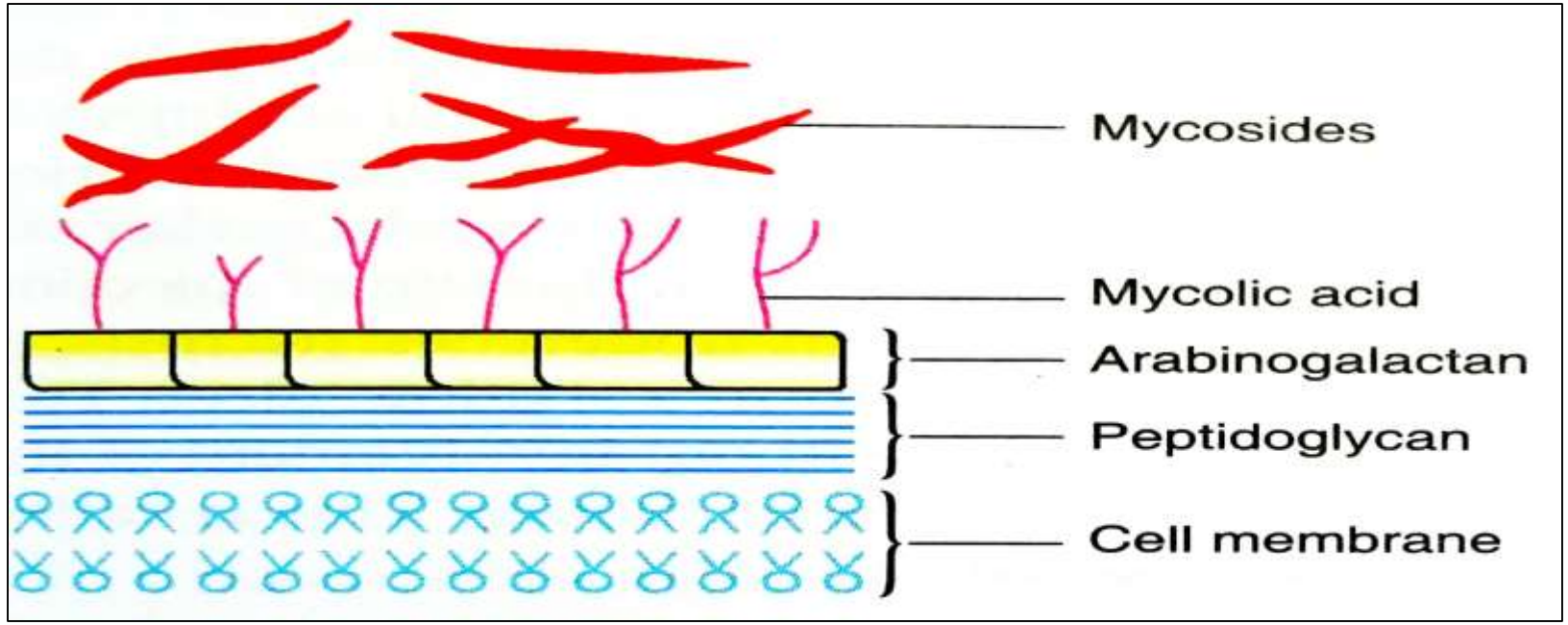
- Sputum – 20-30 hrs
- Droplets - 8-10 days
- Cultures- 6-8 months

Antigenic Structure

- **Cell Wall Antigens:**
  - Peptidoglycan layer
  - Arabinogalactan layer
  - Mycolic acid layer
  - Mycosides

- **Mycolic Acid**
  - Difficult to stain.
  - Difficult to phagocytose.
  - Intracellular survival.
  - Hypersensitivity.
  - Slow growth.
  - Resistant to heat and chemical disinfectants.

- **Cytoplasmic Antigens (Protein antigens)**



Virulence Factor:

- **Cord factor- Trehalose 6-6 dimycolate**, is a glycolipid molecule found in the cell wall of *Mycobacterium tuberculosis* and similar species. It is the primary lipid found on the exterior of *M. tuberculosis* cells.
  - **Serpentine growth** (filaments, cords) grows in close parallel arrangement.
  - Toxic to leukocytes
  - Role in development of granulomatous lesions

- **Sulfolipids-** Sulfated glycolipid (sulfatide) prevent phagosome- lysosome fusion which is important for intracellular survival.

IMMUNITY :

- Following injection by tubercle bacilli, delayed hypersensitivity develops against tuberculoprotein. Antibodies also develop but they don’t have any diagnostic value and not relevant in immunity. Immunity in tuberculosis is mainly cell mediated by sensitized T-lymphocytes and macrophages.

- Tubercle Bacilli do not produce any toxin. Various bacterial components have biological effects.

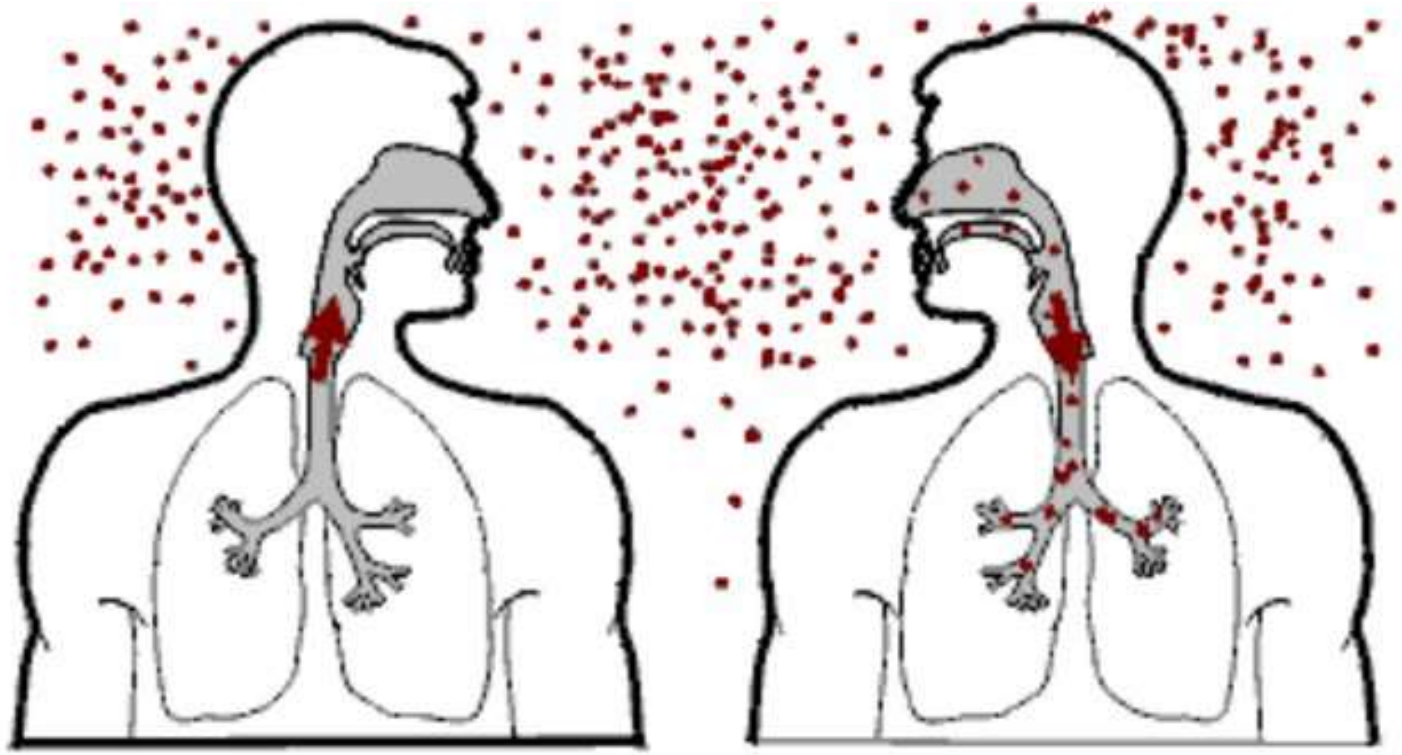
  - **Cell wall** – Causes **Delayed Hypersensitivity**.
  - **Tuberculoprotein** – Induces D.H. Formation of cellular reaction of lymphocytes, monocytes, macrophages, epitheloid cells & giants cells.
  - **Lipids-** Accumulations of macrophages and neutrophils.

Koch's Phenomenon:

- Feature of tuberculous infection in guinea pigs; **helps explain the difference between primary and post-primary lung lesions**.
- When *M. tuberculosis* is injected in a guinea pig, a local nodule develops and draining lymph nodes enlarge with caseation.
- If a second subcutaneous infection occurs after 4-6 weeks, a nodule develops rapidly, ulcerates and sloughs off.
- The tissue reaction is more aggressive the second time round, indicating greater DTH!
- Similar phenomenon occurs if the second injection is not a live bacilli but a sterile tuberculoprotein.

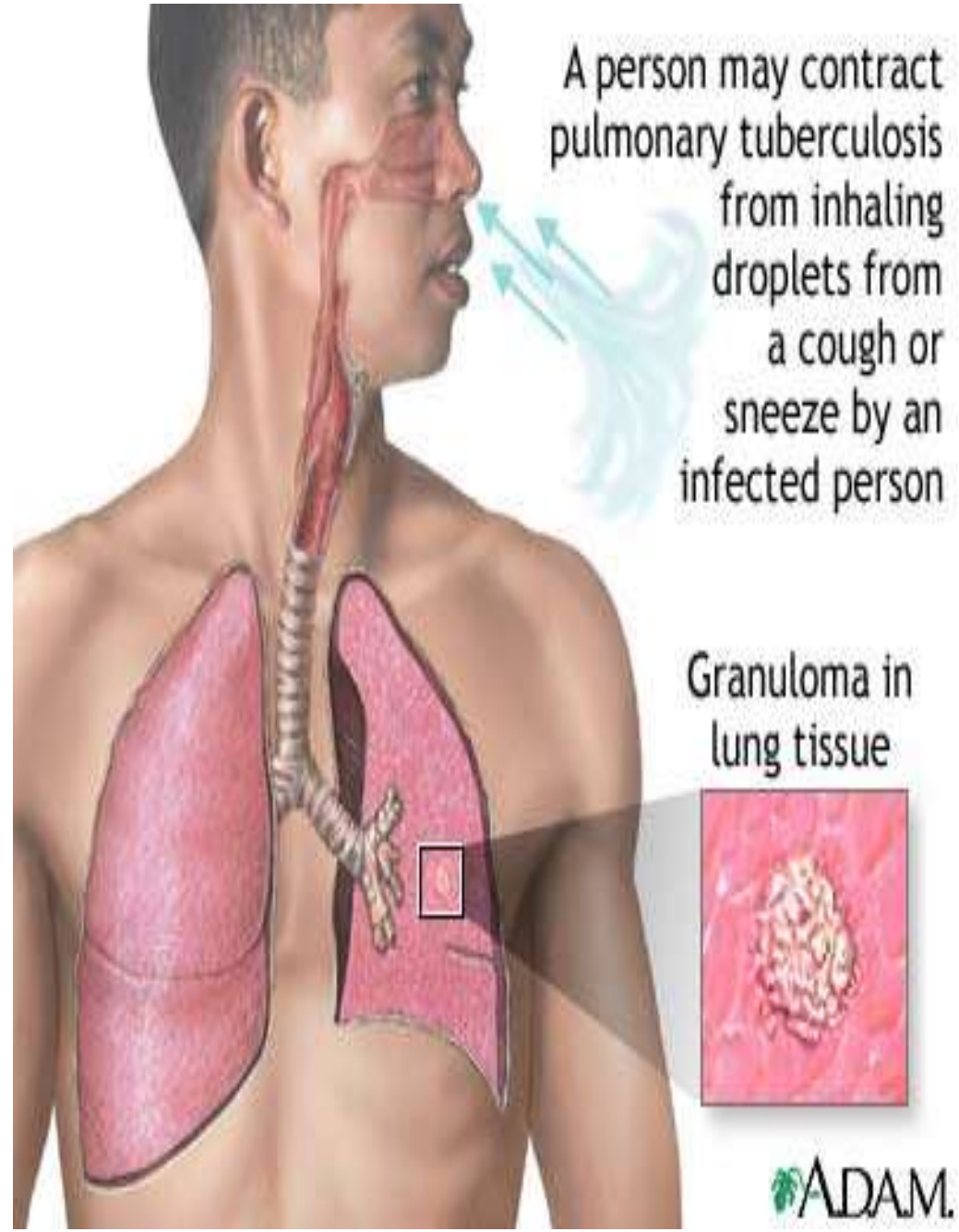
How tuberculosis spreads

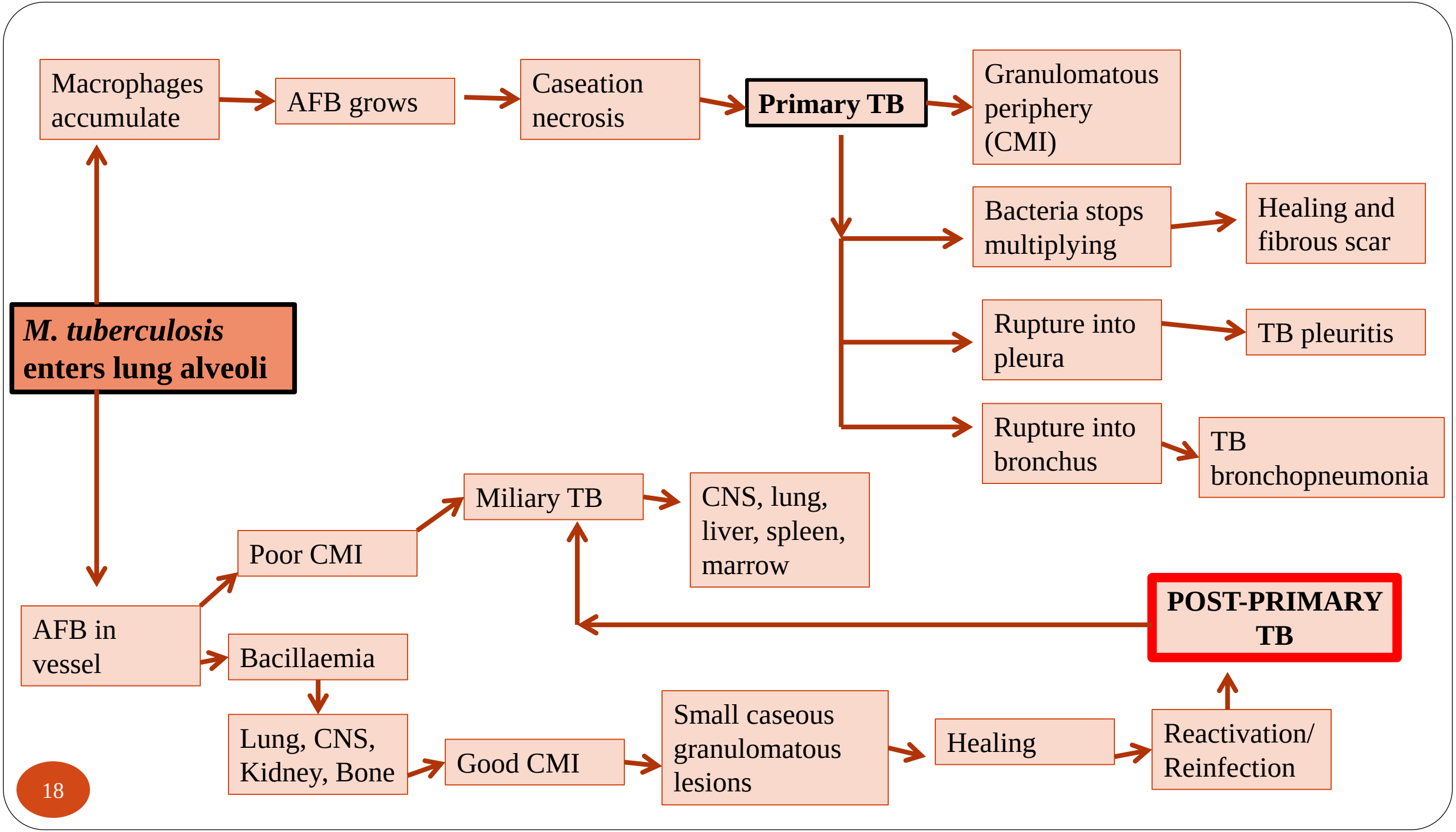
- Tuberculosis (TB) is a contagious disease. Like the common cold, it spreads through the air. Only people who are sick with TB in their lungs are infectious. When infectious people cough, sneeze, talk or spit, they propel bacilli into the air. A person needs only to inhale a small number of these to be infected.



PATHOGENICITY:-

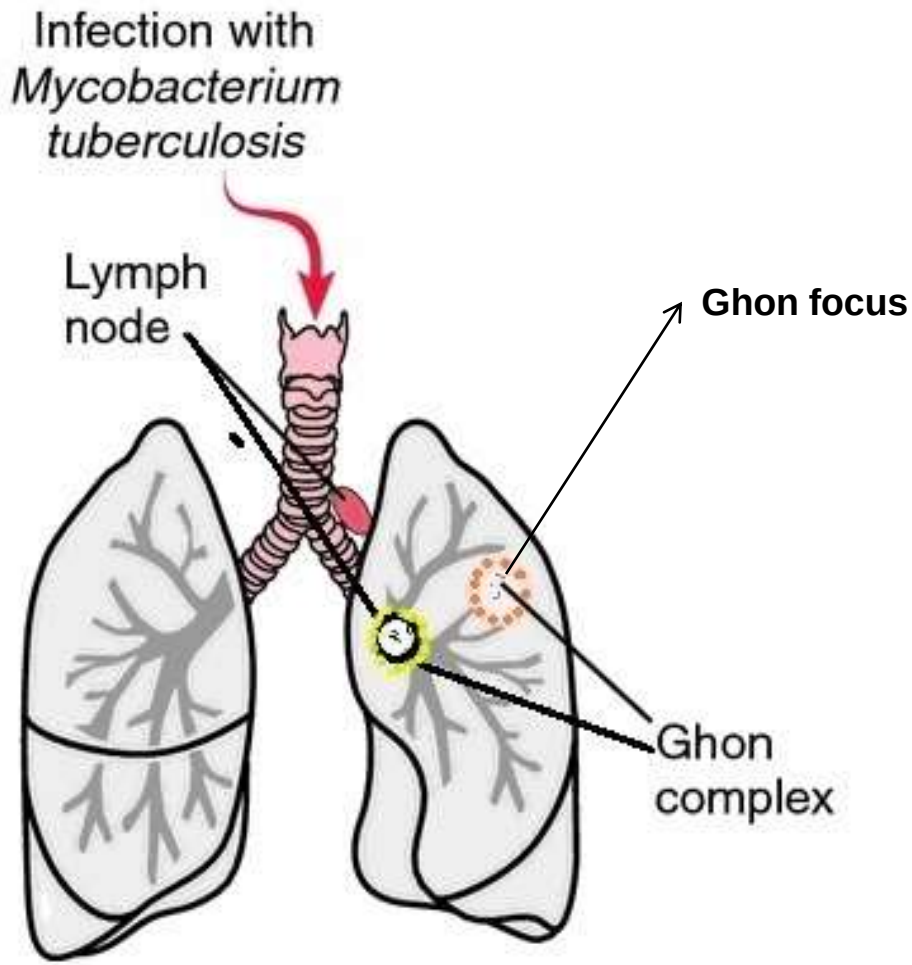
- *M. tuberculosis* can infect any organ or tissue but most commonly lungs are infected; intestines, kidneys, bones, soft tissues, brain etc.
- Infection acquired by inhalation of infected droplets.
- Engulfed by macrophages but survive and multiply.
- Lyses host cell and infect other macrophages.




Macrophages accumulate
AFB grows
Caseation necrosis
Primary TB
Granulomatous periphery (CMI)
Bacteria stops multiplying
Healing and fibrous scar
Rupture into pleura
TB pleuritis
Rupture into bronchus
TB bronchopneumonia
M. tuberculosis enters lung alveoli
Miliary TB
CNS, lung, liver, spleen, marrow
Poor CMI
AFB in vessel
Bacillaemia
POST-PRIMARY TB
Lung, CNS, Kidney, Bone
Good CMI
Small caseous granulomatous lesions
Healing
Reactivation/ Reinfection

Primary Tuberculosis:

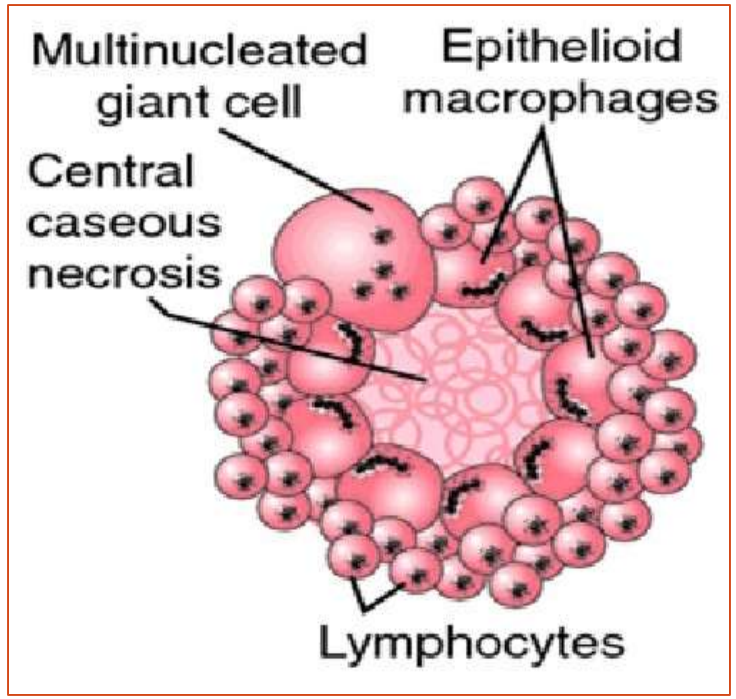
- Mostly asymptomatic.
- Some may have flu like symptoms; chest pain, mild fever and lack of appetite.
- Within 3 weeks, cell mediated immunity checks the bacilli.
- Engulfed bacilli in alveoli forms a lesion called **Ghon focus** in lower lobe. (Anton Ghon, Austrian pathologist)



- Some bacilli are transported **to hilar lymph nodes**.
- Ghon focus together with the enlarged hilar lymph nodes is called **Primary Complex (Ghon Complex)**. (Karl Ernst Ranke, German pulmonologist)

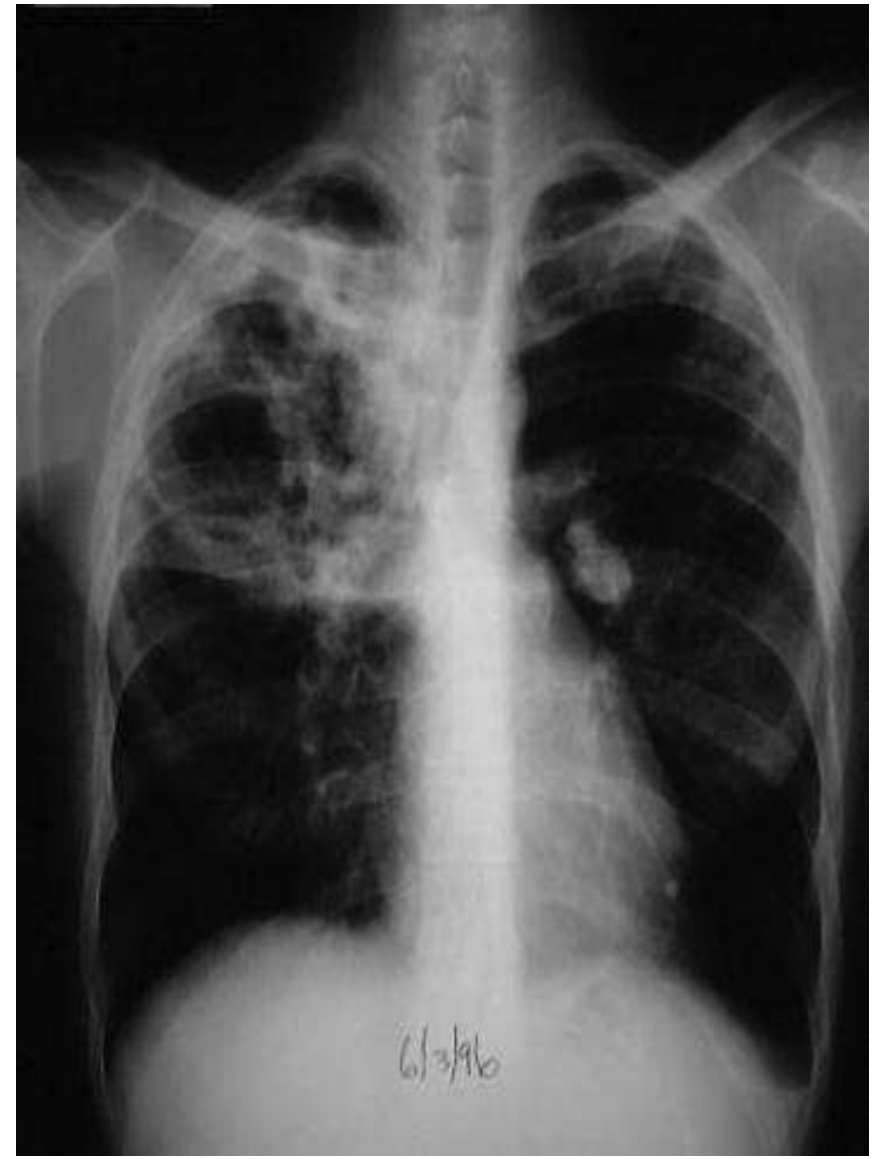
T cell → Th CD4 → (INF ɣ) → Activation of Macrophages → Monocyte aggregation → (Fuse) → Epitheloid cells → (Fuse) → Giant cells (Langhans cells) → Formation of Granuloma → (TNF) → Caseating necrosis

Caseating necrosis → (IL 1, MHC TB-Ag) → **Engulfed Bacilli in Alveolar macrophage**



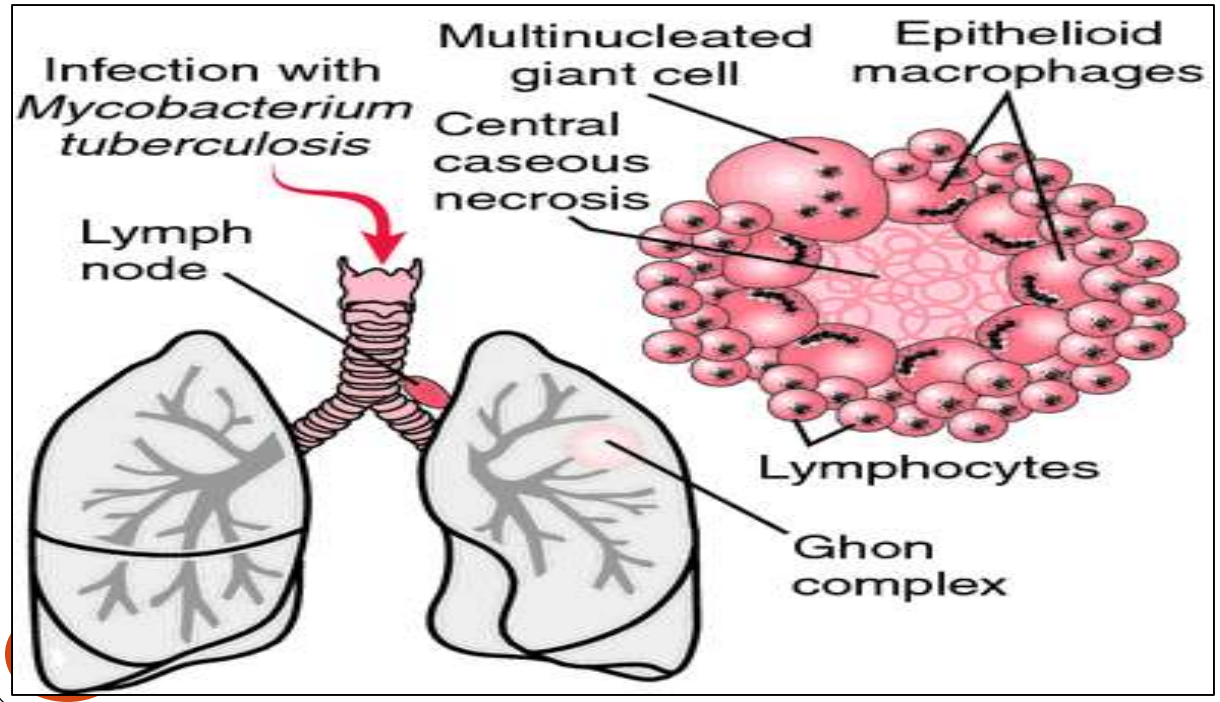
Primary Tuberculosis:

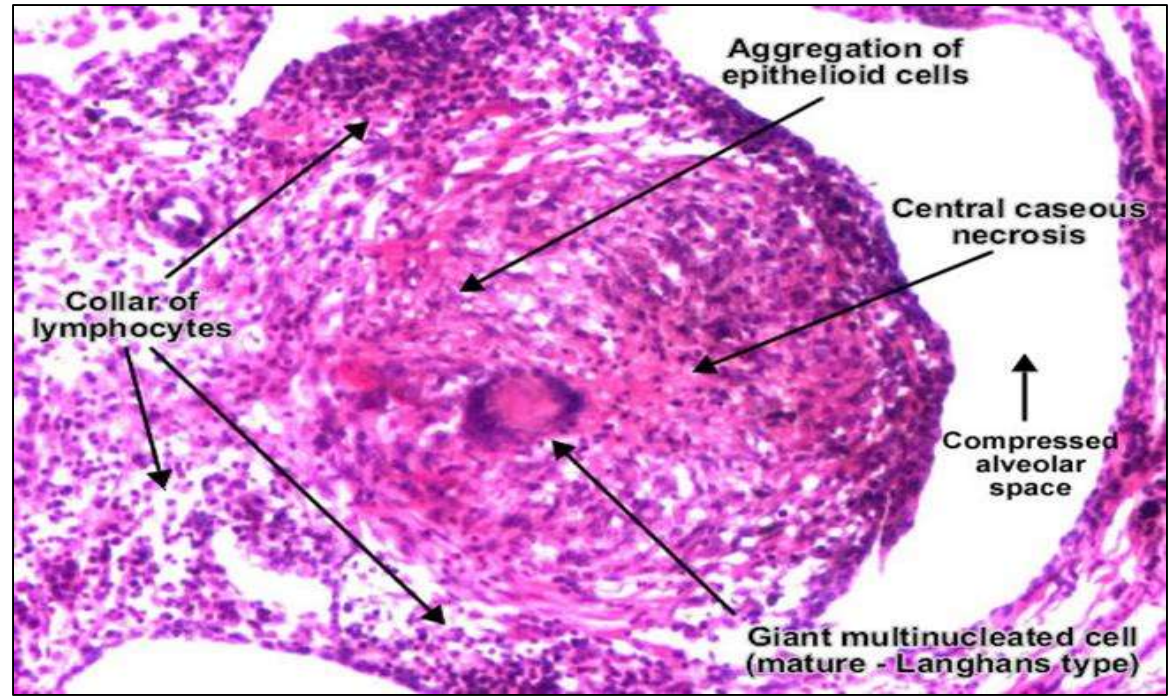
- Primary TB does not progress in most cases.
- Undergoes shrinkage with fibrosis, calcification and sometimes ossification.
- Healed primary complexes are quite small and may be hard to detect.
- Infecting organisms are not totally eradicated and viable bacilli may persist for years and perhaps for life.



Secondary Tuberculosis: (in 10% cases caused by)

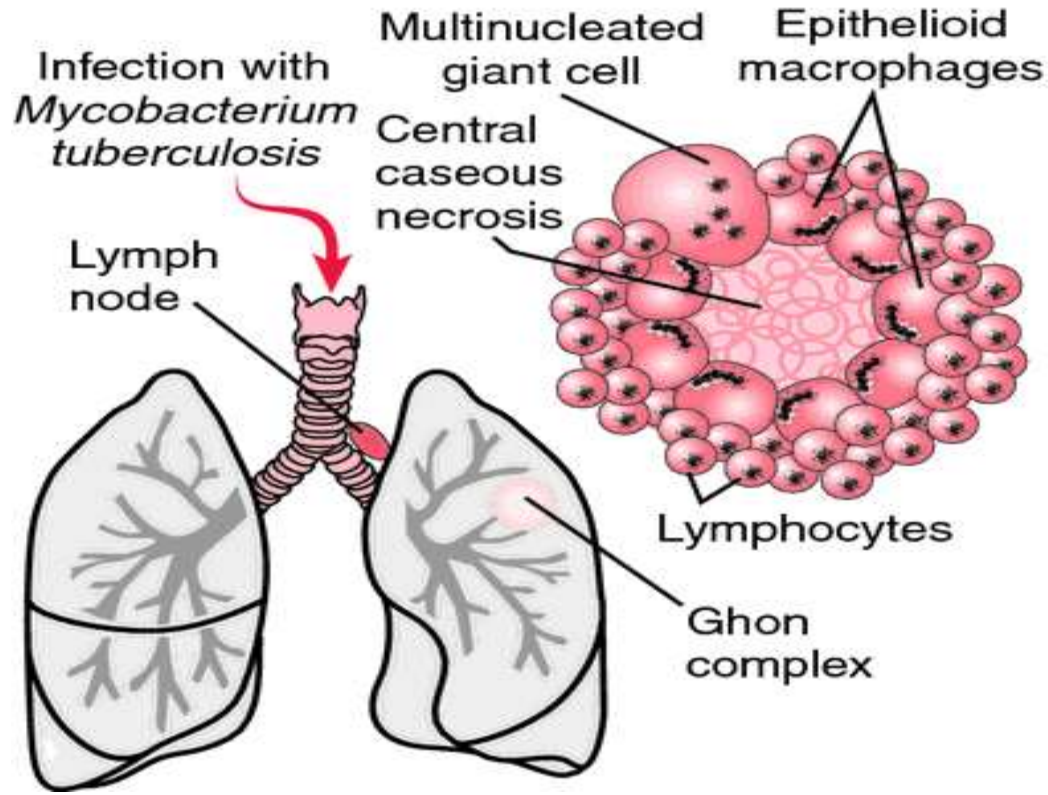
- HIV infection
- Alcoholism and liver cirrhosis
- Malnutrition
- Diabetes
- Steroid and immunosuppressive therapy
- Old age

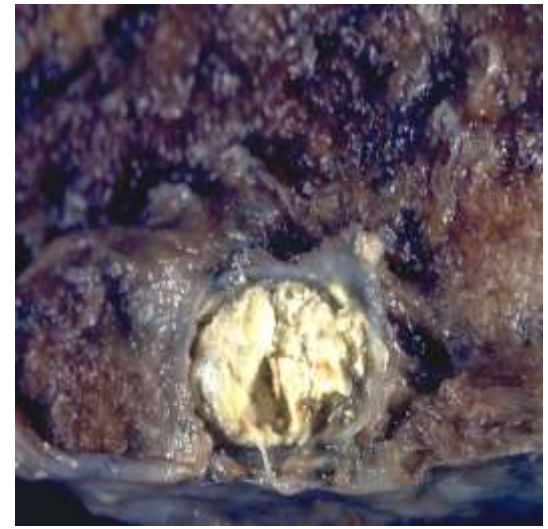


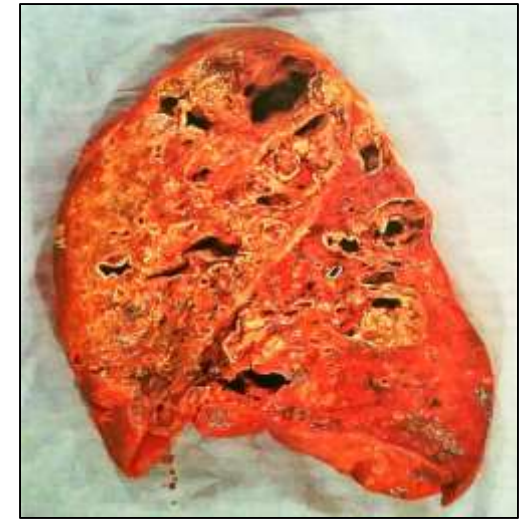


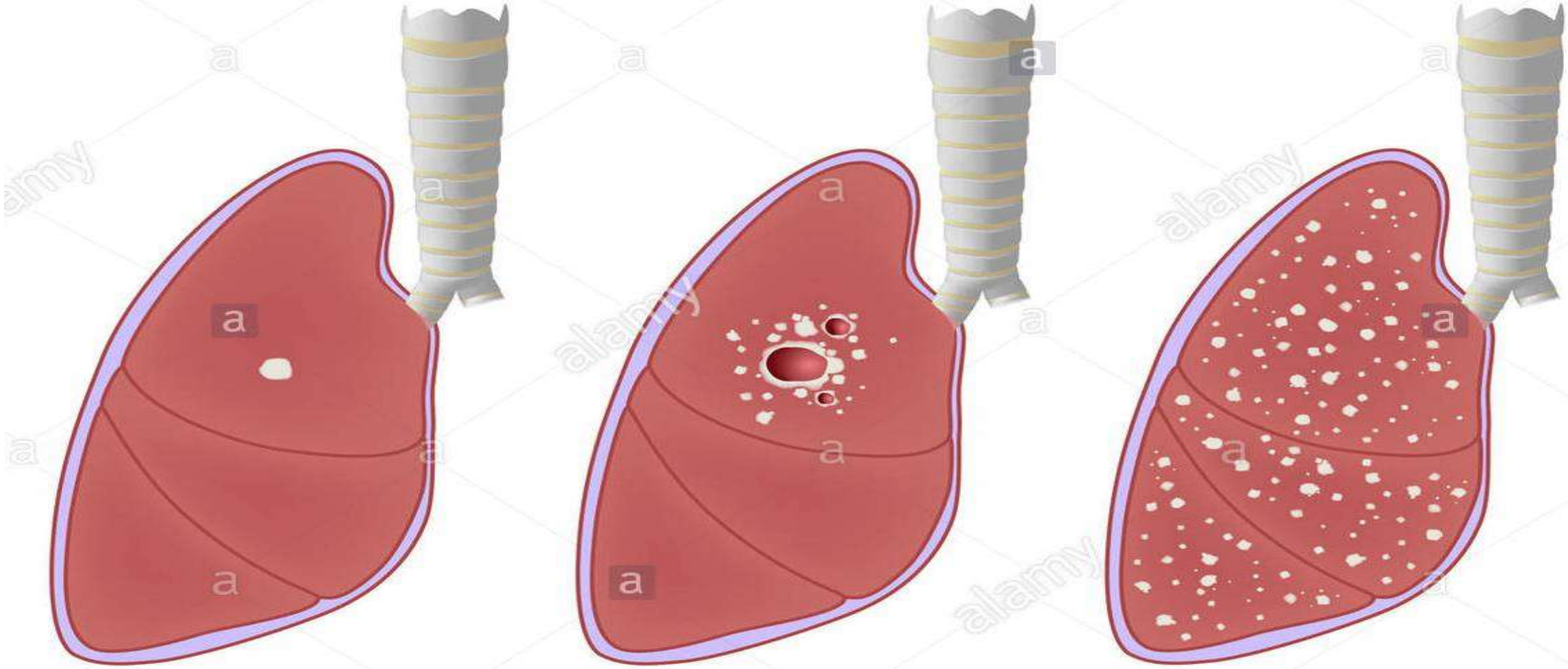
Secondary Tuberculosis:

- Caused by reactivation (**immunosuppression**) of the primary lesion.
- Spreads to upper lobes.
- **Granuloma** occurs in apex of lungs.
- Memory T cells releases cytokines.
- Causes tissue destruction and necrosis called **tuberculomas (caeseous necrosis)**.
- Cavities may rupture into blood vessels, spreading bacilli throughout body and in sputum.
- Causing systemic **Miliary tuberculosis**.









Latent infection

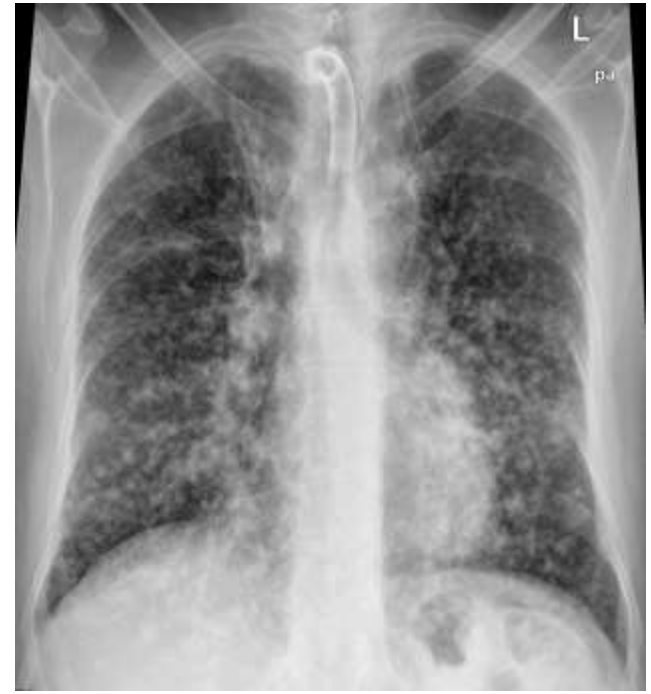
Cavitary tuberculosis

Miliary tuberculosis

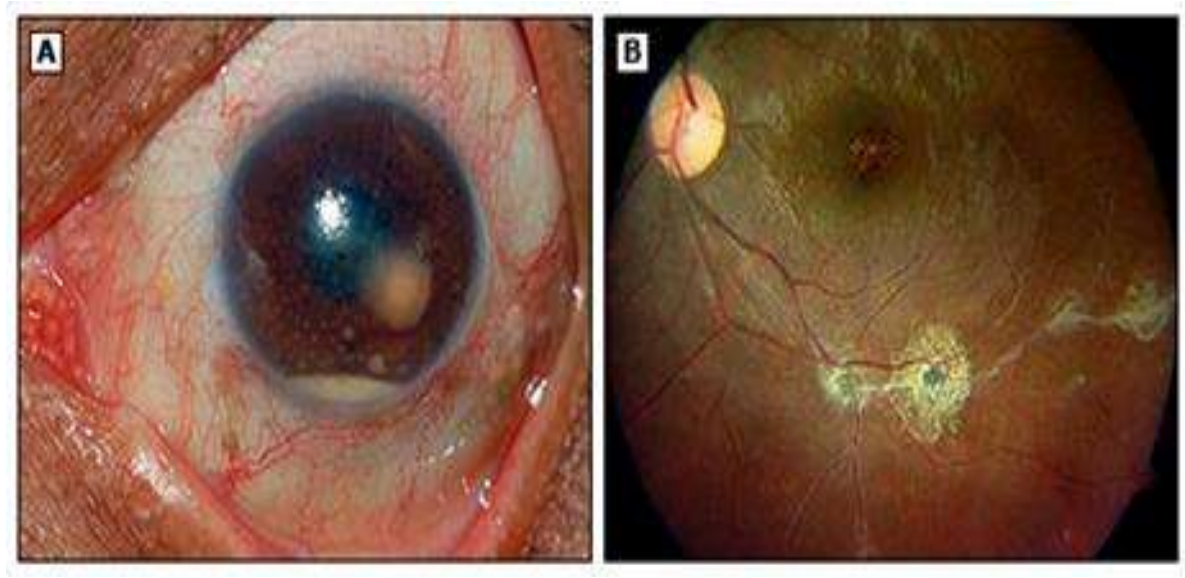
Secondary Tuberculosis:

- Miliary tuberculosis may develop in any organ of the body.
- Certain tissues like heart, striated muscles, thyroid and pancreas are resistant.
- Localization sites are the bone marrow, eye, lymph nodes, liver, spleen, kidneys, adrenal, prostate, seminal vesicles, fallopian tubes, endometrium and meninges.

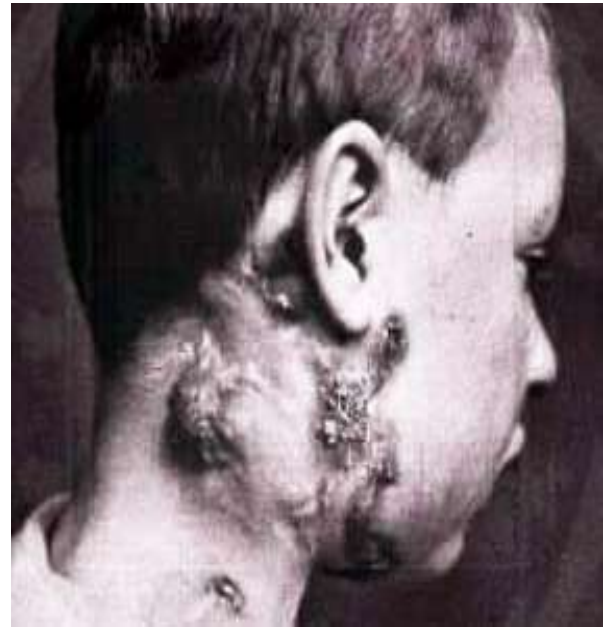
- **Clinical signs:**
  - Temperature elevation usually in mid-afternoon, night sweats, weakness, fatigability, loss of appetite and weight.
  - Productive cough, blood streaked sputum (hemoptysis)



Secondary Tuberculosis:



- Cervical lymph nodes – Scrofula
- Eye - Ocular tuberculosis
- Meninges – Tuberculous meningitis
- Kidney – Renal tuberculosis
- Tuberculosis in Adrenals – causes Addison's disease
- Bones - Tuberculous osteiomyelitis
- Fallopian tubes and epididymis - Genital tuberculosis



LAB DIAGNOSIS:-

- **Specimen** depending on clinical presentation –Sputum, Pus, Urine, CSF, Pleural/ Ascitic fluid.
- **Pulmonary tuberculosis -** Early morning sputum sample on 3 consecutive days. (Bacillary shedding is intermittent).
- Sputum is collected in wide mouth containers.

PROCESSING

Physical exam

Quantity

Colour

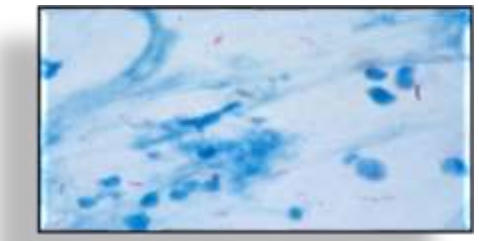
Consistency

Presence of blood

Direct smear stained by Z.N. method

Conc. Petroff's method

Smear



Culture

Interpretation of sputum stained by Z - N Stain (WHO)

More than 10 bacilli / field ---------- +++

From 1 – 10 bacilli / field ---------- ++

From 10 – 99 bacilli / 100 fields ---------- +

From 1 -9 bacilli/100 fields ---------- write the exact no.

No bacilli seen ---------- NEGATIVE

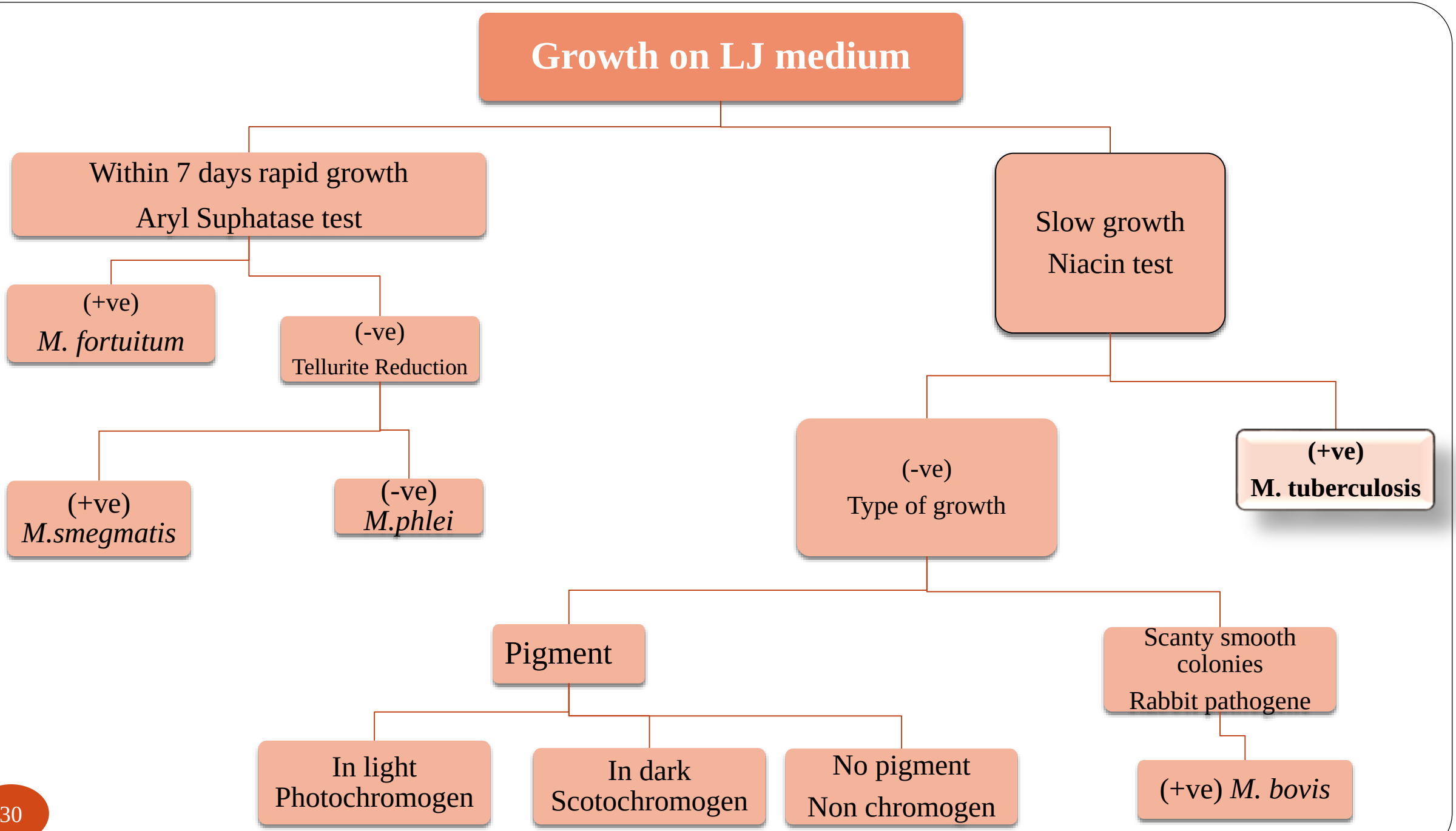
***(10,000 bacilli / ml of sputum): shows positive**

CONC. METHODS: - Petroff's commonly used.

- Sputum + equal volume of 4% NaOH
- Incubate at 37^{o}c for 20min. with frequent shaking till clearing.
- Centrifuge at 3000 rpm for 30min. Decant supernatant.
- Neutralization deposit by N/10 HCl.
- Deposit used for smear, culture, animal inoculation. Conc. Methods destroy other contaminant bacteria.

CULTURE:- Inoculate 2 slopes of LJ medium.

- (Detects as few as 10-100 bacilli/ml) 2 tubes of medium.
- Keep incubator at 37^{o}c with 5-10% CO_2
- Examine every day for growth for rate of culture identification.


Growth on LJ medium
Within 7 days rapid growth
Aryl Suphatase test
(+ve)
M. fortuitum
(-ve)
Tellurite Reduction
(+ve)
M.smegmatis
(-ve)
M.phlei
Slow growth
Niacin test
(-ve)
Type of growth
(+ve)
M. tuberculosis
Pigment
Scanty smooth colonies
Rabbit pathogene
In light
Photochromogen
In dark
Scotochromogen
No pigment
Non chromogen
(+ve) M. bovis

	M. tuberculosis	M. bovis
Morphology	Long, slender and usually curved	Short, stout and straight
Staining	Barred or beaded appearance	Uniform staining
Growth on LJ medium	Eugonic	Dysgonic
Presence of glycerol in medium	Enhances the growth	Inhibits the growth
Colony	Dry, rough, tough, raised & wrinkled, difficult to emulsify	Moist, smooth, flat, white and friable
Biochemical reactions		
Niacin test	+	-
Nitrate reduction	-	-
Animal pathogenicity		
In guinea pig	+ (progressive & fatal)	+ (similar)
In rabbit	- Or mild lesion	+ generalised lesion

TEST PRINCIPLES :-

1. **Niacin test-** suspension of tubercle bacilli +10% cyanogen bromide 4% aniline in ethanol- positive gives yellow colour. (+MTB)

2. **Arylsulphatase test-** Bacteria grown in solution of disulphate + 2N NaOH – pink (-MTB)

3. **Neutral Red Test –** colonies of Tub. Bacilli in neutral red solution in alk buffer –colonies pick up red colour (+MTB)

4. **Catalase Peroxidase test-** 5ml culture suspension + H_2O_2 and 2% catechol – effervescence - Catalase Peroxidase positive. Point mutation is a catalase gene, makes the strains resistant to isoniazide. (weakly + MTB)

5. **Amidase test-** Acetamide, benzamide, carbamide, nictonamide, pyrizinamide. (Split)
   - 0.00164M solutions of amide + tub. bacterial suspension incubate at 37°c.
   - Add solution of phenol, $MnSo_4$, hypochlorite.
   - Boil tube for 20 mins.
   - Blue colour indicates – Positive reaction

Drug sensitivity tests: -

1. **Absolute conc. Method**:-

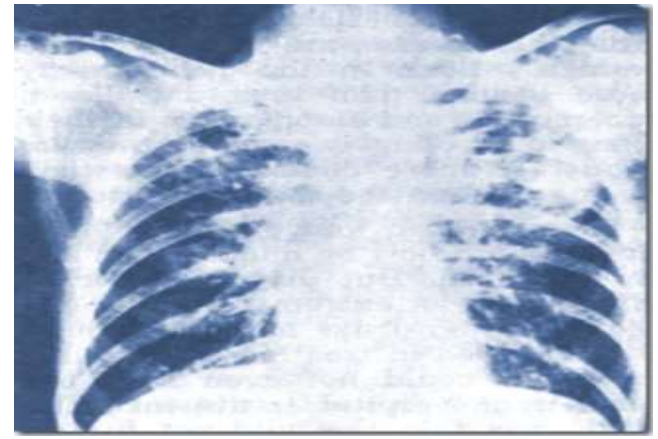
   L.J. media containing serial conc. Of drug are inoculated & minimum inhibitory conc. Noted.

2. **Resistance ratio**:-

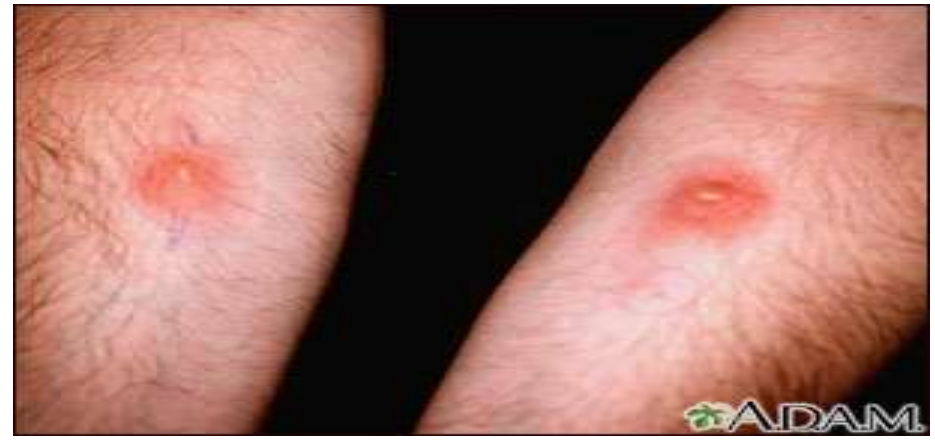
   Two sets of media containing serial conc. Of drugs are inoculated.

- 1st set – test strain
- 2nd set – standard strain

OTHER METHODS OF DIAGNOSIS OF TUBERCULOSIS:-



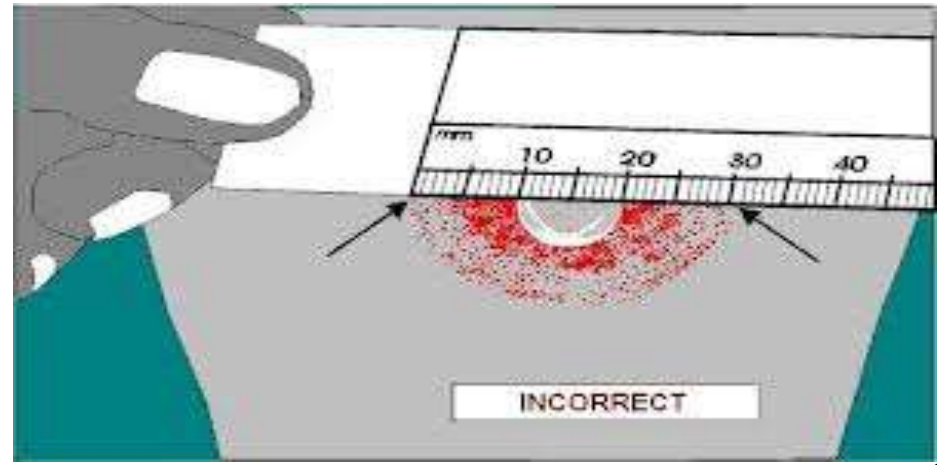
1. **X-ray chest**
2. **Blood exam** – lymphocytosis, increased ESR
3. **Mantoux test** – Tuberculin test.



- Routinely 5TU is used. 0.1 ml of PPD is injected intradermally in forearm. The area is marked by pen do not press or wash.
- Readings taken after 48-72 hrs.
- Erythema & indurations > 10mm – positive

  < 5mm – negative (+ in HIV)

  6-9mm – equivocal



False negative tuberculin test –

Miliary TB, convalescence from measles, lymphoreticular malignancy, impaired CMI, inactive PPD, Improper injection.

False positive tuberculin test –

Infection with atypical mycobacteria.

NEWER METHODS FOR LAB DIAGNOSIS OF TUBERCULOSIS:-

1. **Radiometric methods** –

Advantage:- rapid growth

- Specific identification,
- Result within 7 days

Instrument:-

- **BACTEC**
- Fully automated

2. **PCR** – high sensitivity.

- DNA amplified.
- Cannot differentiate living and dead bacteria; both reported positive.

3. **Serology** – antibodies against *M.tubercule* antigen by ELISA.

TREATMENT

FIRST LINE DRUG:-

- Rifampicin(R) & Pyrizinamide (Z) – kill bacilli in lesions
- Isoniazid (H) – kills replicating bacilli
- Streptomycin (S) – kills extracellular bacilli
- Ethambutol (E) – bacteristatic

- **Intensive phase** – 3 times a week, 2 months – H, E, R, Z
- **Continuing phase** – 3 times a week, 4-5 months – H, R

SECOND LINE DRUG:-

- Quinolones, Aminoglycosides, Macrolides, Thiacetazone, Cycloserine, Capneomycin.

- **MDR-TB** – Resistance to Rifampicin & Isoniazid ; **DOTS** (directly observed therapy under supervision) important.

BACILLUS CALMETTE GUERIN (BCG) :–

- Live attenuated vaccine. Strain of ***M. bovis*** attenuated by serial sub cultures in glycerine bile potato medium over 13 years.
- 0.1ml injected intradermally on deltoid muscle soon after birth.
- Immunity last for about 15 years.

BCG not to be given –

- Infants & children with active HIV disease.
- Babies born to sputum AFB positive mother.