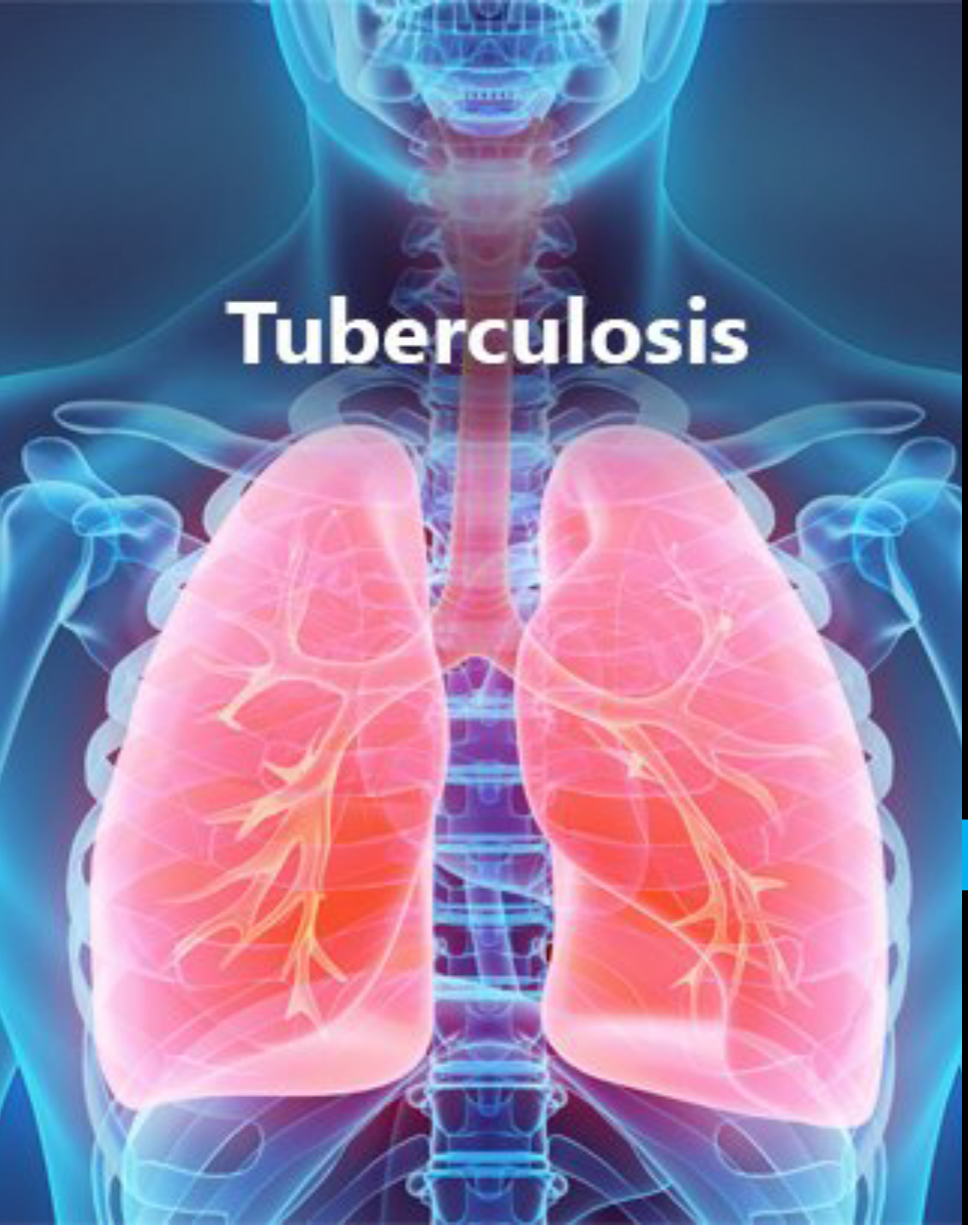


lycobacterium

Features of the genus *Mycobacterium*

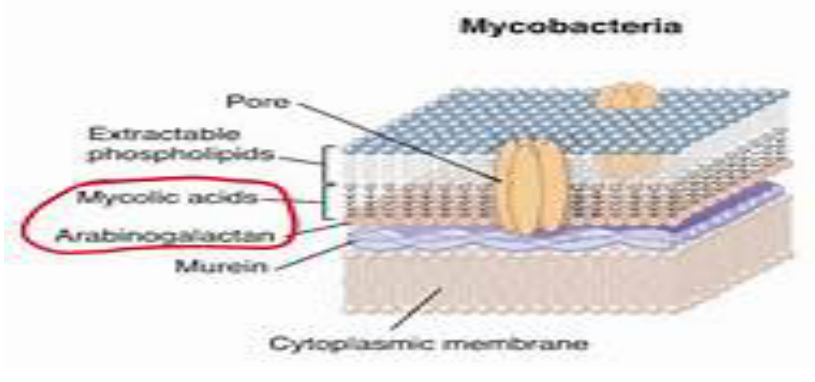
1. **Acid-alcohol fastness or Acid fastness :** Once stained with certain dyes as Ziehl-Neelsen, they retain the dye firmly and resist decolorization even with acid-alcohol or with strong mineral acids.

Due to **high lipid (mycolic acid) content** of the cell wall.

2. **Slow growth rate** due to longer generation time. Rapid growers $<$ 7 days

Slow growers $>$ 7 days

3. **Obligate aerobe**



Lipid-Rich Cell Wall



Ziehl-Neelsen (Z-N) Stain

- Strong carbol-fuchsin + heat

→ All Red



- Acid-alcohol (decolorization) 20% H_2SO_4 or 3% HCL in alcohol



Not decolourized (red) (colourless)

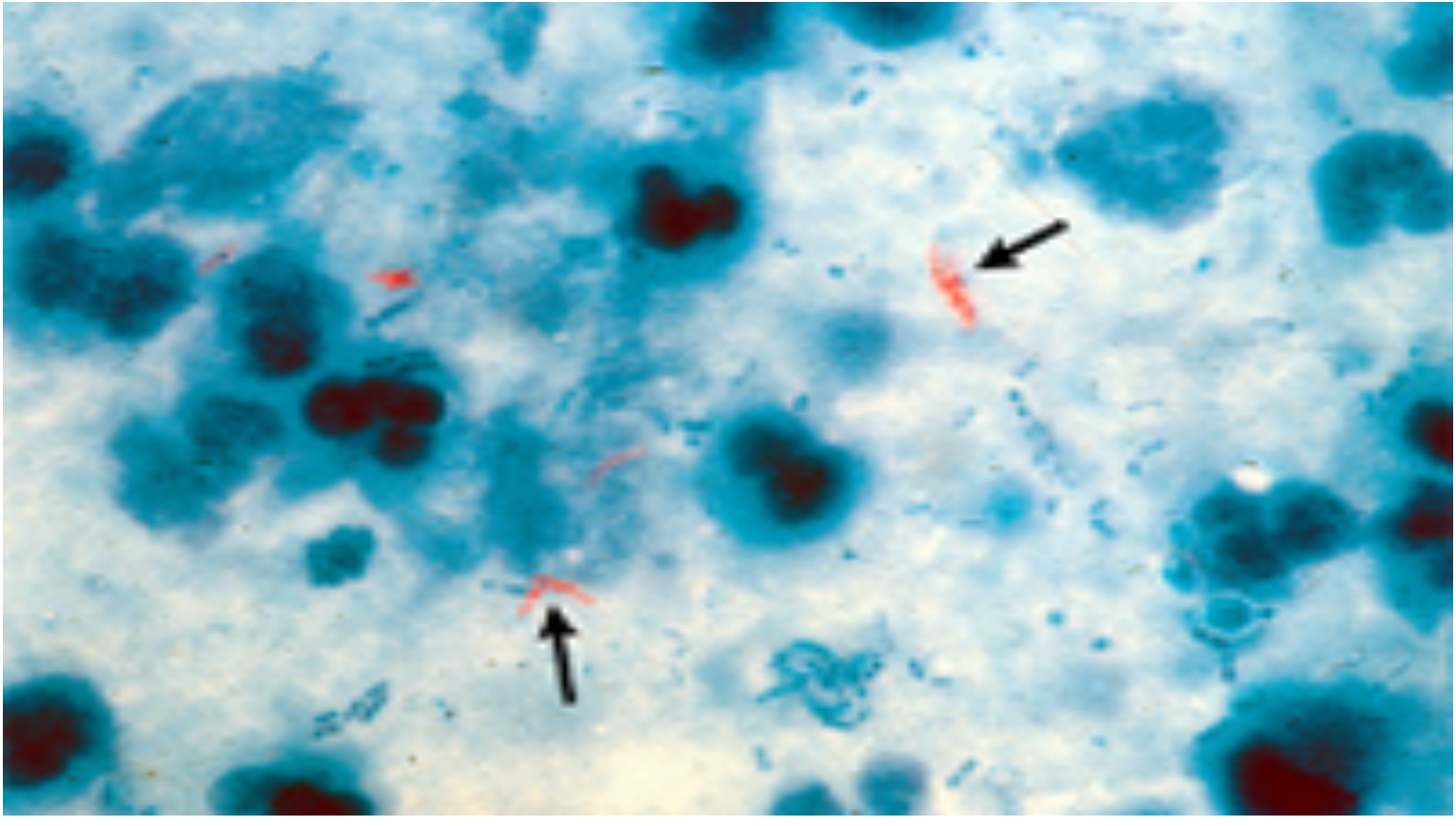
decolourized

- Methylene blue → Colourless →blue (non acid-fast)



acid-fast = *Mycobacteria*

Acid Fast bacilli (Z-N stain)



Features of the genus *Mycobacterium*

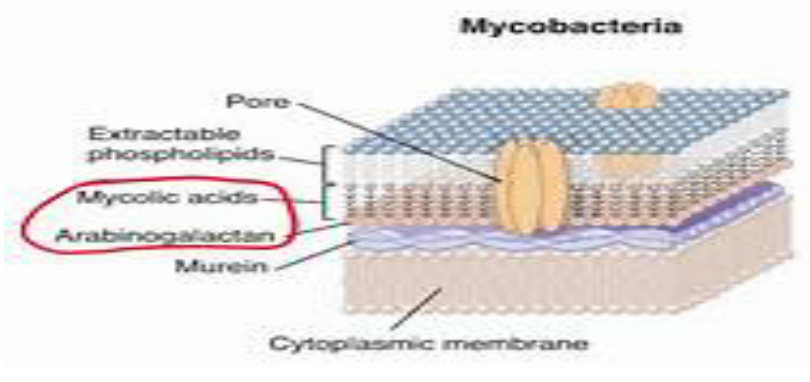
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Members of the genus *Mycobacterium*

1. ***Mycobacterium tuberculosis* complex (tubercle bacilli): *M. tuberculosis* (humans),** *M. bovis* (cows and humans), *M. africanum* & *M. microti*

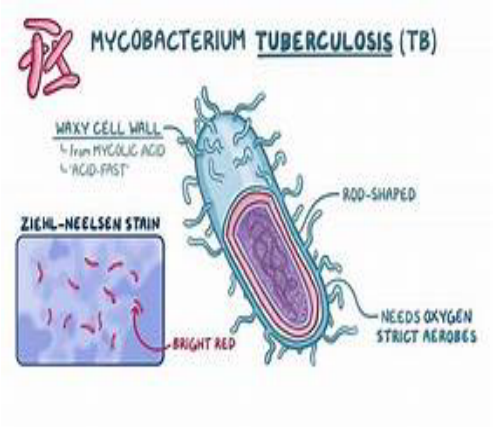
2. **Non-tuberculous mycobacteria (NTM):** e.g. *M. avium* complex & *M. fortuitum* complex

3. ***Mycobacterium leprae:*** causes leprosy

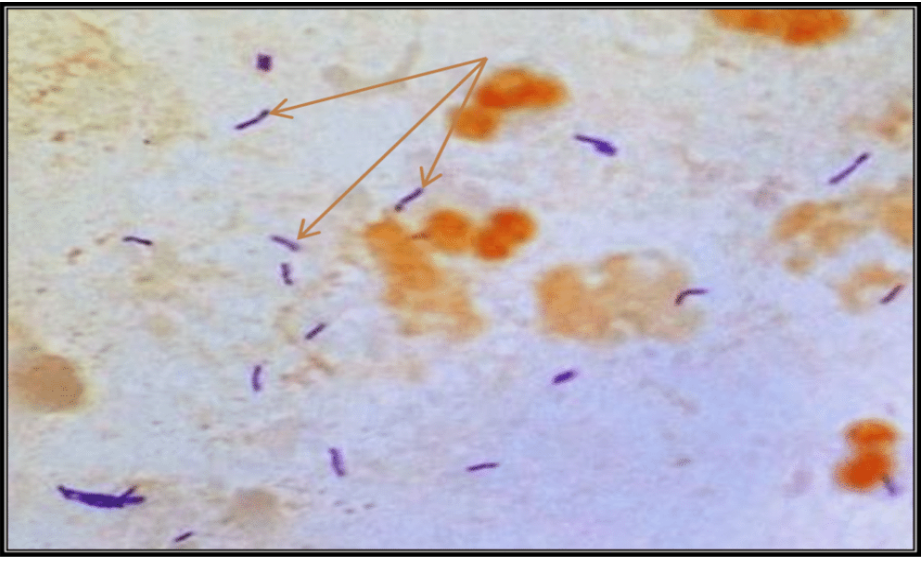
4. **Saprophytic mycobacteria**

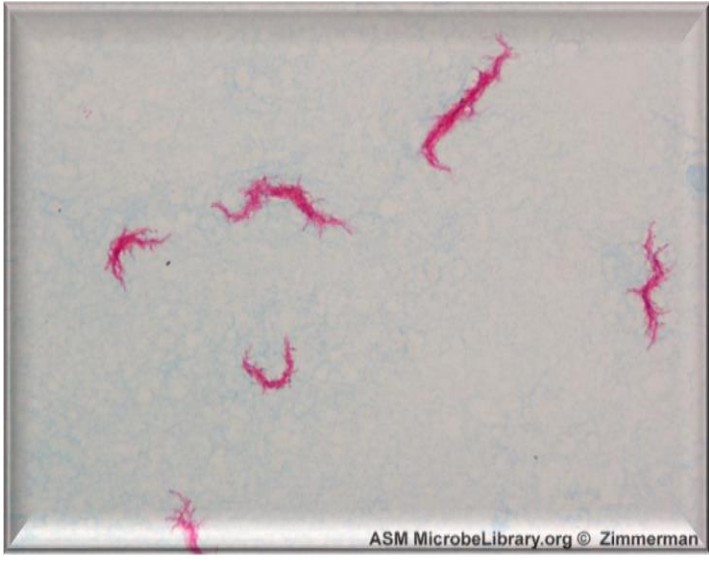
Mycobacterium tuberculosis

Morphology:

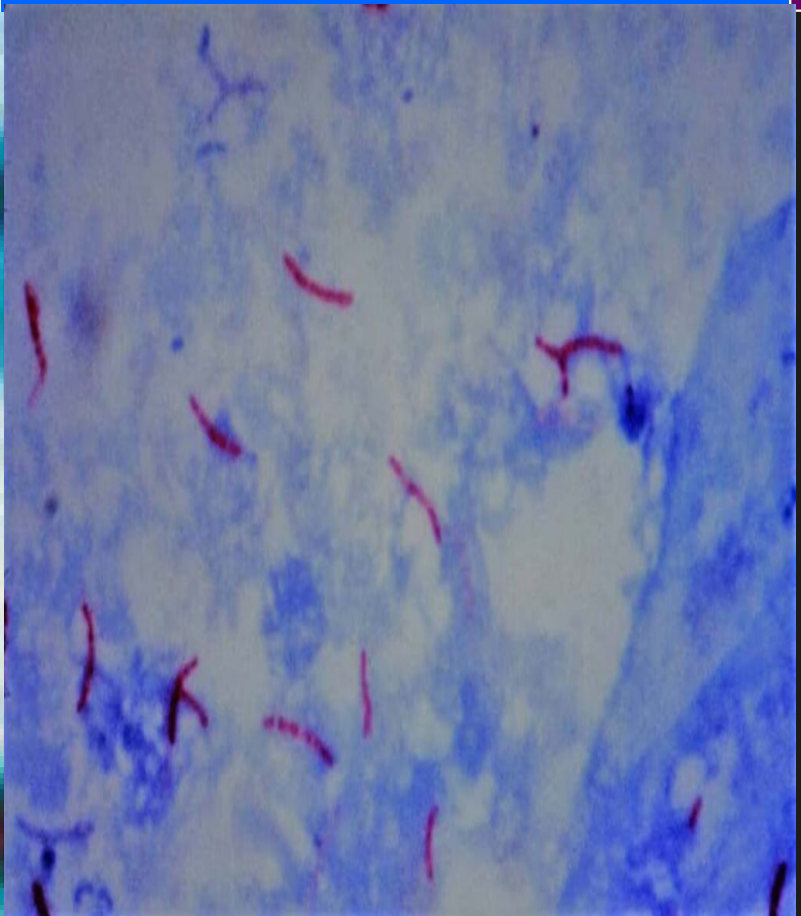


- **Shape:** slender **acid-fast bacilli**
- **Arranged: singly or in small groups/ serpentine cords**



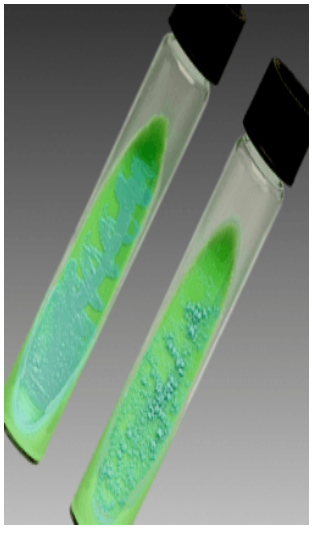


ZN stain / Kinyoun stain	Auramine-Rhodamine
Red in a blue background	Orange yellow in a black background





Culture



1. Strict aerobe, 5-10% CO_2++ growth

2. Special media(selective media):

a) Lowenstein-Jensen (L-J) medium (egg-based)

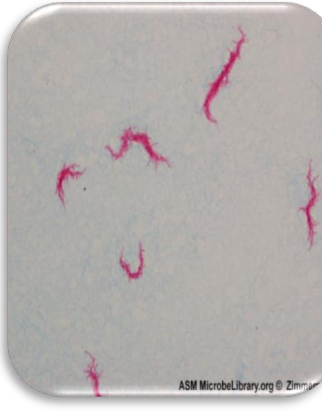
Rendered selective by malachite green dye → non-pigmented rough.

b) Middlebrook's medium

Rendered selective by antibiotics

- **solid** (agar-based)
- **fluid** (Middlebrook's broth)





Serpentine cords

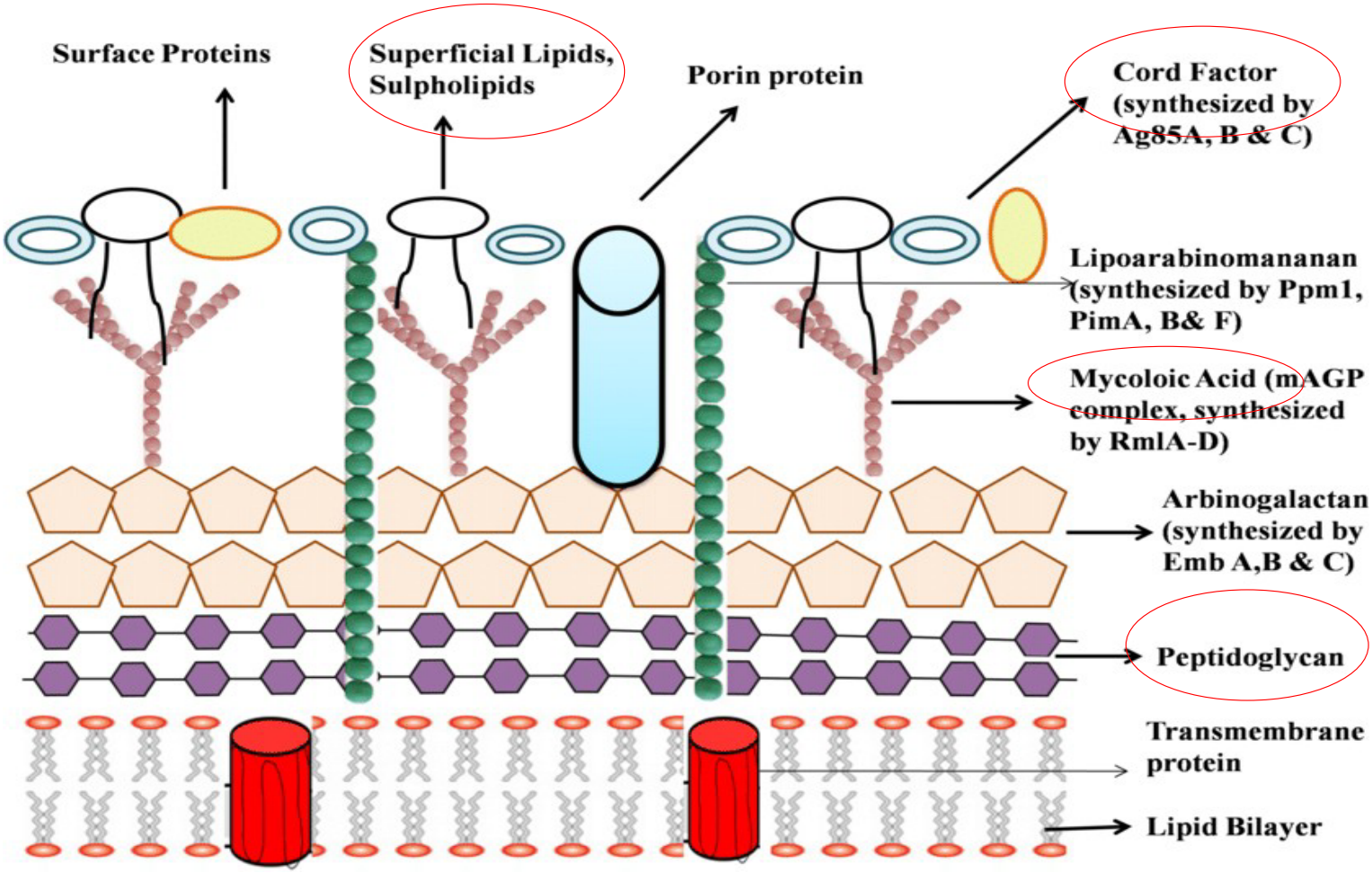
Colonies appear after 2-8 weeks of incubation at 37 °C

Biochemical Reactions

-Used to **differentiate *M.tb.* from other members of the genus**.

- Biochemical reactions include:
  - **Niacin production**
  - **Nitrate reduction**
  - **Heat-sensitive catalase**

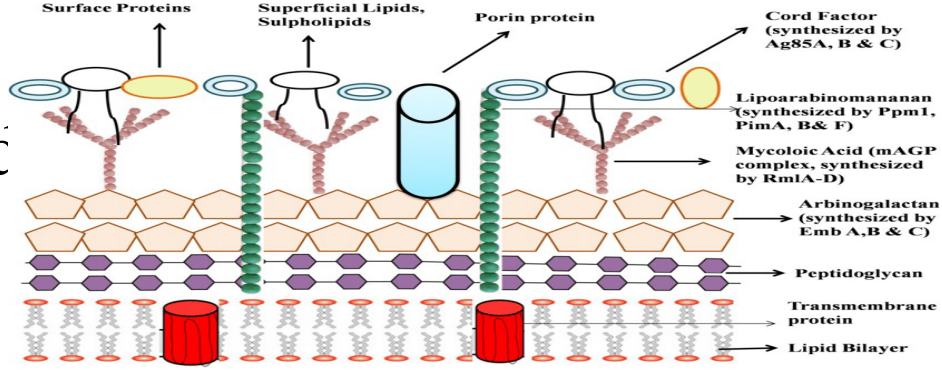
Cell wall structure



Cell wall structure

- **Peptidoglycan** layer resembles that of Gram +ve bacteria.

- A layer of lipic



1. **Mycolic acids:** long-chain fatty acids
2. **Cord factor:** cytotoxin
3. **Sulfolipids:** inhibit phagolysosome formation.

The high lipid concentration has been associated with:

a. Impermeability to **stains & dyes**

b. Resistance to killing by **acids & alkalis**

c. Resistance to **dryness**

d. Resistance to many **antibiotics**

e. Resistance to osmotic lysis *via* **complement** deposition

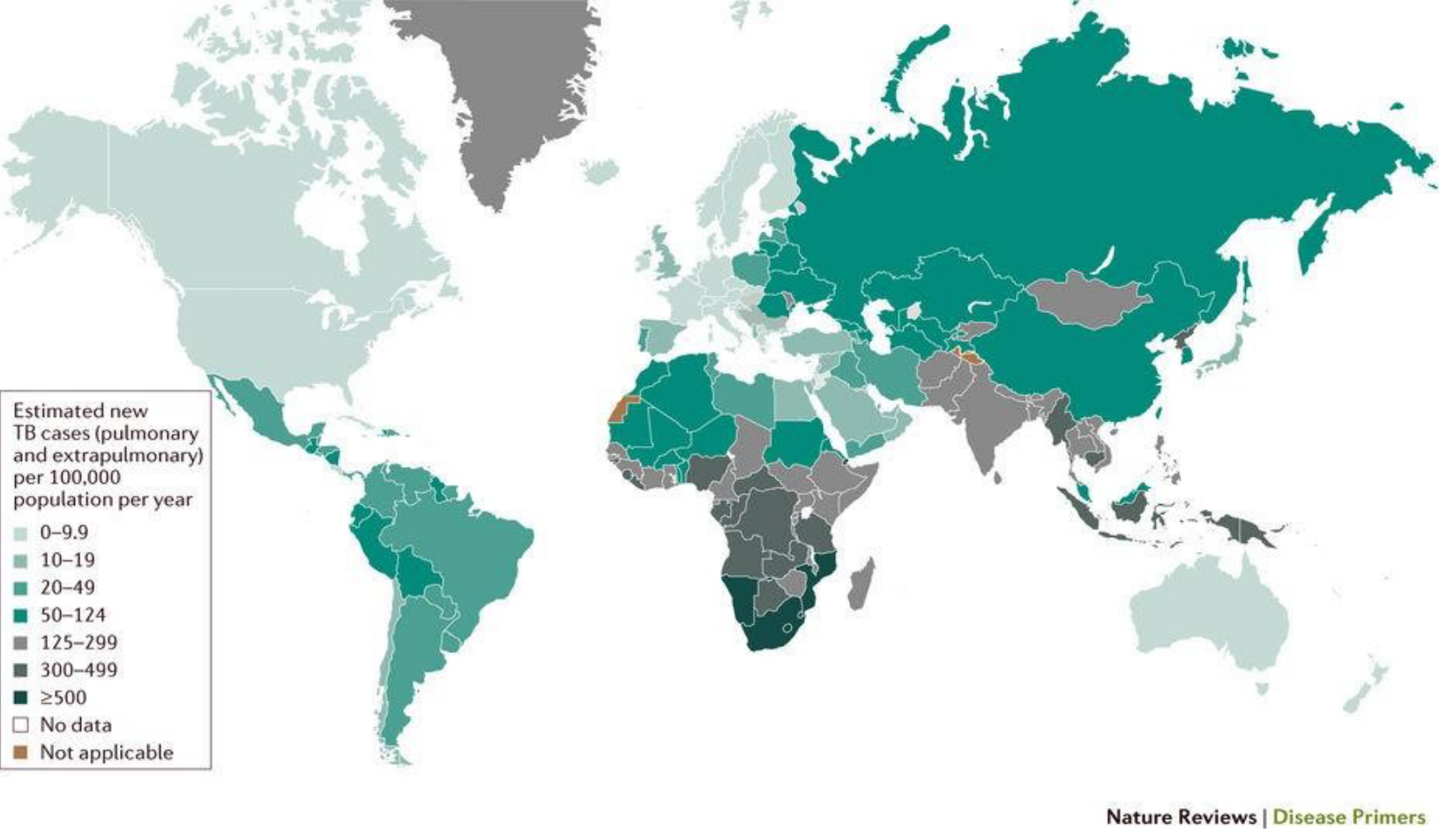
f. Ability to **survive inside** macrophages and induce CMI

Susceptibility to physical & chemical agents

- **Killed by:**

- Heating at 55°C for 1 hr, autoclaving, pasteurization and sunlight.

- Intermediate level disinfection: ethyl & isopropyl alcohol / chlorine



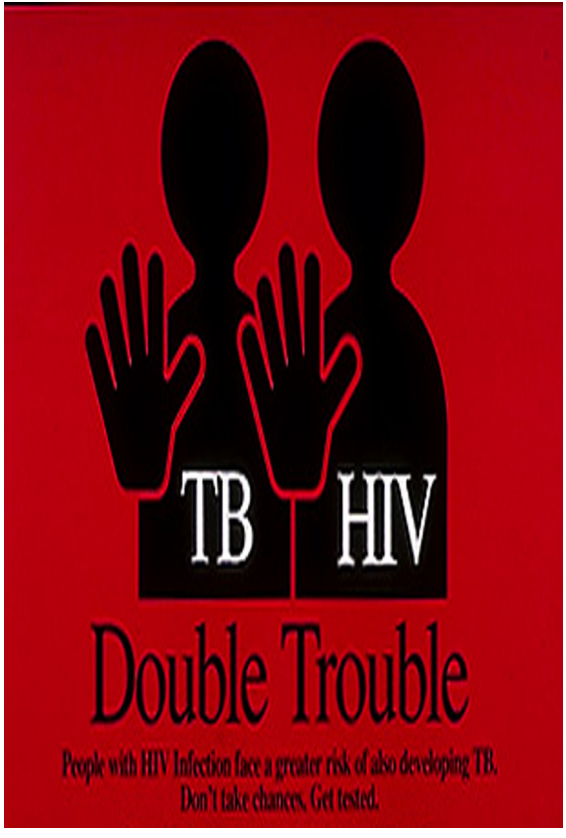
Global incidence of active TB disease

Major cause of death

Human TB

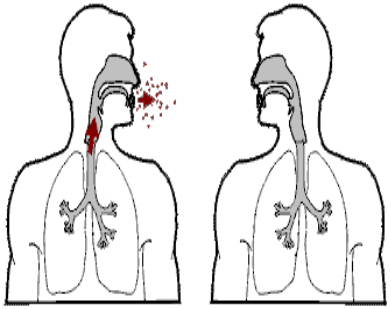
- Number of cases of TB has increased after the start of **AIDS** pandemic

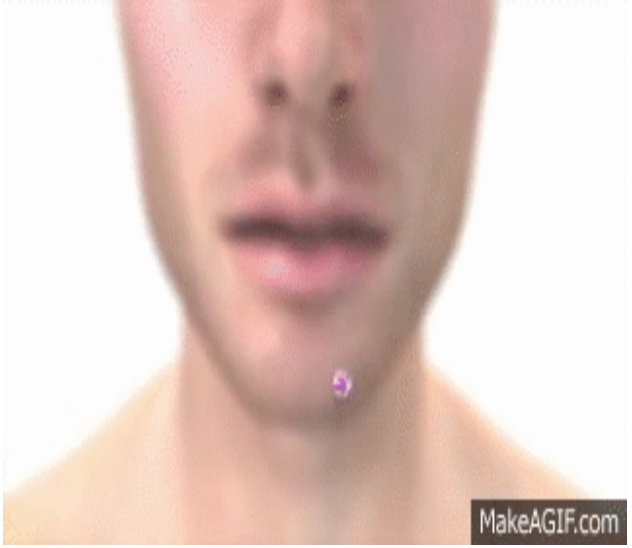
- ++ Number of cases & ++ resistance to drugs (MDR)



Pathogenesis of TB

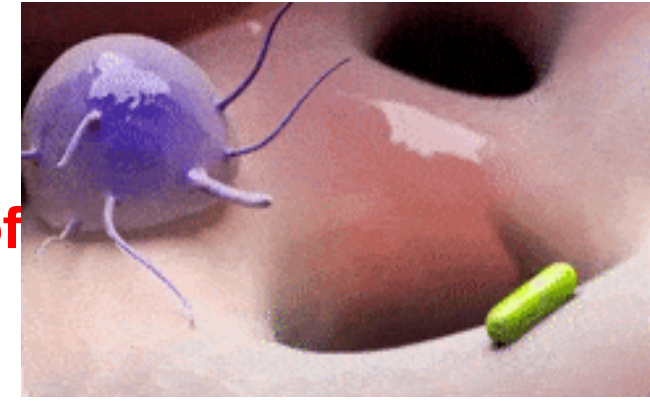
- **Modes of transmission:**





- **Airborne (aerosols)**

from a patient with open pulmonary TB these bacilli are engulfed by alveolar macrophages where **most of killed**.



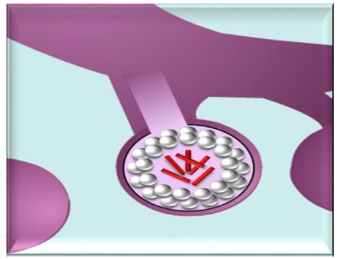
- **Ingestion of milk contaminated with *M. bovis* causing intestinal infection**

The remaining bacilli:

- Survive & multiply intra-cellularly (inhibit phago-lysosomal fusion)……Facultative IP
- CMI(T-helper1,Machrophages,IFN-γ (the main immune response against Mycobacteria) is initiated 4-6 weeks after infection resulting in granuloma (tubercle) formation.

- Within the granuloma, *M.tb*. can survive in small numbers in dormant state (latent TB infection).

Balanced host-parasite relationship



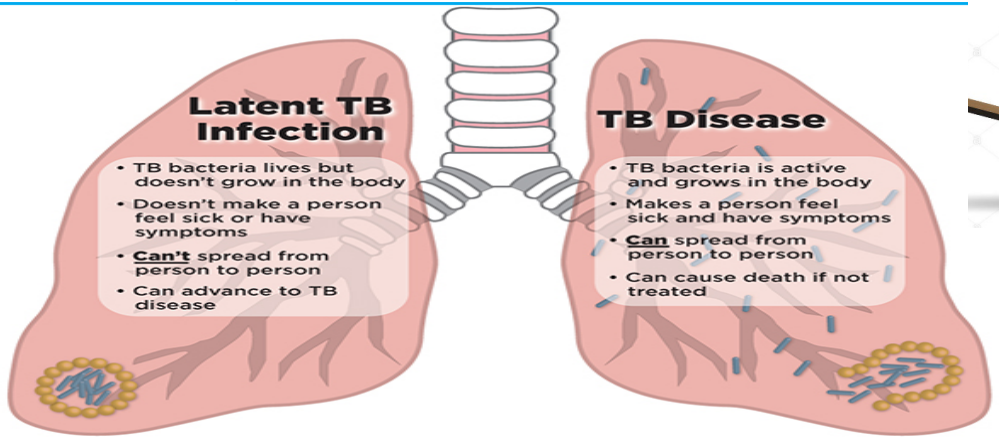


Outcome of primary infection:

- **Latent TB infection:** The above mentioned events can all occur without symptoms. This occurs in 90% of infected people who will be tuberculin +ve.

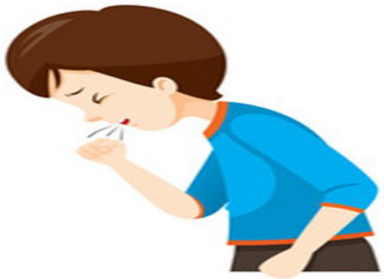
- **TB disease:** In the remaining 10%, *M.tb.* overcome the immune system and multiply resulting in disease. Symptoms: night sweat, night fever, cough, bloody sputum, malaise & fatigue. Spread may occur e.g. to meninges, LN, kidneys & intestine.







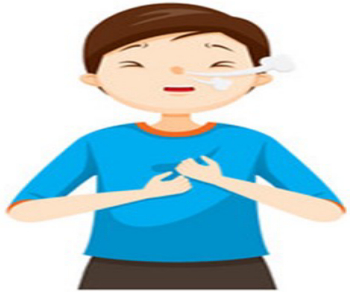
SIGN AND SYMPTOMS OF TUBERCULOSIS



Long-Term Coughing



Coughing up Blood



Chills



Fever



Chest Pain



Fatigue

Reactivation:

- Under certain conditions (e.g. immunosuppression) reactivation of Latent TB may occur resulting in TB disease.
- Reactivation usually occurs 2 years after initial infection in about 5% of people with Latent TB.

- The most common site of reactivation is the lung apex.



Characters	Latent TB	Pulmonary TB disease
M.TB present	Yes	
TST (or QuantiFERON)	Positive	
Sputum smear & culture	Negative	Positive
Symptoms	None	Cough, fever, wt. loss
Communicability	Not infectious	Infectious before ttt
Case definition	Not a TB case	TB case

Laboratory diagnosis of open pulmonary TB

A) Specimens: sputum, BAL,…etc.

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

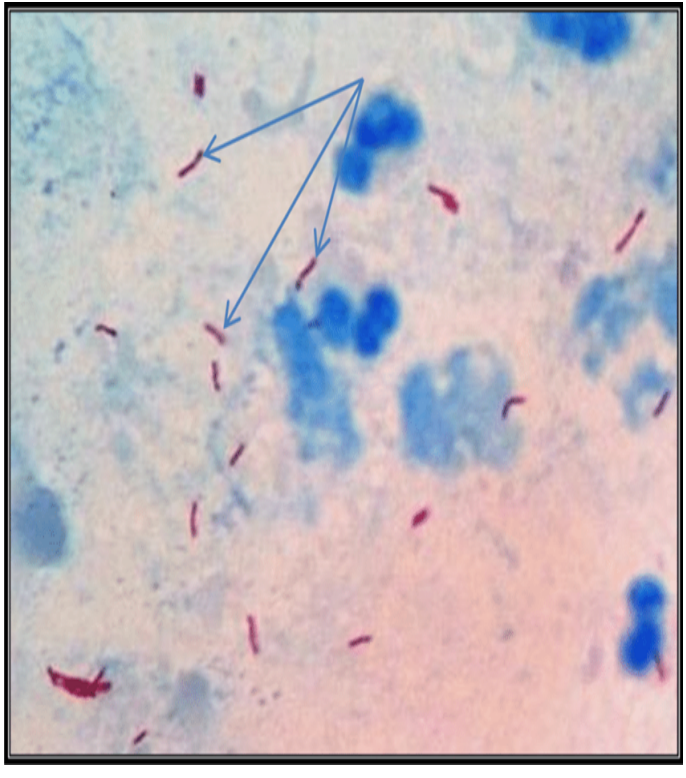


B) Direct Detection:

1.Smears:

- **Ziehl- Neelsen (Z-N) or Kinyoun method.**

Under the ordinary light microscope, AFB appear **red** in a **blue** background.



- Fluorochrome staining with auramine-rhodamine stain

Under the UV microscope, AFB fluoresce orange yellow in a black background.

It's faster and more sensitive than Z-N, but less specific.



Positive AFB sputum smear has the following advantages:

- Early presumptive diagnosis → rapid patient care
- Monitoring infectivity & response to anti-tuberculosis therapy.

However,

Positive AFB: Does Not differentiate MTB from other mycobacterium.

Negative AFB: Does Not exclude T.B. infection.



2. Molecular tests (PCR):

- For **rapid** detection
- Expensive

C) Processing of Sputum

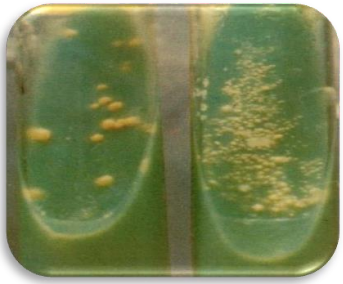
(Liquefaction, Decontamination & Concentration)

N-acetyl L-cysteine (NALC) / NaOH method

Problem	Solution
Trapped?	Liquefaction (NALC / NaOH)
Numerous flora?	Decontamination (NaOH)
Few?	•Concentration (centrifugation) • 3 morning samples

Before culture of any potentially contaminated specimen

D) Culture (definitive diagnosis):



a) Conventional culture media:

L-J or Middlebrook's agar (2-8 weeks)

b) Fluid media (4-14 days)



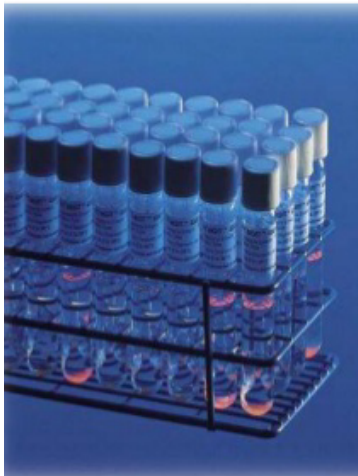
MGIT +ve tubes

- **Bactec AFB system**
  - Radio-labeled palmitate as sole source of carb
  - Bacterial growth → liberates C-labeled CO_2
- **M**ycobacteria **G**rowth **I**ndicator **T**ube **(MGIT):**

  M. tuberculosis growth → oxygen depletion

  ↓

  fluorescence (UV light)

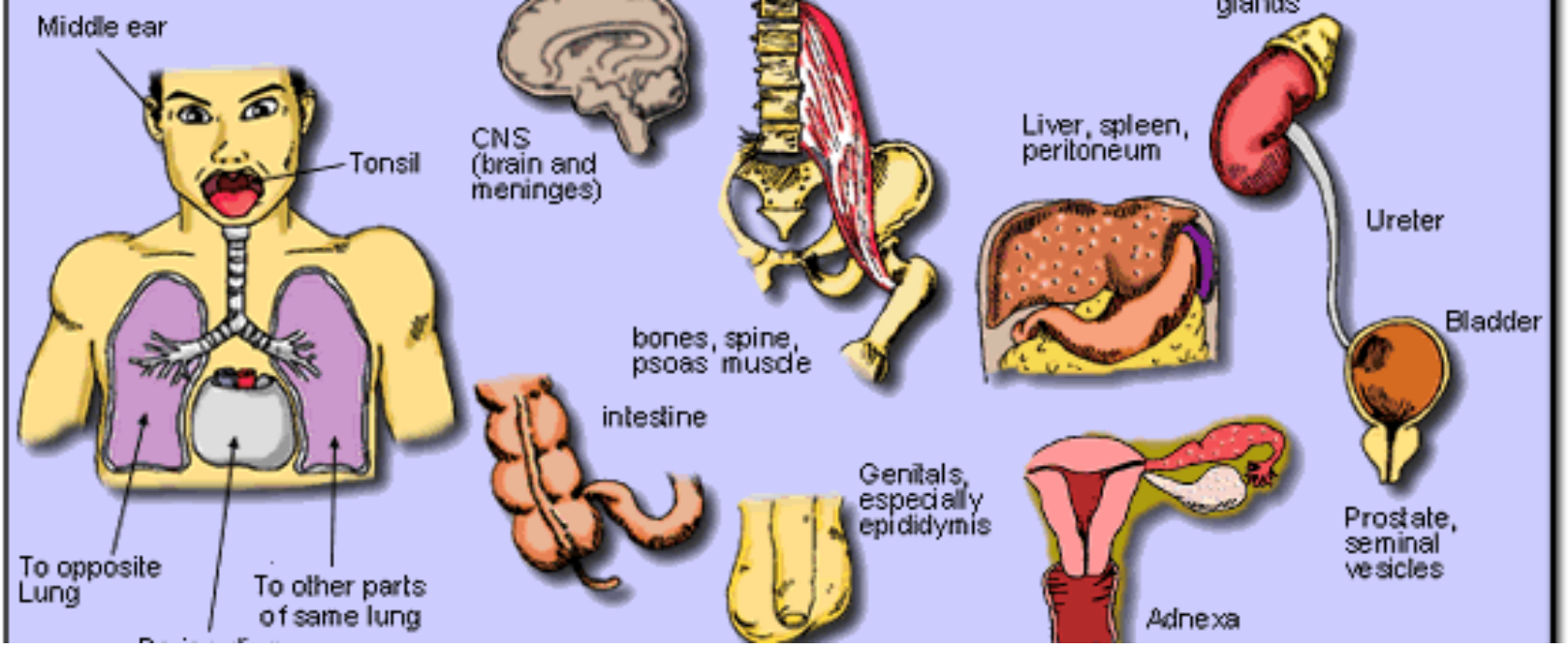


E) Identification:

1. **Cultures examined twice weekly.**

2. **Colonies appear after about 2- 4 weeks of incubation up to 8 weeks. Then tested for:**

   - **Colony morph.:** *M. tuberculosis* are non-pigmented.
   - **Microscopy: acid-fast stain** (*M. tuberculosis* are AFB).
   - **Biochemical reactions:** (*M. tuberculosis* are positive for niacin production & nitrate reduction).

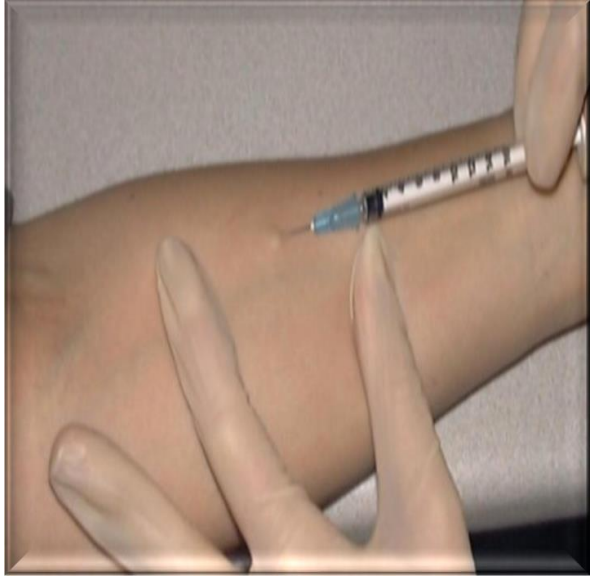


- **Lab. diagnosis of extra-pulmonary TB:**

As before but with appropriate samples.

Sterile specimens e.g. CSF don't require decontamination.

Tuberculin Skin Test (TST)



Principle:

- Detects a hyper delayed type sensitivity response to previous exposure of the host to tubercle bacilli.



- It's one of the main tests to diagnose latent TB infection.

- Agused: Purified protein derivative (PPD).

TS
T

Underlying mechanism:

Previous exposure to Mtb activated & clonally expanded. → Th1 cells are sensitized,

In +ve reactors, the injected tuberculin stimulates the presensitized Th1 cells → secretion of cytokines → recruit inflammatory cells esp. macrophages → raised indurated area around the injection site.

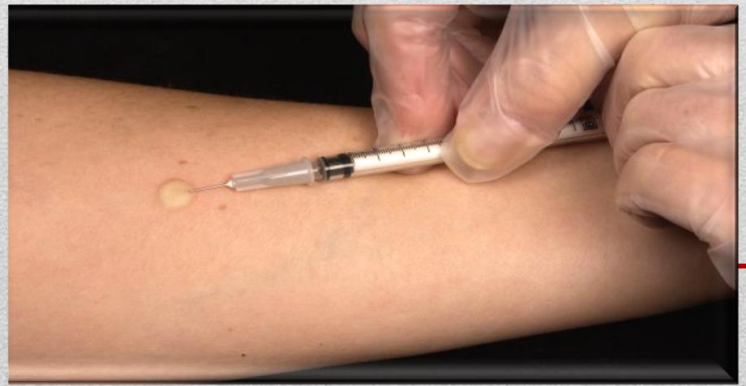
NO reaction in those not sensitized to Mtb.

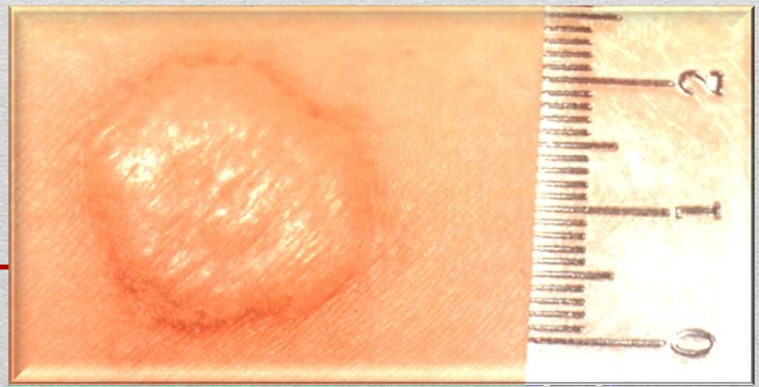
TS

T

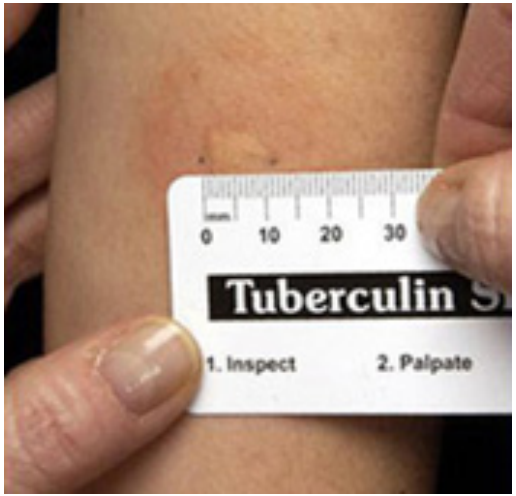
Technique:

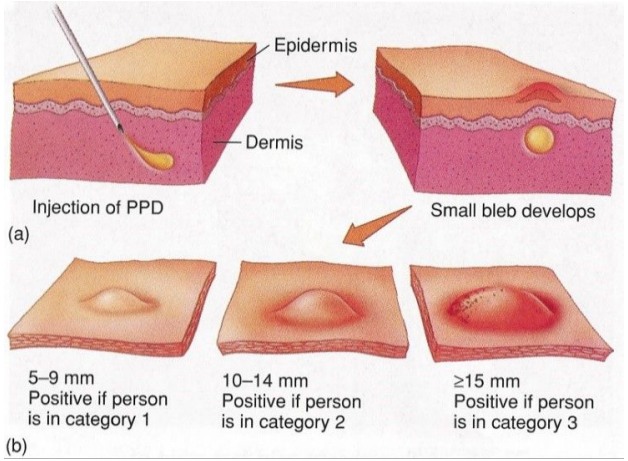
- **0.1 ml of PPD containing 5 tuberculin units (TU) is injected intradermally in the anterior aspect of the forearm (Mantoux method).**
- **Result read after 48-72 hrs by palpating for induration. The diameter is measured in mm. The induration (not the erythema) is measured.**





Tuberculin Skin Test

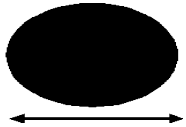




Interpretation of TST







Induration ≥ 5 mm	Induration ≥ 10 mm	Induration ≥ 15 mm
Positive in case of: 1. History of **TB disease.** **2. Close contacts** of people with infectious TB **3. HIV** infection.	**Positive** in: **1. Endemic areas.** 2.People with certain **medical conditions** (diabetes, malnutrition, steroid therapy). **3. Unvaccinated children < 4** years.	**Positive** in: People with **no risk factors for TB (low probability).**

TST

False negative results:

1. Anergy:
a weakened immune system
- Severe TB disease
- HIV infection
- Cancer

2. Recent TB infection:
(4 to 6 weeks)

False positive results:

1 Infection with NTM:
cross-reaction

2 BCG vaccination:
for up to 5 years.

QuantiFERON TB (IFN-γ release assay)

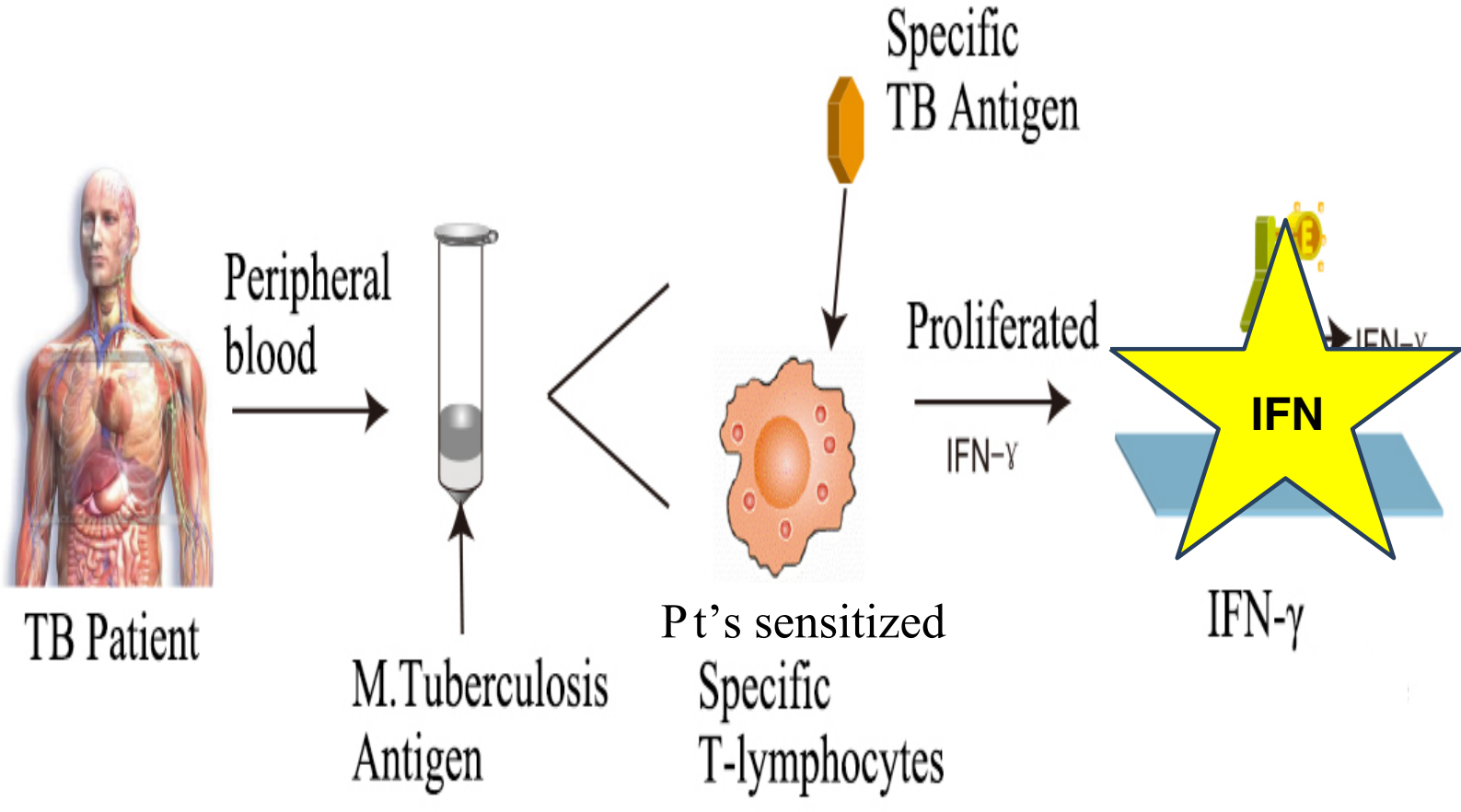
- **Principle:** exposing the person to M.tb.

  ⟹ antigens measuring the amount of IFN-γ

  released from the patient's sensitized T-cells

- Compared to TST, it's **specific for** diagnosis of **Latent TB**, because the antigens used are not present in NTM or BCG vaccine.

QuantiFERON-TB test



Treatment:

- To be successful:

1. **Combined** therapy: **≥ 4** drugs to reduce toxicity & prevent emergence of resistance.
2. **Prolonged** therapy: **≥ 6** months due to:

- Intracellular location
- Blocking of antibiotic penetration by the caseous material
- Slow growth rate
- Presence of persisters (metabolically inactive organisms)

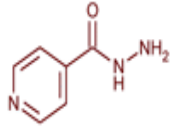
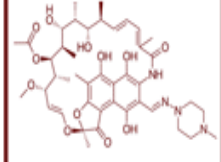
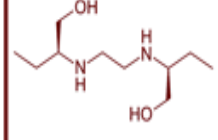
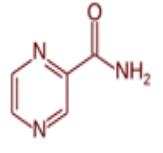
The patient usually becomes non-infectious within2-3 weeks.

Treatment:

- 1st line drugs: INH, RIF, PZA, EMB & streptomycin.

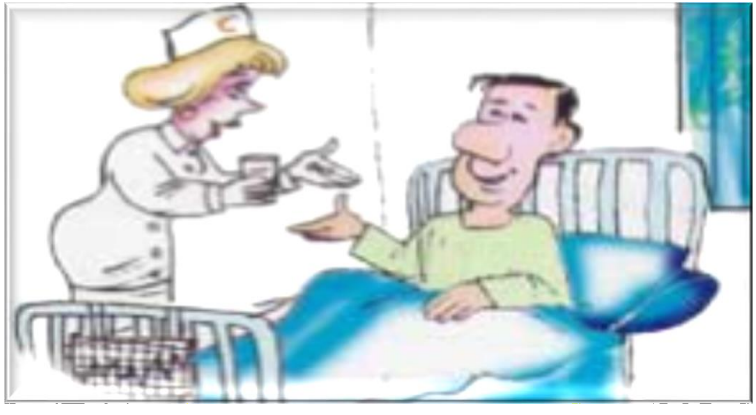
- 2nd line drugs (only if resistance occurs to 1st line drugs): FQs, PAS, ethionamide, cycloserine, capreomycin, amikacin, kanamycin & rifabutin.

The Four Principal First-Line TB Drugs

Drug Name	Formulation	Chemical Structure
Isoniazid		
Rifampin		
Ethambutol		
Pyrazinamide		

Treatment:

- Due to patient's non-compliance, directly-ob served therapy short course (DOTS) should be followed.





- Antibiotic sensitivity testing should be done.
- MDR= R to both INH & RIF
- XDR= MDR + resistance to FQ & at least an additional drug

Prevention is Always Better than Treatment

1- General measures



- Early case finding & ttt
- Proper infection control (N95)
- Avoid over-crowding
- Pasteurization of milk.

2- Treatment of latent TB (INH for 6-9 m) for:

- Recent converters: asymptomatic people whose TST or QuantiFERON test recently converted to +ve
- TSTor QantiFERON +ve people who are subject to immunosuppression.

3- BCG vaccine

- Live attenuated *M. bovis*
- To newborns ID over the left deltoid
- Controversial





Members of the genus *Mycobacterium*

1. ***Mycobacterium tuberculosis* complex (tubercle bacilli): *M. tuberculosis* (humans), *M. bovis*** (cows and humans), ***M. africanum* & *M. microti.***

2. **Non-tuberculous mycobacteria (NTM):** e.g. *M. avium complex* & *M. fortuitum complex*.

3. ***Mycobacterium leprae:*** causes leprosy.

4. **Saprophytic mycobacteria.**

Non-tuberculous Mycobacteria (NTM)
= Mycobacteria other than tuberculosis (MOTT)

- Include *Mycobacterium* speciesthat are not members of *M. tuberculosis* complex.
- Recovered from tap water, water of hemodialysis.

General features:

- **Morphology:** similar to M.tb.
- **Grow well on media of Mtb.**
- **Growth characteristics:** for grouping (I-IV)
- **No person-to-person spread.** Transmission is from environmental sources
- **Cause opportunistic infections.**
- **Highly resistant to disinfection**
- **More resistant to drugs used for ttt of TB. Combinations must be used (rifabutin, clarithromycin & azithromycin).**

Non-tuberculous Mycobacteria (NTM)
Examples of species & diseases

Microorganism	Disease
Slow growers	
M. avium- intracellulare complex	Pulmonary TB-like disease, particularly in AIDS patients
M. kansasii	Chronic lung disease resembling TB
M. ulcerans	**Skin** ulcer (Buruli ulcer)
Rapid growers:	
M. fortuitum-chelonae complex	**Skin** and soft tissue infection
M. abscessus	Chronic lung disease which can disseminate to skin, bone, and joints

Good Luck

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is the result of

good planning