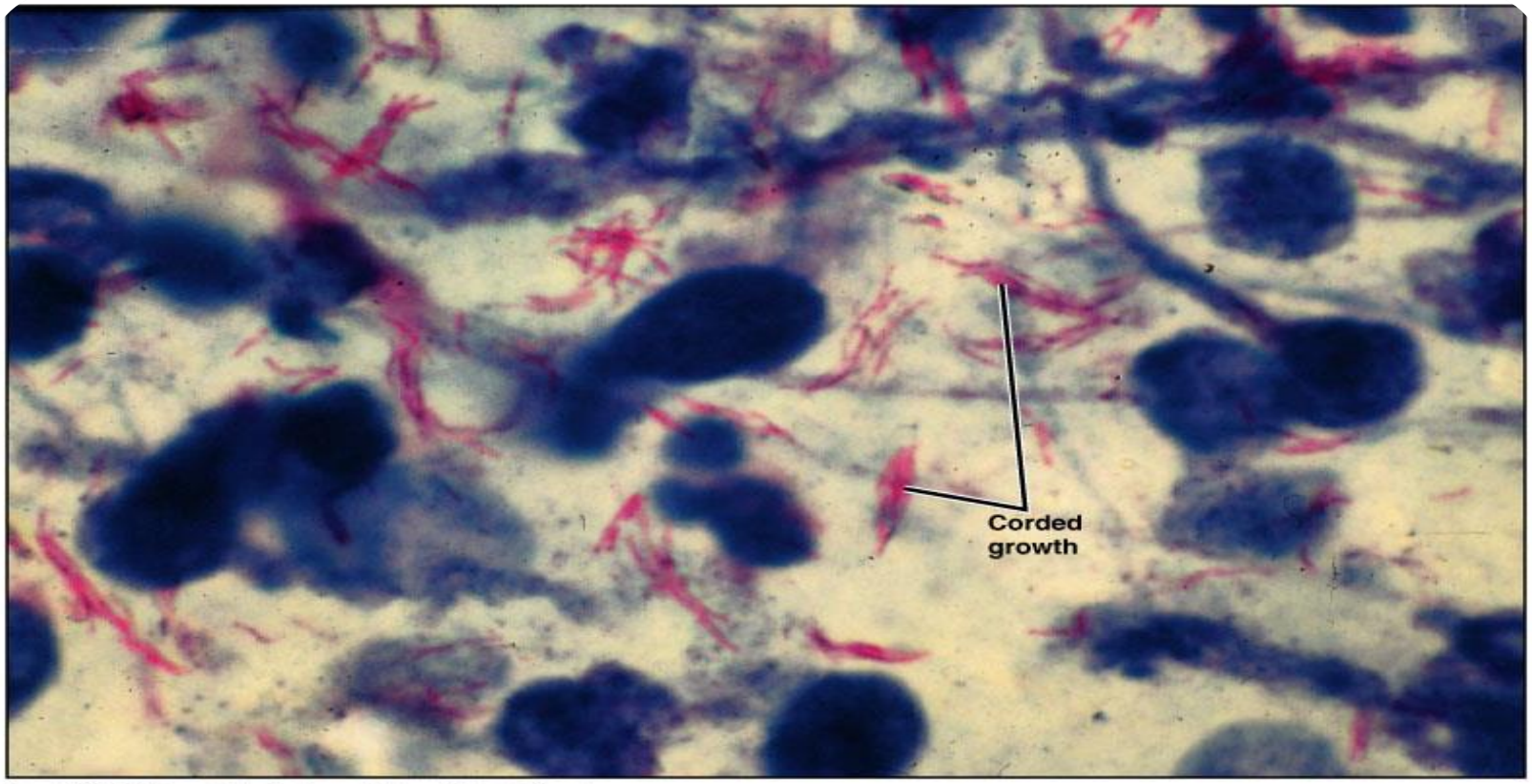
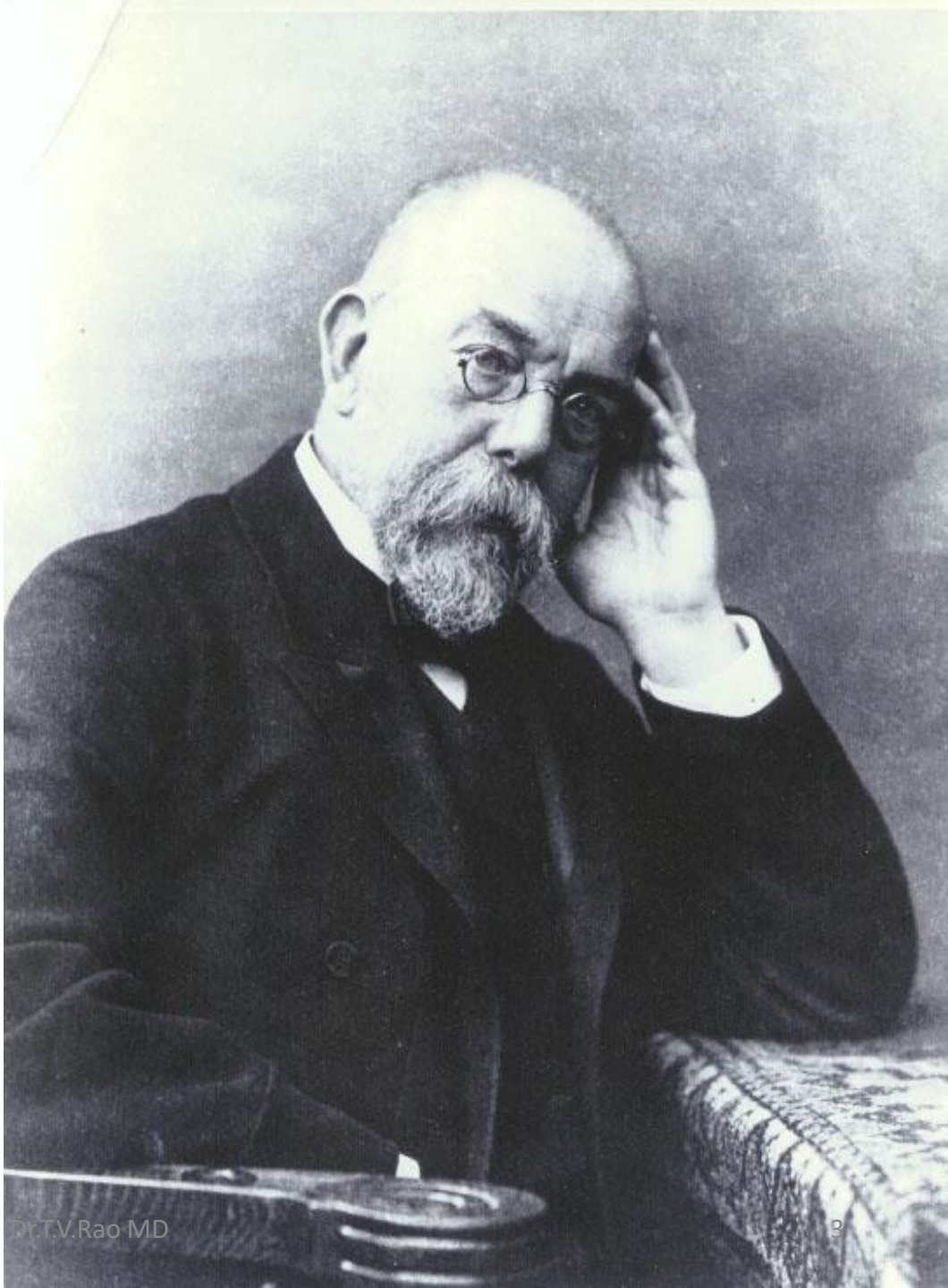
Mycobacteria



Learning objectives

- To know the epidemiology, classification and imp features of Mycobacteria
- To recognize antigenic determinants , transmission and pathogenesis of Tuberculosis
- To interpret the lab diagnosis of Mycobacteria

Robert Koch Discoverer of Mycobacterium Tuberculosis in 1882



Tuberculosis is a diseased caused by bacteria mycobacterium tuberculosis.

Egyptian mummies from 3500 BCE have the evidence of *Mycobacterium tuberculosis*

- TB has remained an enemy of human society for all age.
- TB is not only a problem for the person suffering from it or their families but a public health problem of the entire world.

DISEASE CLASSIFICATION

INFLAMMATORY DISEASE

- It is a Chronic specific Inflammatory infectious Disease caused by Mycobacterium Tuberculosis In human.
- Usually attacks the lungs but It can also effect any part of The body.

TB Infection vs. TB Disease

- There is a difference between TB "infection" and TB "disease"

- TB **infection**: TB germs stay in your lungs, but they do not multiply or make you sick
  - You cannot pass TB germs to others

- TB **disease**: TB germs stay in your lungs or move to other parts of your body, multiply, and make you sick
  - You can pass the TB germs to other people

Mycobacterium tuberculosis

- One of the most serious infectious diseases in the developing world.
- One third of world's population infected with *M. tuberculosis.*
- Each year, it is estimated that 1.7 million people die of tuberculosis .
- Nine million new cases occur.
- An estimated 500,000 people are infected with a multidrug-resistant strain of *M. tuberculosis.*

CLASSIFICATION

Mycobacteria can be classified as :

1-Tuberculous complex group:

- *Mycobacterium tuberculosis*
- *Mycobacterium bovis*
- *Mycobacterium africanum*
- *Mycobacterium microti* (animal pathogen)

2- Atypical mycobacteria

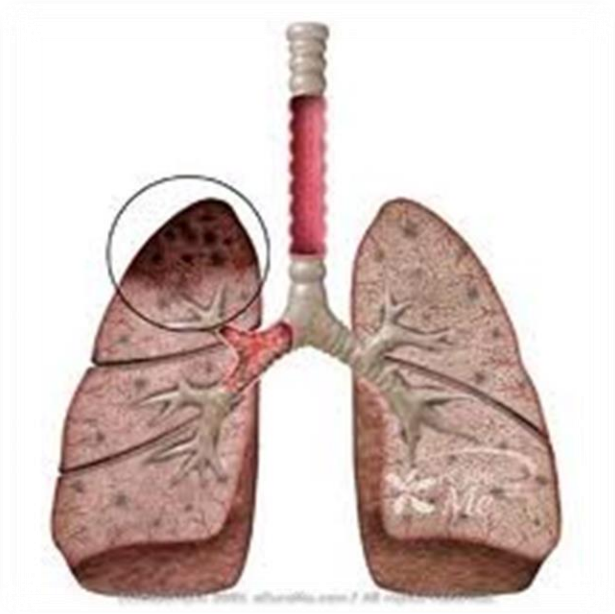
3- *Mycobacterium leprae*

Here's a flow chart to help keep track of whom we are talking about.

Genus *Mycobacterium*

- *Mycobacterium tuberculosis* complex
  - *Mycobacterium tuberculosis*
  - *Mycobacterium bovis*
  - *Mycobacterium microti*
  - *Mycobacterium africanum*
- *Mycobacterium ulcerans*
- *Mycobacterium leprae*
- Nontuberculous Mycobacteria
  - Runyon I → *M. kansasii* and *M. marinum*
  - Runyon II → *M. scrofulaceum*
  - Runyon III → *Mycobacterium avium* complex
  - Runyon IV → *M. chelonei* and *M. fortuitum*

MYCOBACTERIUM TUBERCULOSIS



MYCOBACTERIUM

- Slender, often slightly curved, rods
- Obligate aerobic, non-motile, non-spore forming
- Acid fail to wash the stain out ➡ acid fast bacilli .
- The acid-fast property of M. tuberculosis(and other mycobacteria) is due to long-chain (C78–C90) fatty acids called mycolic acids in the cell wall.

MYCOBACTERIUM

- 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.
  - Heating is required for stain penetration because of the high lipid content of the cell wall (mycolic acid and waxD).
    - Once the stain penetrates and binds to the mycolic acid in the cell wall, it cannot be removed even with acid alcohol treatment.

MYCOBACTERIUM

- *M. tuberculosis* grows slowly(i.e., it has a doubling time of 18 hours, in contrast to most bacteria, which can double in number in 1 hour or less).
- The growth is so slow, cultures of clinical specimens must be held for 6 to 8 weeks before being recorded as negative.

MYCOBACTERIUM

- *M. tuberculosis* can be cultured on bacteriologic media, where as *M leprae* cannot. Media used for its growth contain complex nutrients and dyes (malachite green) known as Lowenstein Jensen media. The dyes inhibit the unwanted normal flora present in sputum samples.

MYCOBACTERIUM

- *M. tuberculosis* is relatively resistant to acids and alkalies. NaOH is used to concentrate clinical specimens, it destroys unwanted bacteria.

MYCOBACTERIUM

- *M. tuberculosis* is resistant to dehydration and therefore survives in dried expectorated sputum.
- Humans are the natural reservoir of *M. tuberculosis*

Transmission

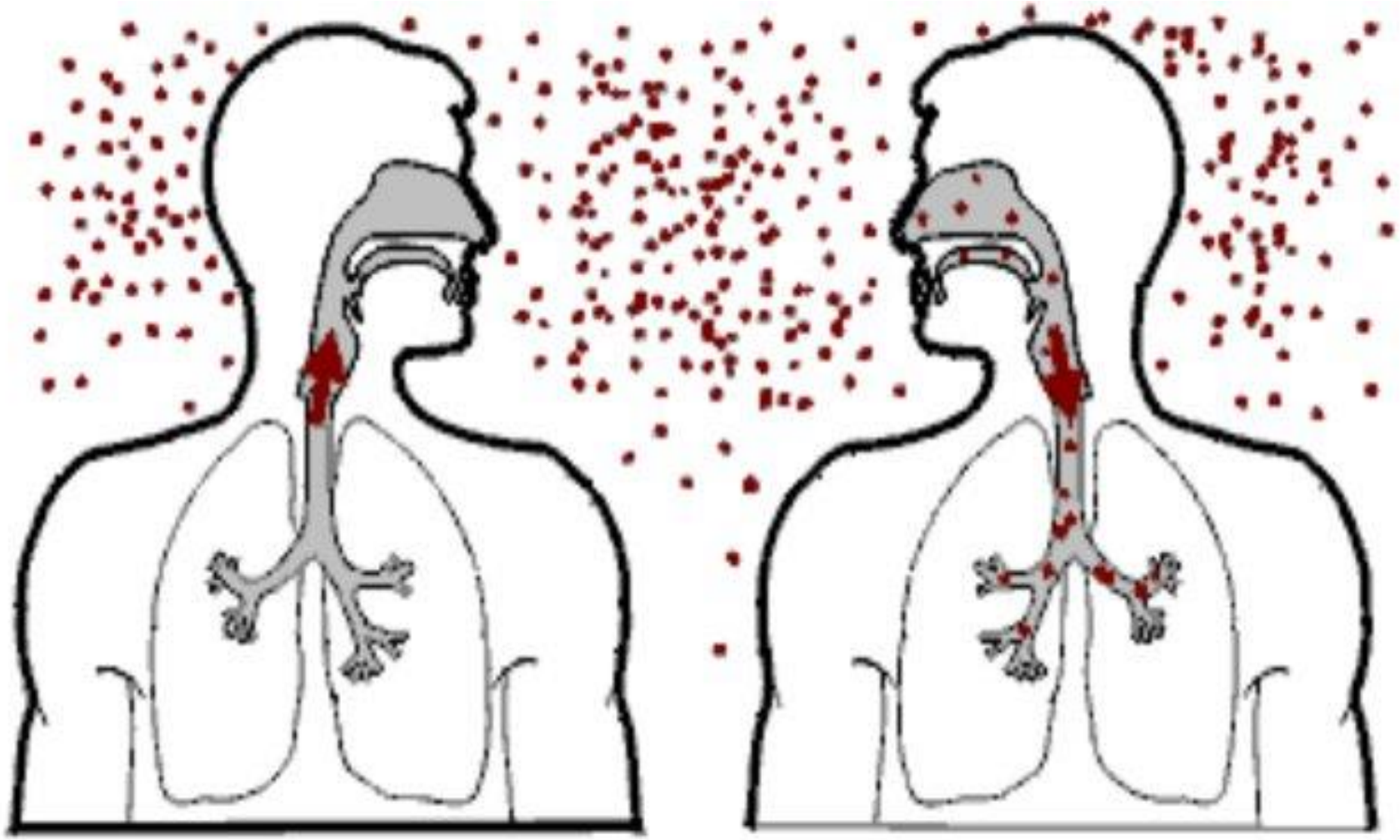
1) Droplet infection

- Person to person by inhalation of aerosols
- Cough, sneeze, speak or singing

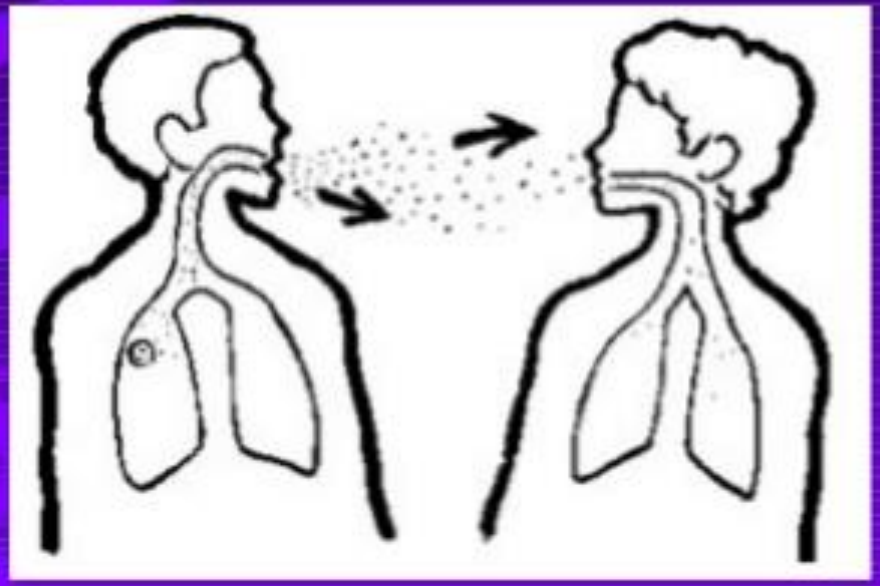
2) Ingestion of milk

- Infected cattle
- *Mycobacterium bovis* (Intestinal tuberculosis)

Tuberculosis spread by Respiratory route

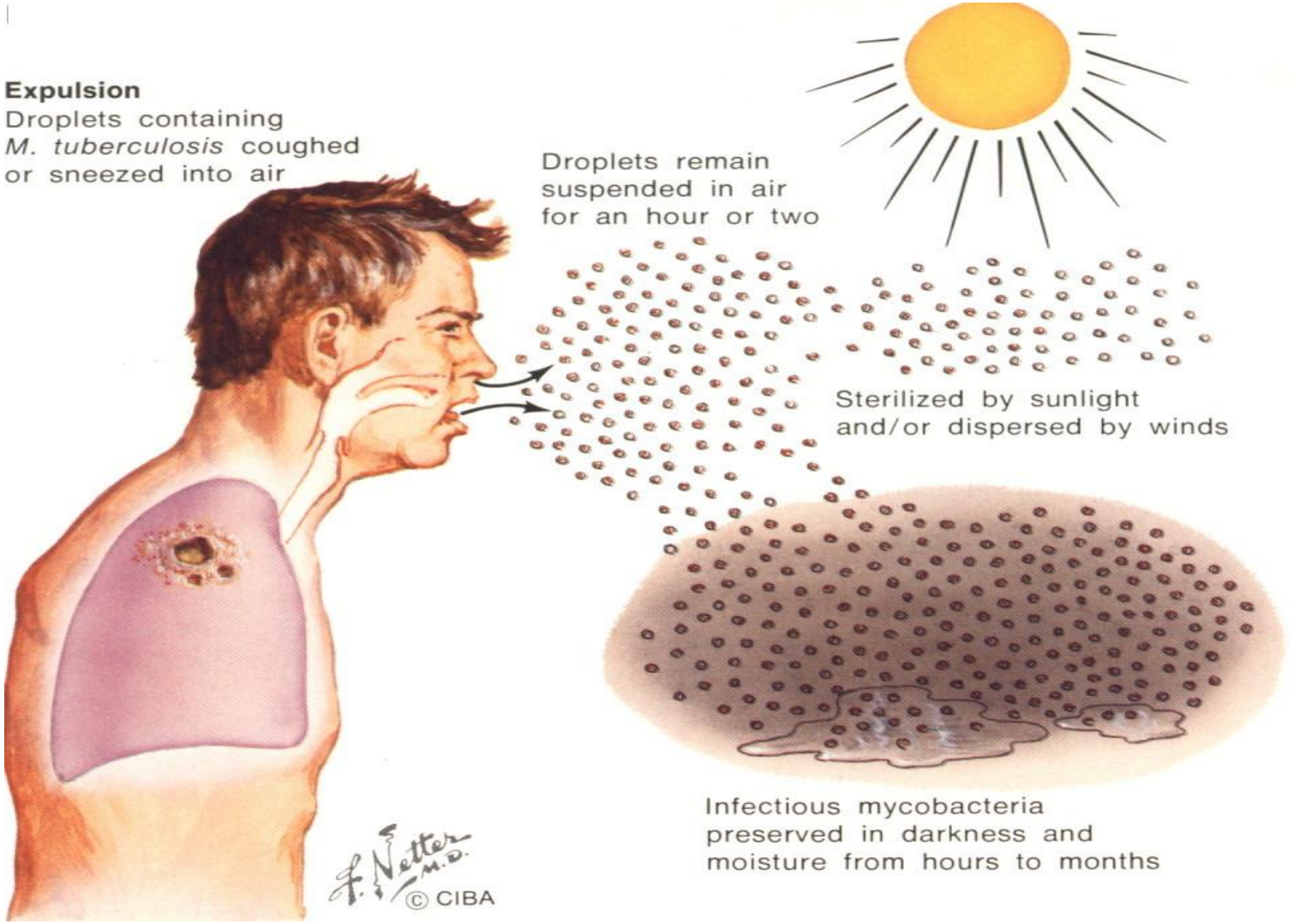


- TB spread from person to person by airborne transmission. Infected person release droplet nuclei (1-5 micro meter in diameter) through,
  - Talking
  - Coughing
  - Sneezing
  - Laughing
  - Singing

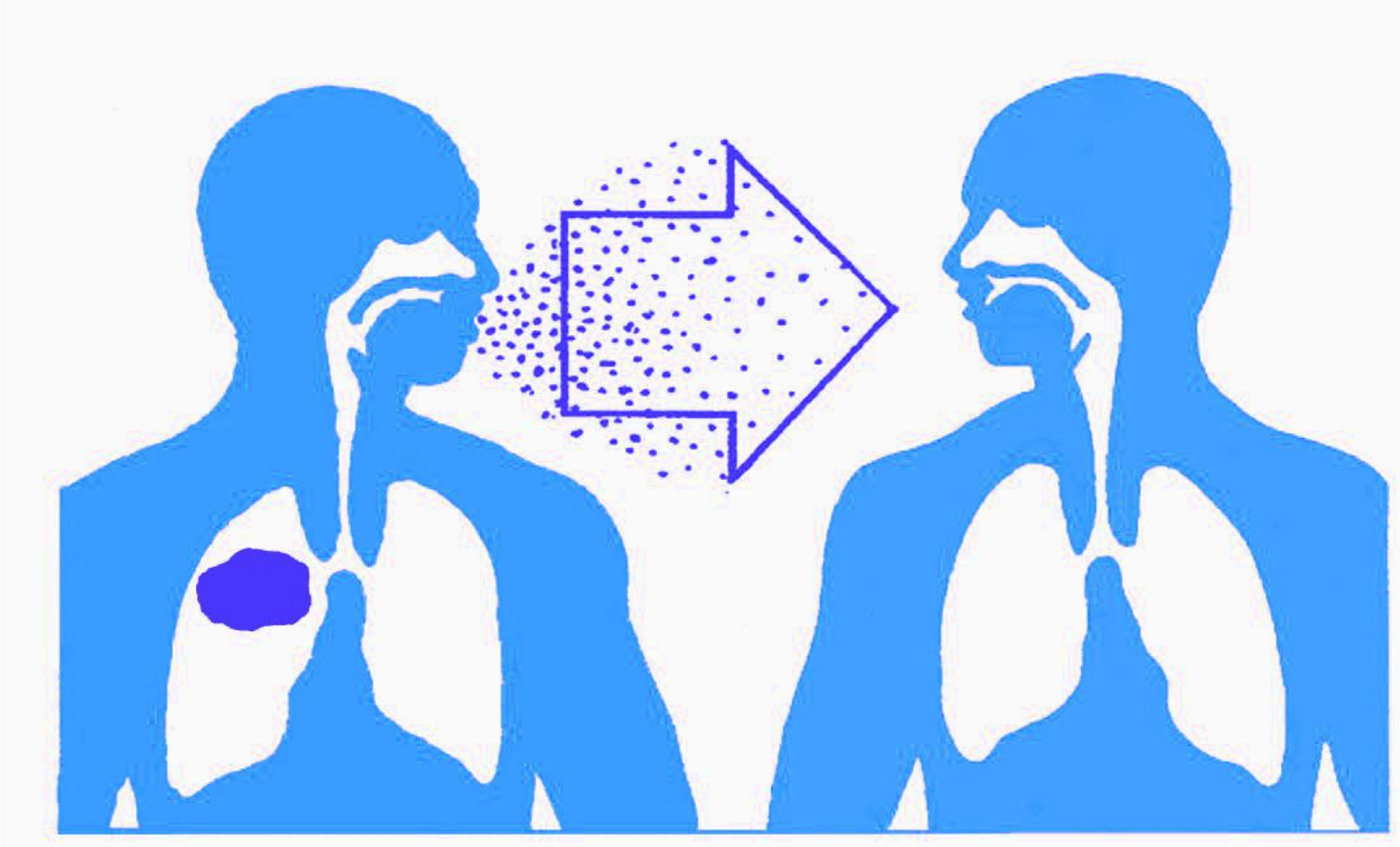


- If not treated properly, TB can be fatal.

TB droplet nuclei



How Are TB Germs Spread?



Transmission

- Most transmission occurs by aerosols generated by the coughing of “smear-positive” people (i.e., those whose sputum contains detectable bacilli in the acid-fast stain).

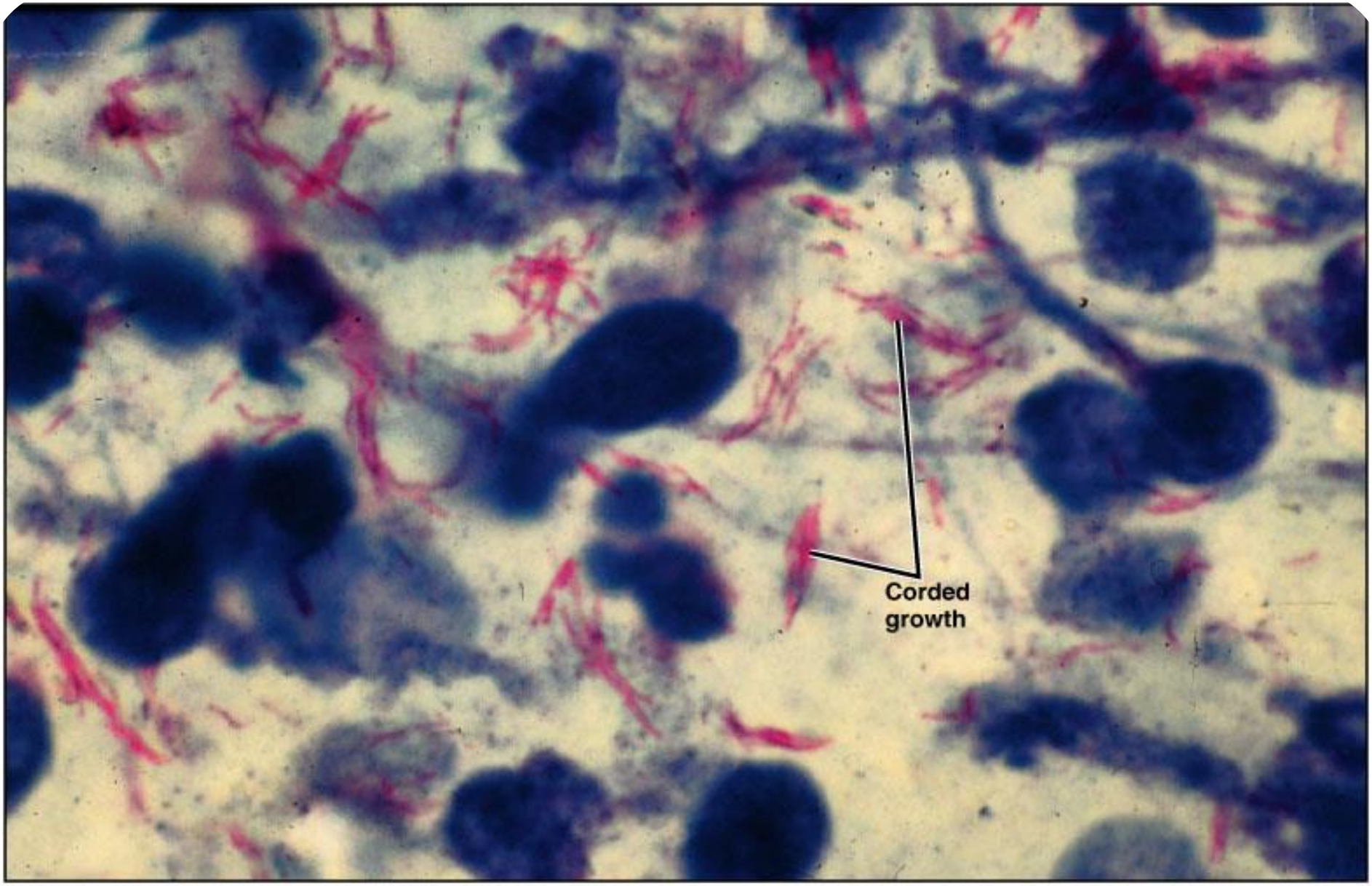
- However, about 20% of people are infected by aerosols produced by the coughing of “smear negative” people.

Virulence Determinants

- M. tuberculosis produces no exotoxins and does not contain endotoxin in its cell wall.
- The organism preferentially infects macrophages and other reticuloendothelial cells. *M. tuberculosis* survives and multiplies within a cellular vacuole called a phagosome.

Virulence Determinants

- Cord factor(trehalose dimycolate) is correlated with virulence of the organism.
- Virulent strains grow in a characteristic “serpentine” cordlike pattern, whereas avirulent strains do not.



Antigenic Determinants

- **Proteins:**
  - Act as immunogen:
  - Cause antibody production
  - Elicit tuberculin reaction

- **Polysaccharides:**
  - Arabinogalactan & arabinomannans
  - Induce immediate type of hypersensitivity.

Other Virulence Mechanisms

Slow generation time

– Immune system cannot recognize TB, or cannot be triggered to eliminate TB

High lipid concentration in cell wall

– accounts for impermeability and resistance to antimicrobial agents.

It's a facultative intracellular pathogen and thus evades the immune system very effectively.

- Inside the macrophage it can inhibit phagosome-lysosome fusion by secretion of a protein(PKnG) that modifies the phagosome membrane.

Pathogenesis

- Droplet nuclei are inhaled, that are generated by talking, coughing and sneezing.
- Once nuclei are inhaled, the bacteria are non-specifically taken up by alveolar macrophages.
  - The large droplet nuclei reaches upper respiratory tract, and the small droplet nuclei reaches air sacs of the lung (alveoli) where infection begins.

Pathogenesis

- **facultative intracellular pathogen**
  - **inhibits phagosome-lysosome fusion**
  - **resists lysosomal enzymes**

- The organism produces a protein called “exported repetitive protein” that prevents the phagosome from fusing with the lysosome.

Pathogenesis

- TB multiplies within the inactivated macrophages until macrophages burst.
- Other macrophages diffuse from peripheral blood, phagocytose TB and are inactivated, but are unable to destroy TB.
- These macrophges loaded with TB migrate through lymphatics to the hilar lymph nodes.
- These initial stages are asymptomatic and non specific defense mechanism is involved.

Pathogenesis

- About 2–4 weeks after infection, two host responses to *M. tuberculosis* develop:

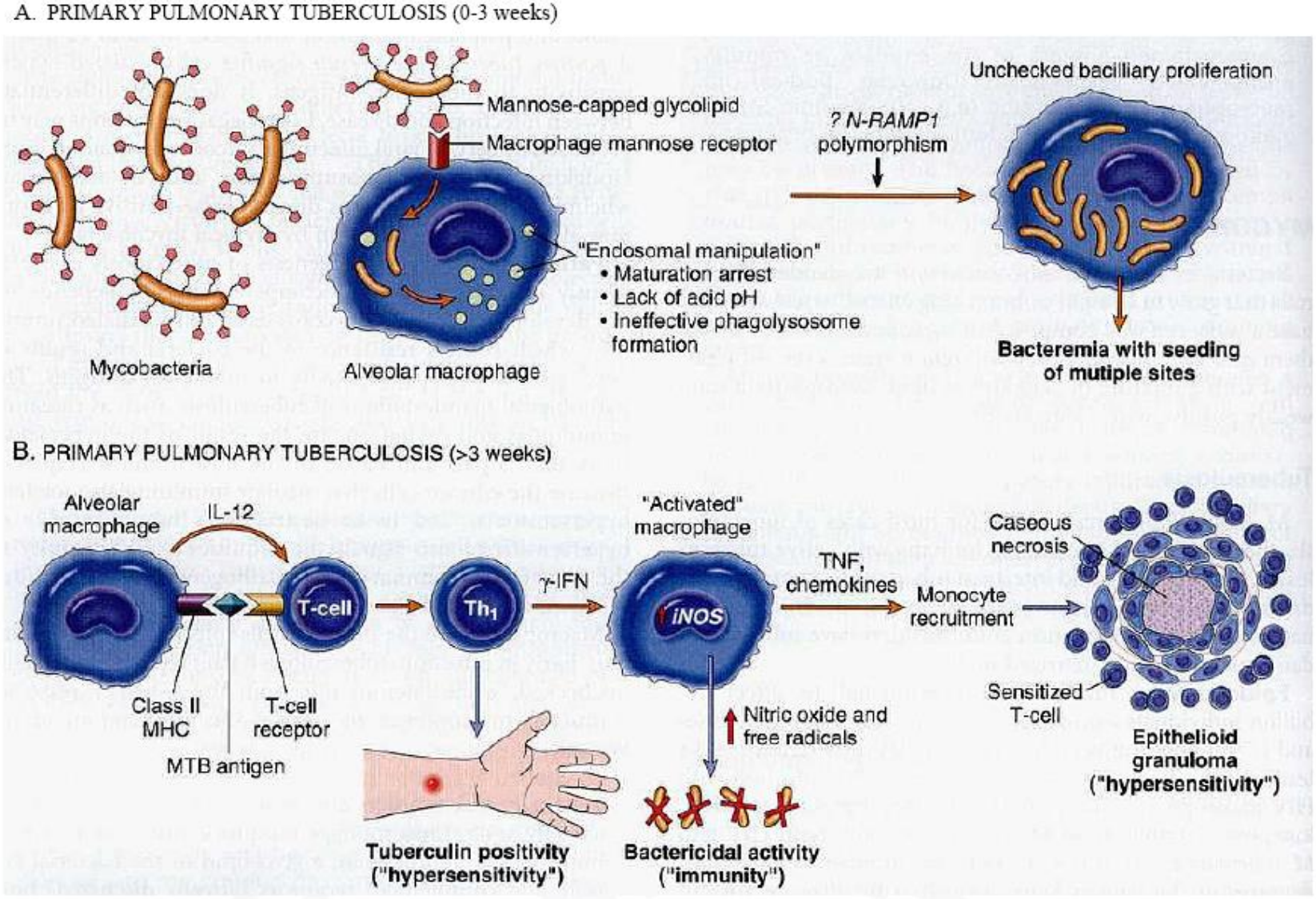
1) A macrophage-activating CMI response ,resulting in the activation of macrophages that are capable of killing and digesting tubercle bacilli.

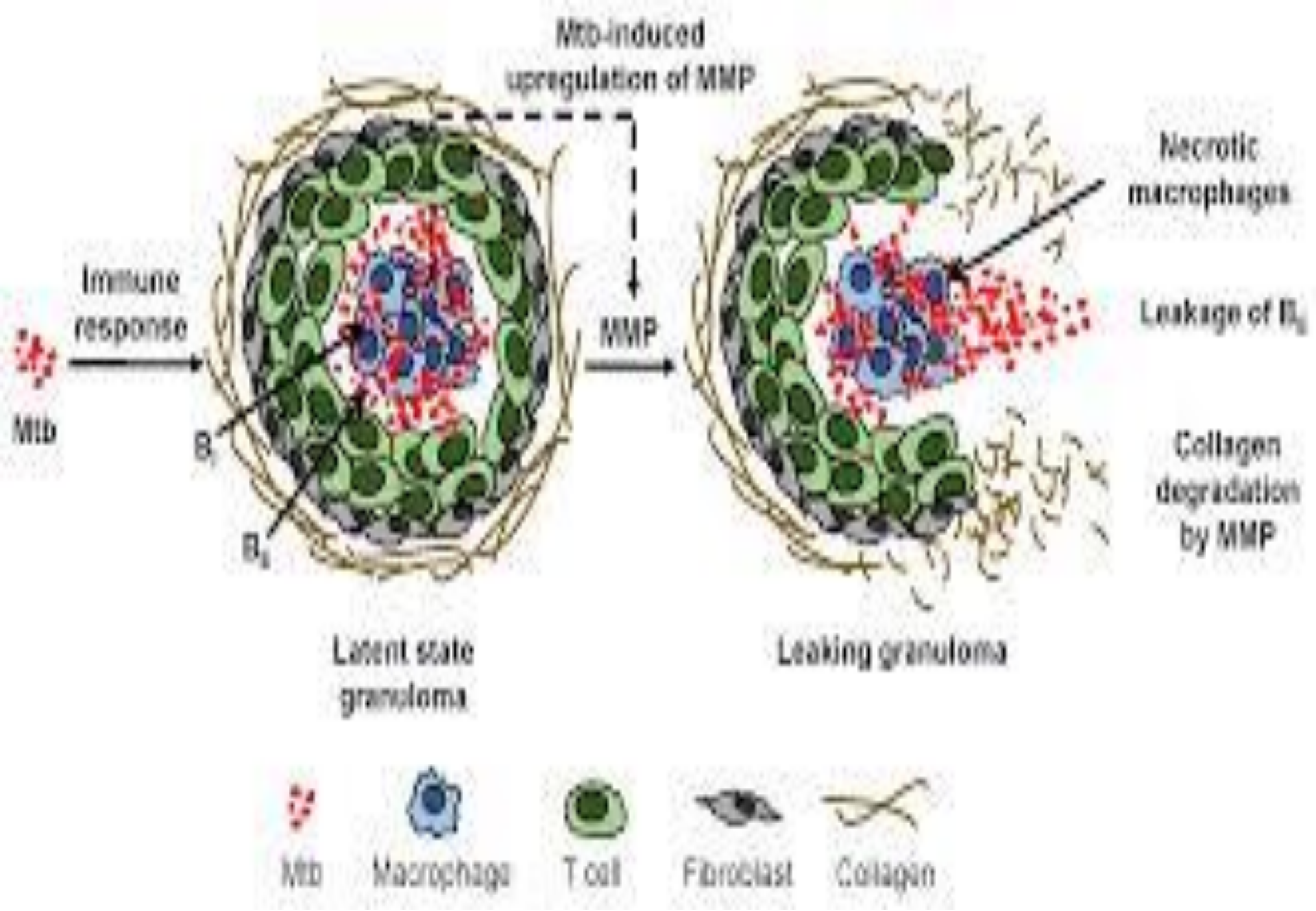
2) The *tissue-damaging response* is the result of a delayed-type hypersensitivity (DTH).

Pathogenesis

- For persons with intact cell-mediated immunity, the next defensive step is formation of granulomas around the *M tuberculosis* organisms.
- These nodular-type lesions form from an accumulation of activated T lymphocytes and macrophages, which creates a micro-environment that limits replication and the spread of the mycobacteria.
- This environment destroys macrophages and produces early solid necrosis at the center of the lesion; however, the bacilli are able to adapt to survive.

Pathogenesis




Mtb-induced
upregulation of MMP
Immune
response
Mtb
MMP
Necrotic
macrophages
Leakage of
Collagen
degradation
by MMP
Latent state
granuloma
Leaking granuloma
Mtb
Macrophage
T cell
Fibroblast
Collagen

Pathogenesis

- TB cannot multiply within tubercles due to low PH and anoxic environment, but TB can persist within these tubercles for extended periods.

- It is the balance b/w two responses that will determine the form tuberculosis that will develop subsequently.

Types of tuberculosis

- **Primary tuberculosis**: is a form of disease that develops in a previously unexposed and therefore unsensitized person.

- **Secondary tuberculosis**: is the pattern of disease that arises in previously sensitized or infected host.

Lesions Formed

- Lesions are dependent on the presence of the organism and the host response. There are two types of lesions:
- (1) **Exudative lesions**, which consist of an acute inflammatory response and occur chiefly in the lungs at the initial site of infection.
- (2) **Granulomatous lesions**, which consist of a central area of giant cells (Langhans cell) containing bacilli surrounded by a zone of epithelioid cells.

Lesions Formed

- A **tubercle** is a granuloma surrounded by fibrous tissue that has undergone central **caseation necrosis**. Tubercles heal by fibrosis and calcification.
- A tubercle can erode into bronchus, empty its caseous contents and thereby spread the organism to other parts of the lung and other persons if expectorated.

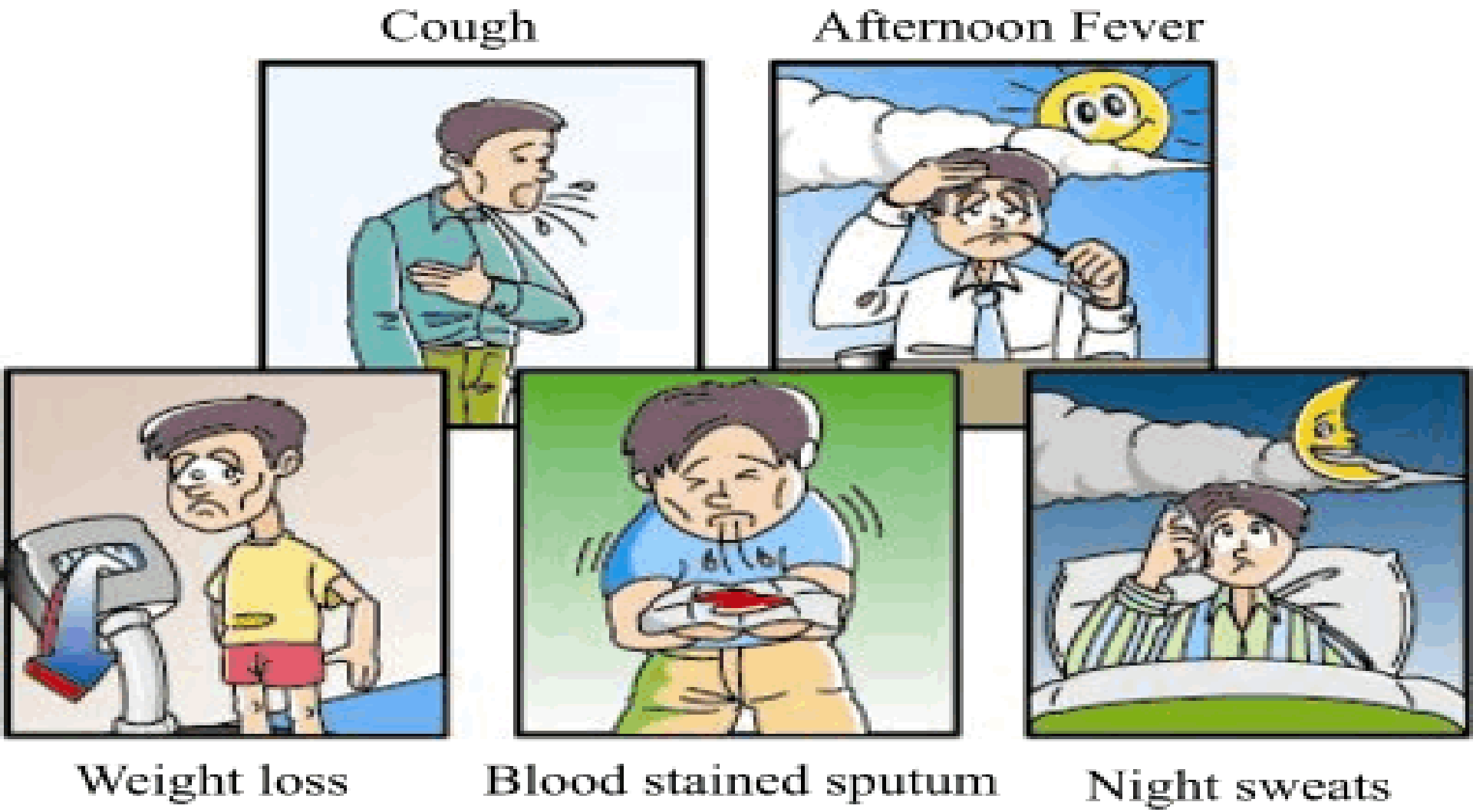
Lesions Formed

- The primary lesion of tuberculosis usually occurs in the lungs. The parenchymal exudative lesion and the draining lymph nodes together are called **Ghon complex**. Primary lesions usually occurs in the lower lobes, where as reactivation lesion usually occurs in the apices.

Symptoms and Signs of Tuberculosis

- Most (approximately 90%) infections with *M. Tuberculosis* are asymptomatic. Asymptomatic infections, also known as latent infections, can reactivate and cause symptomatic tuberculosis.

Symptoms and Signs of Tuberculosis



Clinical Findings

- Pulmonary tuberculosis causes cough and hemoptysis.
- **Scrofula is mycobacterial cervical lymphadenitis that** presents as swollen, nontender lymph nodes, usually unilaterally. Both *M. tuberculosis and Mycobacterium scrofulaceum* cause scrofula. Lymphadenitis is the most common extrapulmonary manifestation of tuberculosis.

Clinical Findings

- **Oropharyngeal tuberculosis** typically presents as a painless ulcer accompanied by local adenopathy.
- **Erythema nodosum,** characterized by tender nodules along the extensor surfaces of the tibia and ulna, is a manifestation of primary infection seen in patients who are controlling the infection with a potent cell-mediated response

Clinical Findings

- **Miliary tuberculosis** is characterized by multiple disseminated lesions that resemble millet seeds.
- **Tuberculous meningitis and tuberculous osteomyelitis,** especially vertebral osteomyelitis (Pott's disease), are important disseminated forms.

Clinical Findings

- **Gastrointestinal tuberculosis** is characterized by abdominal pain and diarrhea accompanied by more generalized symptoms of fever and weight loss. Intestinal obstruction or hemorrhage may occur. The ileocecal region is the site most often involved. Tuberculosis of the GI tract can be caused by either *M. tuberculosis* when it is swallowed after being coughed up from a lung lesion or by *M. bovis* when it is ingested in unpasteurized milk products.

Clinical Findings

- In **renal tuberculosis,** dysuria, hematuria, and flank pain occur. “Sterile pyuria” is a characteristic finding. The urine contains white blood cells, but cultures for the common urinary tract bacterial pathogens show no growth. However, mycobacterial cultures are often positive.

Fate of primary tuberculosis

- Heal by fibrosis→ calcification
- Progressive primary tuberculosis
- Primary miliary tuberculosis

Secondary tuberculosis

- Definition: the infection of an individual who has been previously infected or sensitized
- The infection may be acquired from
  - Endogenous source: reactivation of dormant primary complex
  - Exogenous source

- Primary lesions usually occur in the lower lobes, whereas reactivation lesions usually occur in the apices.
- Reactivation lesions also occur in other well-oxygenated sites such as the kidneys, brain, and bone.
- Reactivation is seen primarily in immuno compromised or debilitated patients

Differences Between Primary and Secondary TB

Primary tuberculosis	Secondary tuberculosis
Lesion in lower and middle lung lobes (base of lung)	Lesion in apex of lung lobes
Involvement of regional lymph nodes with caseation	Less marked regional lymphadenopathy
Cavitation rare	Cavitation prominent
Immune reaction not prompt	Prompt immune reaction is mounted
Occurs in persons with normal immunity	Occurs in patients with waning immunity

The co-epidemic(HIV & TB)

- **HIV is the most powerful factor known to increase the risk of TB**
- **HIV promotes both the progression of latent TB infection to active disease and relapse of the disease in previously treated patients.**
- **TB is one of the leading causes of death in HIV-infected people.**

Tuberculosis and HIV infection

- HIV association has become a threat to the developed countries too. HIV association will lead to rapid spread of tuberculosis.
- HIV is the strongest risk factor for progression to active disease
- HIV kills $CD4^+$ T Helper cells which normally inhibit *M. tuberculosis*
- HIV interferes with PPD skin test

LABORATORY DIAGNOSIS

- **1- Microscopy**
- **2 -Culture**
- **3 -Immunodiagnosis**
- **4- Molecular Techniques**

Immunodiagnosis

a) Myco dot
b) Tuberculin skin test
c) Gamma interferon release assay
    1) Quantiferon test
    2) T-spot test

Molecular diagnosis

a) PCR
b) DNA fingerprinting

Specimen collection

- **Fresh sputum:**

  3 consecutive early morning samples
- **Gastric washings-**

  3 fasting morning samples
- **Urine**

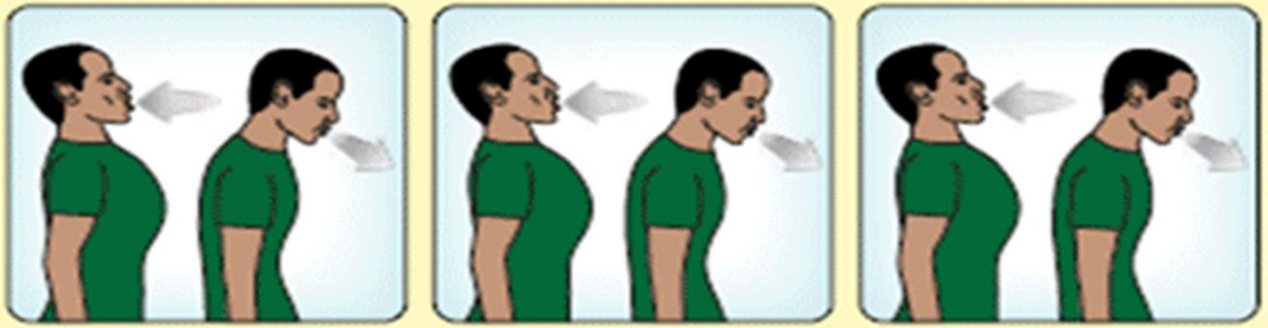
  3 early morning MSU samples
- **Stool**

Specimen collection

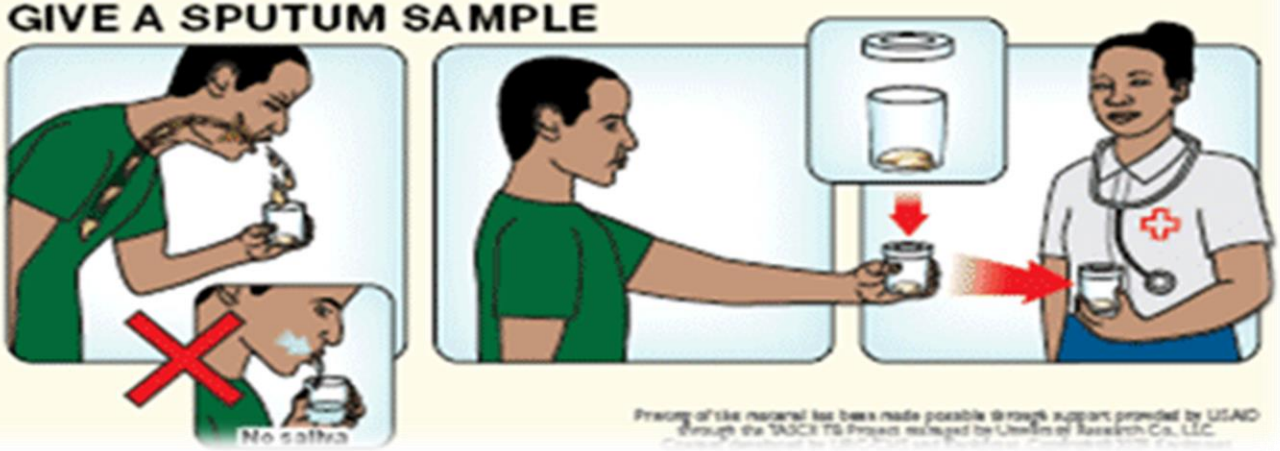
- **Pleural fluid, Ascitic fluid-**
  with anticoagulant (trisodium citrate)
- **CSF-**
  without anticoagulant
- **Joint fluid**
- **Biopsy material-**
  no addition of formalin
- **Blood:** *in hematogenous TB*

1 CLEAR YOUR MOUTH

2 BREATH IN AND OUT 3 TIMES



3 GIVE A SPUTUM SAMPLE



Preparation of this material has been made possible through support provided by USAID through the TASCH TB Project managed by Llewellyn Research Co., LLC

Specimen

* **According to site of infection :**

  - Sputum - Urine - Body fluids
  - Gastric lavage - Blood - Tissue biobsy

* **Specimens need appropriate processing**

Sputum

- Liquefaction with N-acetyl-L- cysteine
- Decontamination with NaOH
- Centrifugation

MICROSCOPY

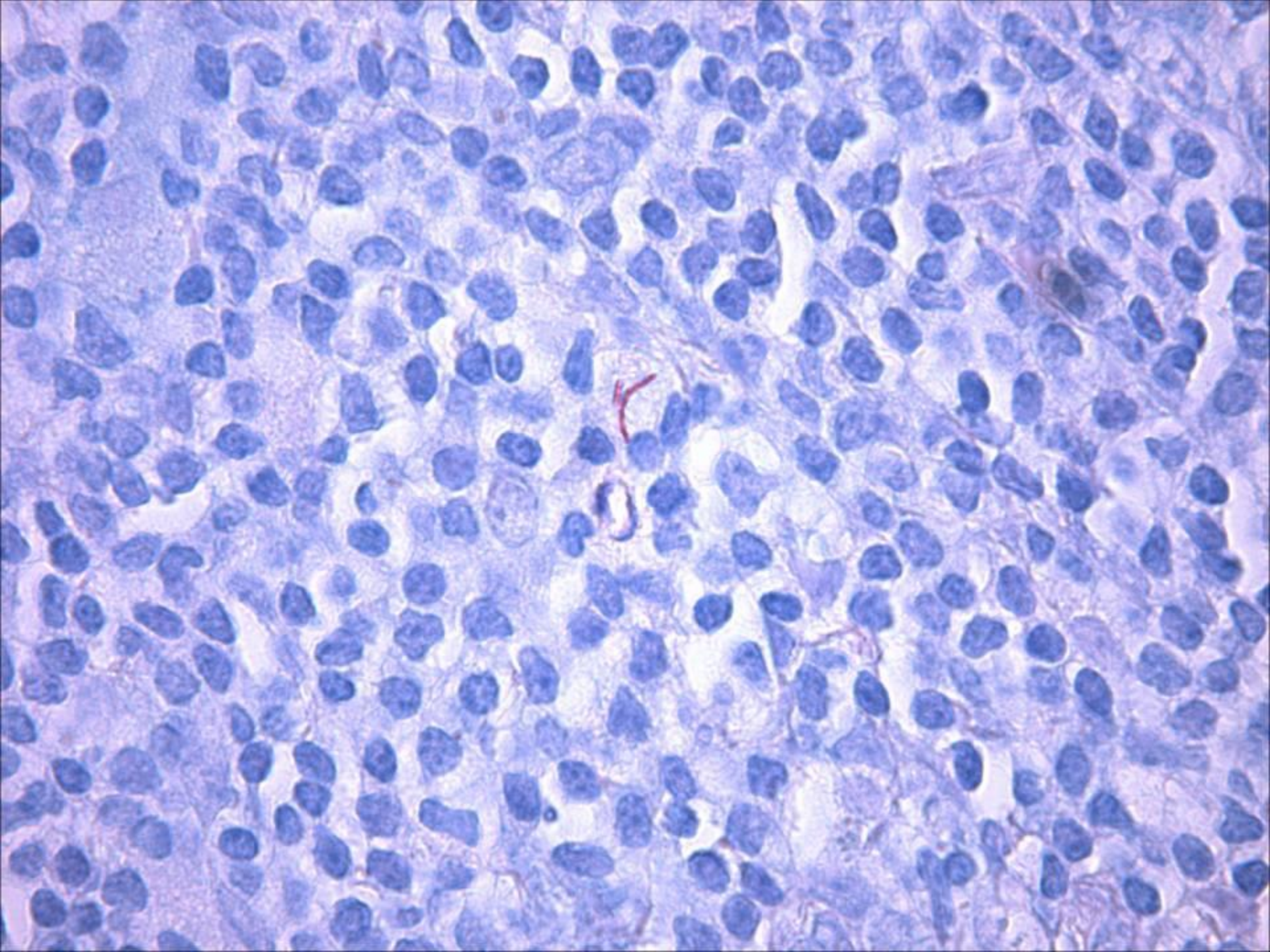
a) Zn stain/ Kinyoun stain

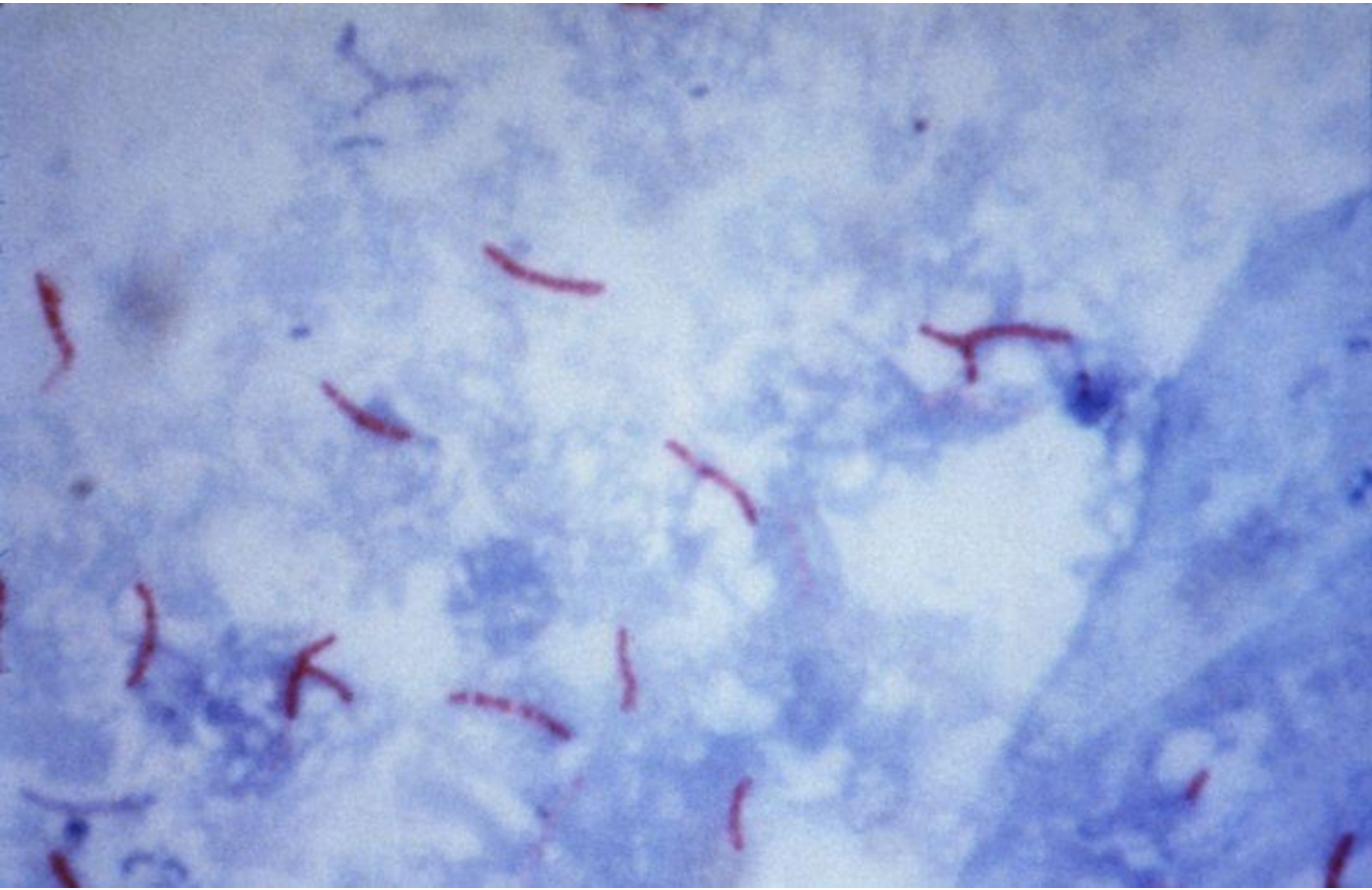
b) Fluorochrome stain

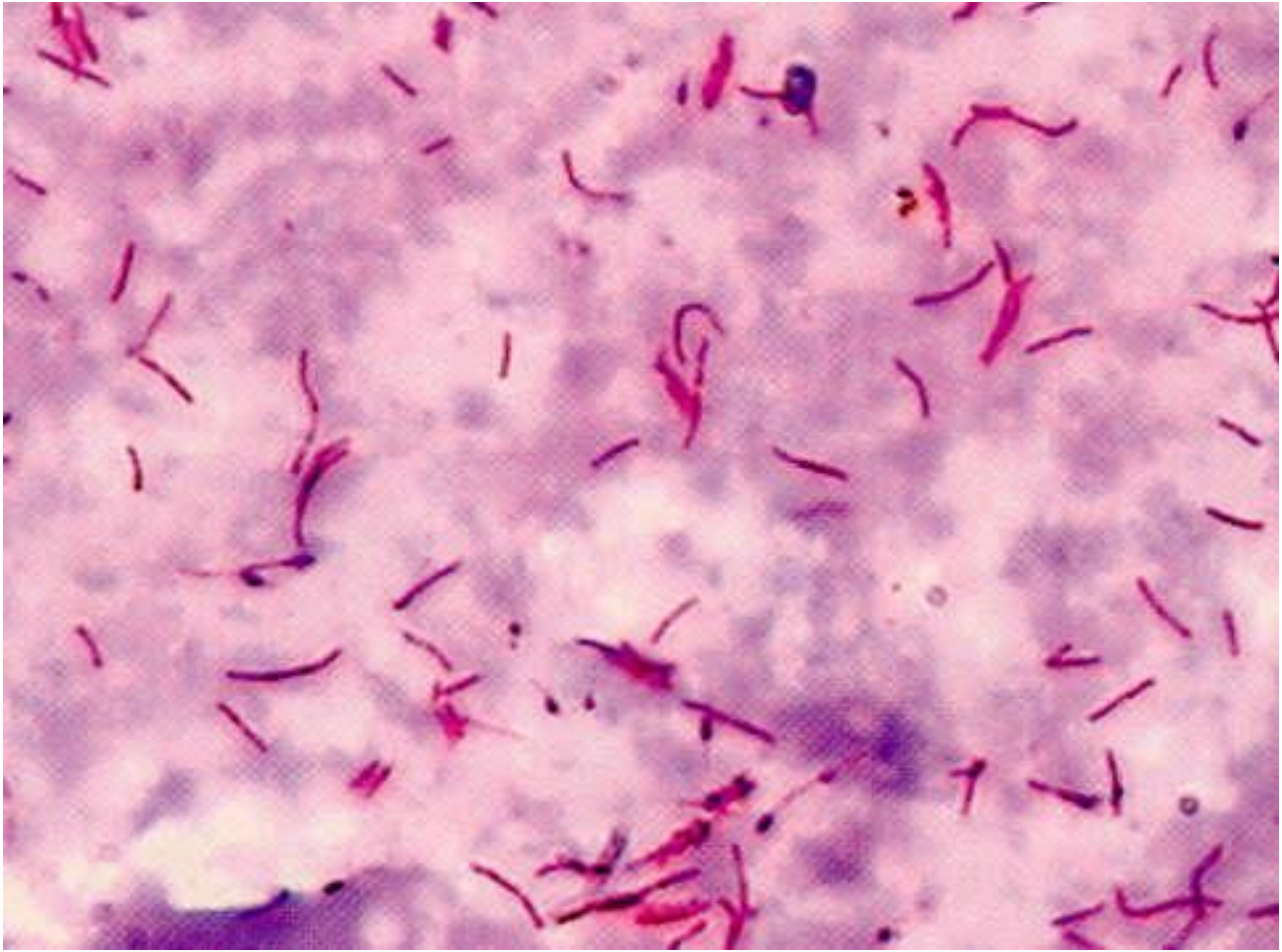
Kinyoun stain

- Similar to the Ziehl-Neelsen stain, but does not involve heating the slides being stained.

- Kinyoun carbol fuchsin has a greater concentration of **phenol** and **basic fuchsin** so does not require heating in order to stain properly

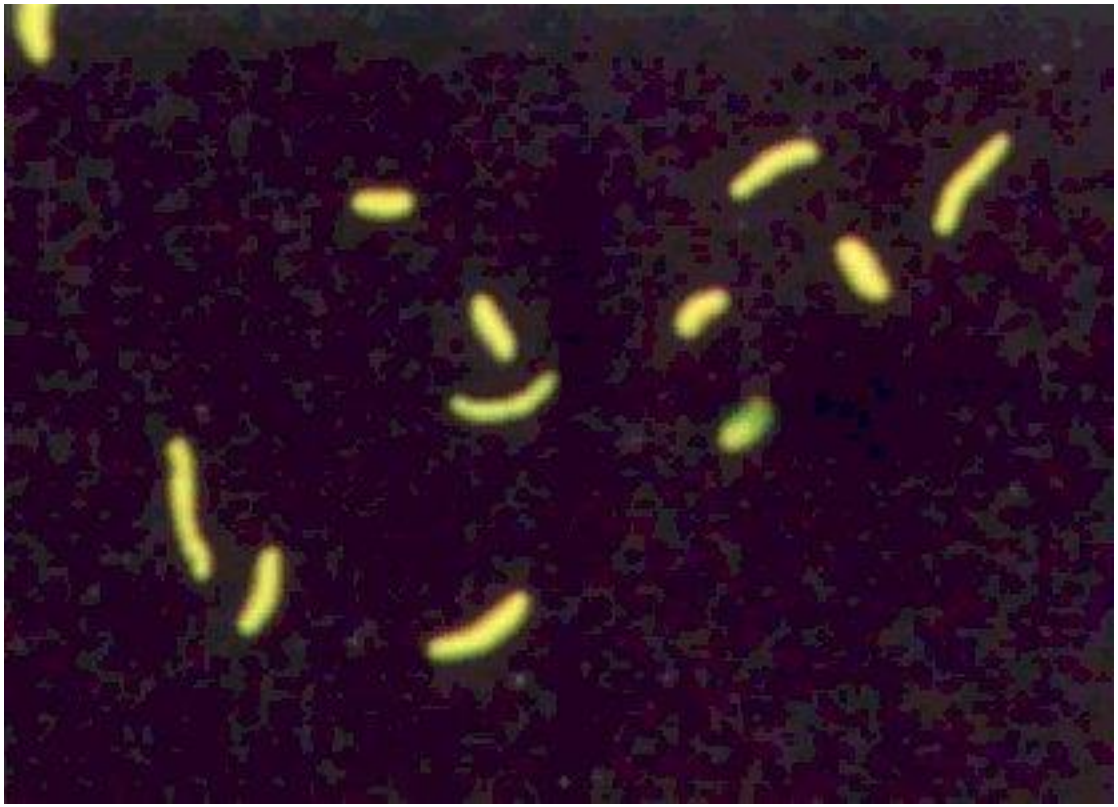






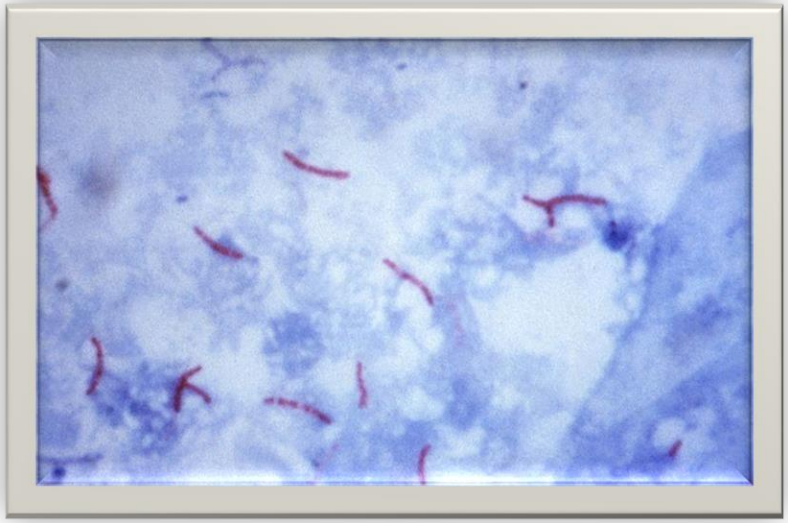
Acid fast Bacilli seen as in Florescent Microscope

AFB seen on flourescent microscopy



Interpretation of Sputum Stained by ZN Stain (WHO)

No. of AFB seen on average	No. of fields to screen	Report
No. of AFB / 100 immersion fields	100	No AFB observed
1–9 AFB / 100 immersion fields*	100	Record exact figure
10–99 AFB / 100 immersion fields	100	+
1–10 AFB / 1 immersion field	50	+ +
> 10 AFB / 1 immersion field	20	+ + +



Culture

- Culturing for isolation of Mycobacterium spp continues to be a ***Gold standard***, particularly in Developing countries.
- Need only ***10 – 100 bacilli / 1 ml*** of sputum

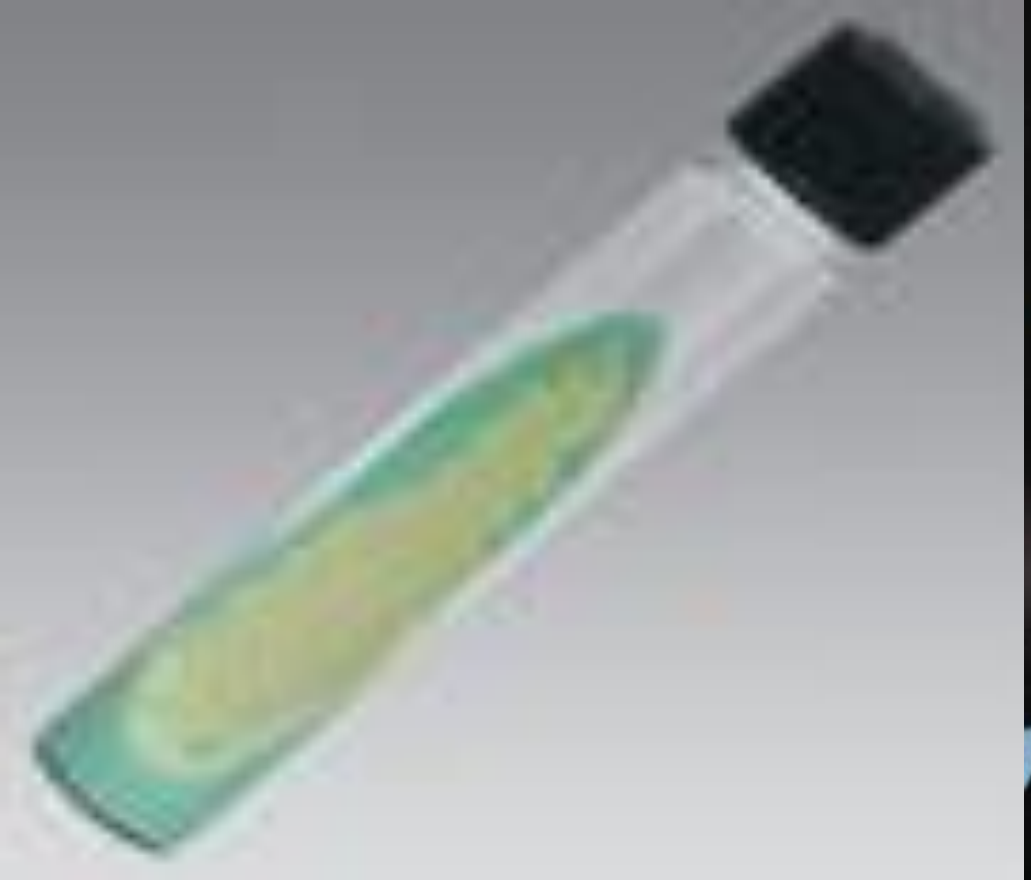
CULTURE

Steps

- Collection of Specimens
- Transportation
- Digestion/Decontamination Process
- Inoculation
- Incubation
- Detection

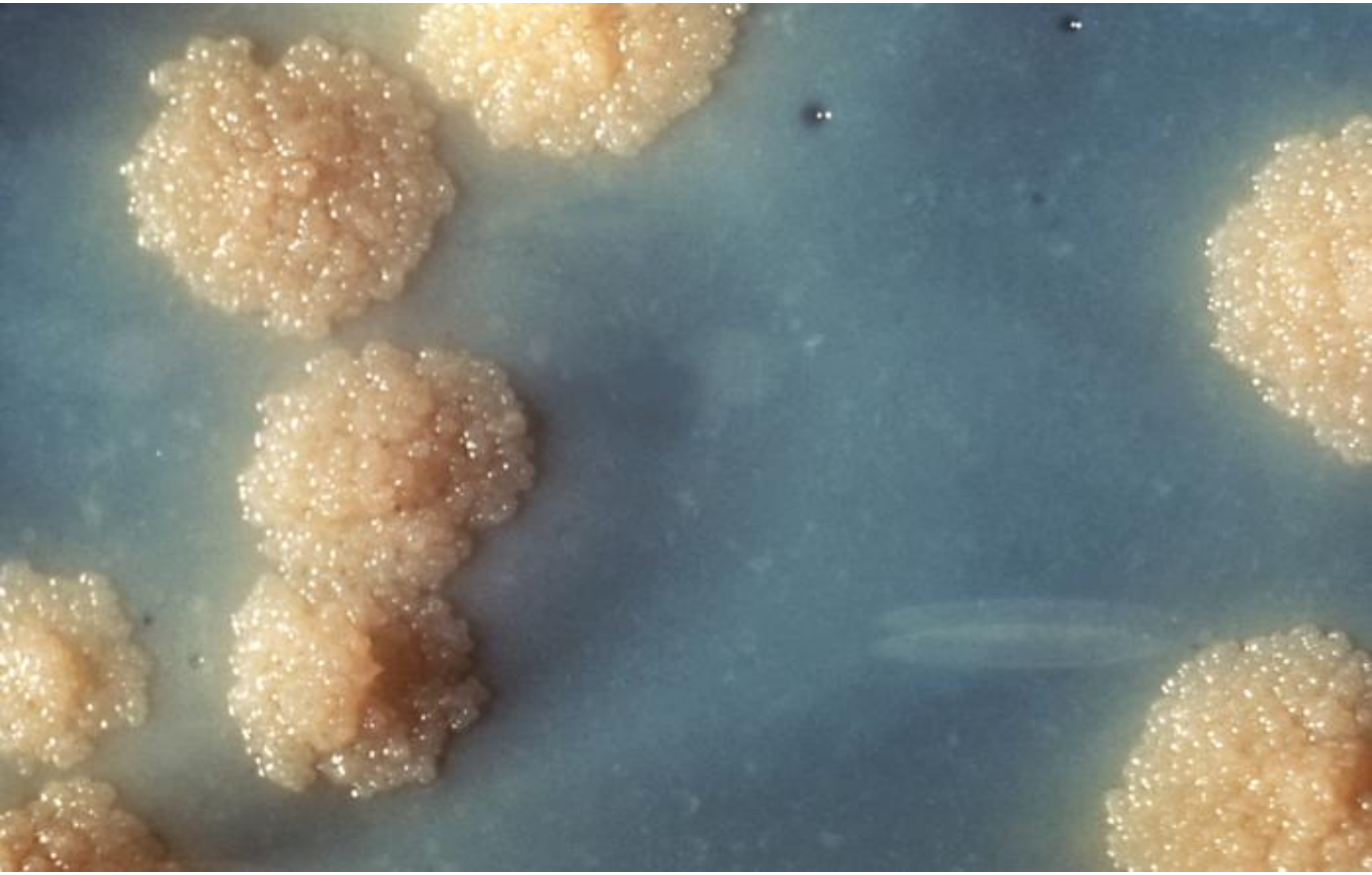
Medium Selection

- **Agar Based Medium**
  - Middlebrook 7H10
  - Middlebrook 7H11
- **Egg Based Medium**
  - Lowenstein Jensen
- **Liquid Medium**
  - BACTEC 12B/13A
  - Middlebrook 7H9
  - Dubos Tween Albumin Broth



Lowenstein Jensen Medium –

Selective. Always in screw capped bottle. Bluish Green.
Contains – Egg protein – Solidifying agent
Mineral salts – Mg Sulphate, Mg citrate
Asparagine
Malachite Green – Selective agent
Sterilized by - Inspissation



- BACTEC 460
- Mycobacteria growth indicator tube (MGIT)
- BACTEC MGIT 960 (continuous growth monitoring systems)

Tuberculin skin test
Mantoux Test

Tuberculin test

TB infection

↓

cellular immunity

↓

delayed type hypersensitivity

↓

tuberculin reaction

PURPOSE OF TST

A. Purpose of TST is to detect Latent TB Infection
B. TST is useful when:
   - Examining close contacts to active TB cases
   - Target testing those at high risk for TB infection
   - Examining person with active TB symptoms

Mantoux tuberculin skin test

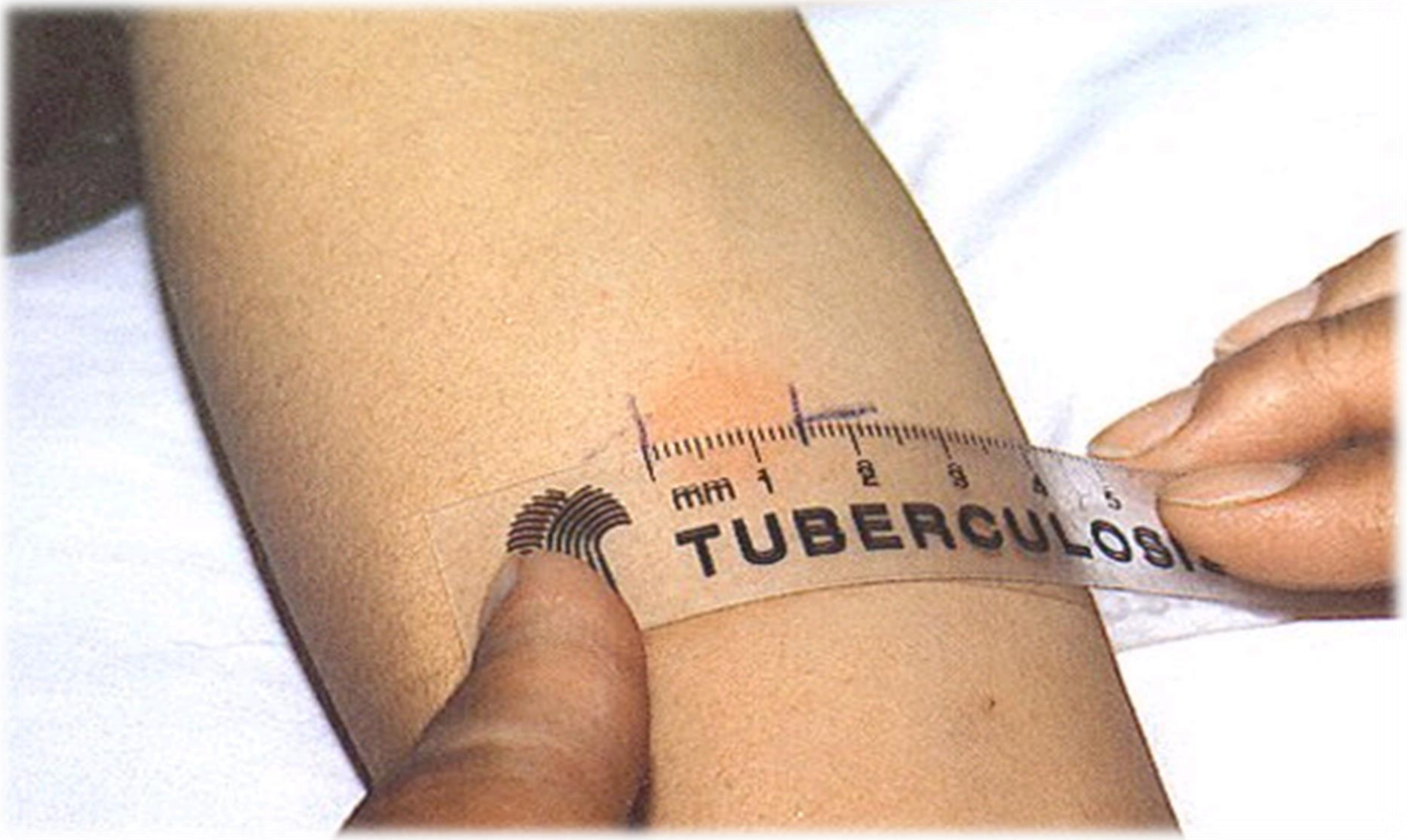
- *0.1 ml of PPD* containing *5 TU* is injected intradermally over flexor aspect of forearm .
- Examine after *48 – 72* hrs .
- Measure diameter of induration.

- Visually inspect site under good light

Erythema (reddening of the skin) – do not measure

Induration (hard, dense, raised formation)

Tuberculin Test
(Mantoux Test)



Interpretation

Induration diameter :

- 0 - 5 mm : negative
- 5 - 9 mm : doubt
- $\geq$ 10 mm : positive
- Induration of 5 mm or more is positive in a person who has deficient cell-mediated immunity (e.g., AIDS patients) or has been in close contact with a person with active tuberculosis

Tuberculin positive

1. TB infection :
   - infection without disease / latent TB infection
   - infection AND disease
   - disease, post therapy
2. BCG immunization
3. Infection of *Mycobacterium atypic*

Tuberculin positive

- A positive skin test result indicates previous infection by the organism but not necessarily active disease.

Tuberculin negative

1. No TB infection
2. Anergy
3. Miliary TB

Anergy

Patient with primary complex do not give reaction to TST due to supression of CMI :

- Severe TB: miliary TB, TB meningitis
- Severe malnutrition
- Steroid, long term use
- Certain viral infection: morbili, varicella
- Severe bacterial infection: diphteria, pertussis
- Viral vaccination: morbili, polio
- Malignancy: Hodgkin, leukemia, ...

TREATMENT

- **Multidrug** therapy is used to prevent the emergence of drug-resistant mutants during the long (6- to 9-month) duration of treatment. (Organisms that become resistant to one drug will be inhibited by the other.)

Treatment

Drugs used :

1- First line drugs :

- Isoniazid - Rifampicin - Pyrazinamide
- Ethambutol - Streptomycin

2- Second line drugs (more toxic and less effective):

- Kanamycin - capreomycin - Cycloserin
- ethionamide - ciprofloxacin - Ofloxacin

* Noncompliance (failure to complete the course):

Directly observed therapy (DOT)

Health care workers observe the medication

What is MDR-TB ?

- It is a mutated form of the TB microbe that is extremely resistant to at least the two most powerful anti-TB drugs - isoniazid and rifampicin.

- People infected with TB that is resistant to first-line TB drugs will confer this resistant form of TB to people they infect.

- MDR-TB is treatable but requires treatment for up to 2 years.

MDR

- The treatment of MDR organisms usually involves the use of four or five drugs, including ciprofloxacin, amikacin, ethionamide, and cycloserine.

XDR

- The strains of *M. tuberculosis resistant to INH, rifampin, a* fluoroquinolone, and at least one additional drug are called extensively drug resistant (XDR) strains. XDR strains emerged in 2005 among HIV-infected patients in South Africa.

Immuno-prophylaxis

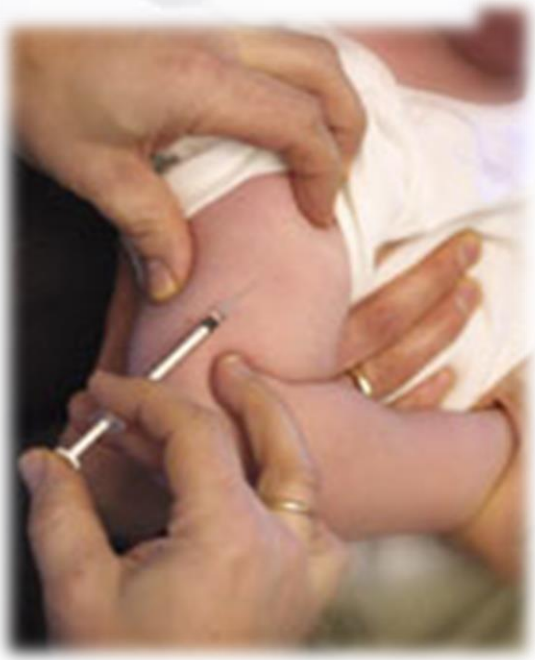
- Intradermal injection of live attenuated vaccine Bacille Calmette-Guerin (BCG).
- The strain causes self limited lesion and induces hypersensitivity & immunity.
- Converts tuberculin negative person to positive reactor.
- Immunity lasts for 10-15 years. Immunity 60-80%

BCG

- Given at birth without tuberculin testing
- Protects against TB, the disease runs milder course in protected, prevents skeletal, meningeal & miliary forms.
- Also found useful in leprosy, leukaemias and other malignancies by non-specific stimulation of RE system.

BCG Vaccine

- Bacille Calmette Guerin(BCG) is an attenuated strain of *M.bovis*
- Live attenuated vaccine
- Given anytime from birth to 15 days of life as part of EPI schedule
- Measles can depress cell mediated immunity & as BCG induces cell mediated immunity BCG should not be given along with **MMR**



- **Procedure:**
  - 0.1 ml of vaccine is given intradermal over left deltoid area.
  - Immediately there is a small swelling (6mm) at the injection site which persists for 6-8 hours.
  - Swelling disappears & the injection site looks normal.
  - After 6-8 weeks a swelling reappears

- **Procedure:**
  - It grows in size & forms a nodule which breaks open, discharges some fluid & forms an ulcer.
  - The ulcer heals by forming a scar which remains for lifetime.
  - It may take 3-6 months for the scar to form. If no scar is visible do PPD test.
    - **If negative**: give repeat BCG.
    - **If positive**: BCG is not necessary
  - If ulceration occurs within **7 days** of injection, it may be a sign of tuberculosis in the child.

- **Indication:**
  - PPD negative persons exposed to multidrug resistant TB
  - Healthcare workers from low-risk countries working in endemic areas
  - In children to prevent Meningitis and Disseminated TB , but it cant prevent primary infection or reactivation of latent pulmonary infection

- **Contraindication:**
  - Immunocompromised patient
  - Vaccination of tuberculin positive patient results in superficial ulceration at site of injection

- **Not recommended** in countries with
  - Meningitis rate in children < 1/10 million per year
  - Risk of TB infection < 0.1%

- **Complications:**
  i. Local lymphadenitis-involving axillary lymph nodes or rarely supraclavicular gland

  ii. Progressive systemic disease in children with immune compromised state like AIDS

- **Duration of immunity** : 10-15 yrs

- **Efficacy in preventing active TB:** approximately 70%