

Mycobacteria

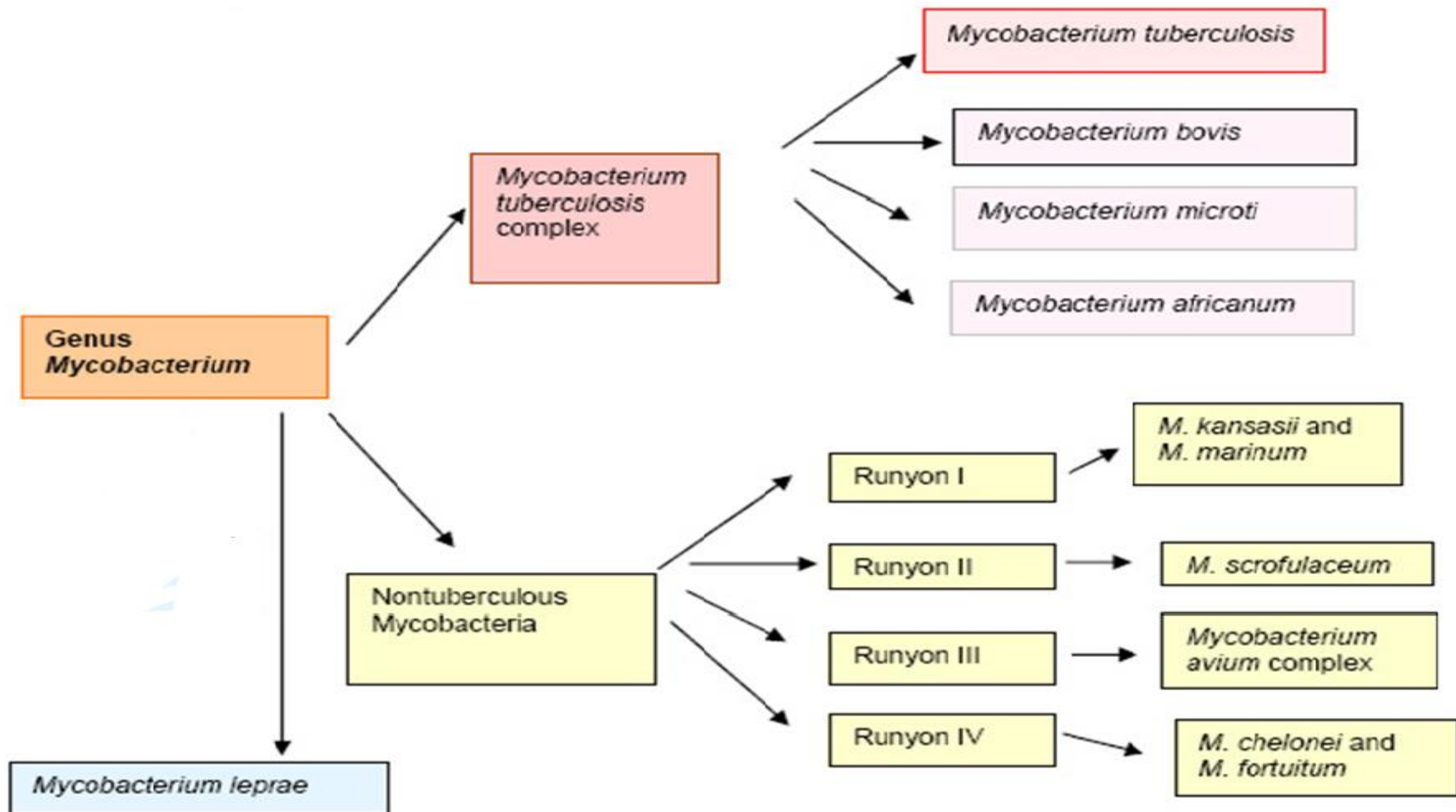
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Mycobacteria

- M. tuberculosis complex
 - slow-growing, no pigmentation
- Non-tuberculous mycobacteria (NTM)/ MOTT/Atypical
- *Mycobacterium leprae*



Mycobacteria

- Non-tuberculous mycobacteria (NTM)/ MOTT/ Atypical
 - Runyon group I (Photochromogens)
 - slow-growing, yellow pigment (+) in light
 - Runyon group II (Scotochromogens)
 - slow-growing, yellow pigment (+) in dark
 - Runyon group III (Nonchromogens)
 - slow-growing, nonpigmented
 - Runyon group IV (Rapid growers)
 - rapidly growing

Important human pathogens

- Most common
 - ***Mycobacterium tuberculosis***
- MOTT/ Atypical
 - Mycobacterium avium-intracellulaire Complex (MAC)
- *Mycobacterium leprae*

Mycobacterial Clinical Syndromes

Mycobacterium tuberculosis

Pulmonary tuberculosis

Extrapulmonary tuberculosis

Tuberculosis in HIV-infected patients

Mycobacterium avium-intracellulare complex

Asymptomatic colonization

Pulmonary disease

Disseminated disease in HIV-infected patients

Mycobacterium leprae

Tuberculoid leprosy

Lepromatous leprosy

Other mycobacteria

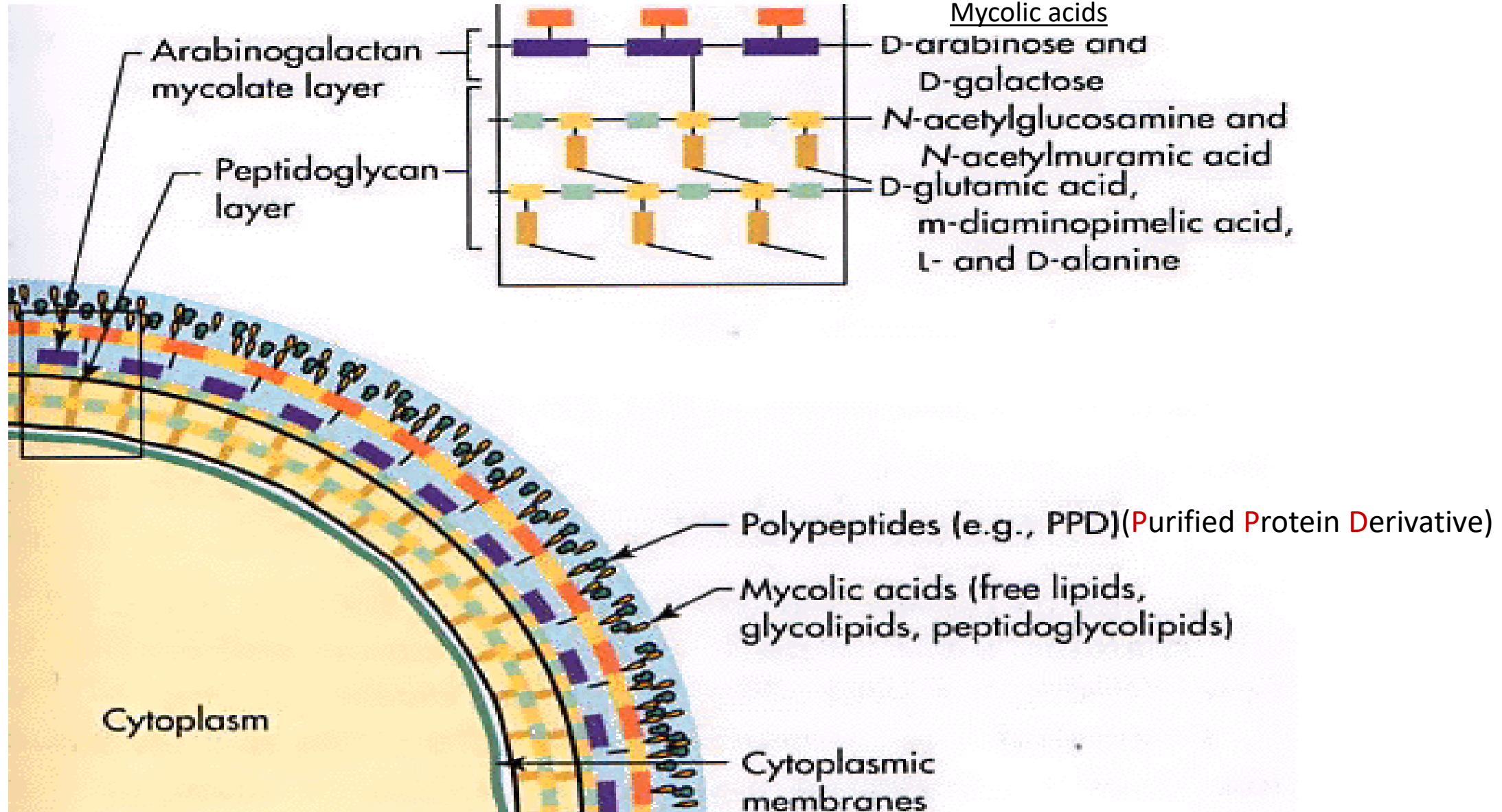
Pulmonary disease (e.g., *M. kansasii*)

Cutaneous disease (e.g., *M. marinum*, *M. ulcerans*,
M. fortuitum-chelonae)

Mycobacterium

- “Myco” – like a fungus
- Slightly curved, slender rods with beaded appearance
- Can not differentiate TB from MOTT by microscopy
- Different culture characteristics

Lipid-Rich Cell Wall of Mycobacterium



Mycobacteria- cell wall

- **Rich in lipids:**

- responsible for many characteristic properties (acid-fastness, resistant to disinfectants and antibiotics, antigenicity, slow growth)
- Due to thick cell wall with mycolic acids
 - Difficult to stain by to common laboratory stain.
 - Resist de-colorization by acids and alcohols
 - Once stained, cannot be decolorized with acid/alcohol solutions

Acid and Alcohol Fast

M. tuberculosis

- Intracellular pathogen
- Slightly curved, rod shaped bacilli
- Infect alveolar macrophages
- lifelong infections (latent)
- Obligate aerobe and grow very slowly (18-24 hrs doubling time)
 - 6-8 weeks to grow on plates
- Acquired by inhalation of aerosolized infectious particles to alveoli

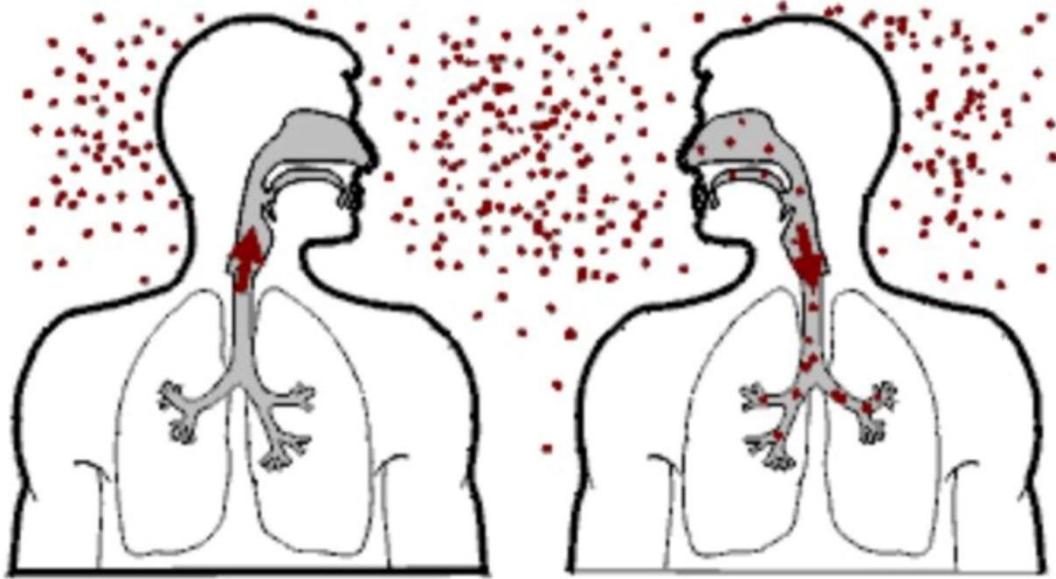
Global TB burden

- 1/3rd of world population is infected with TB
- Commonest cause of death due to an infection in adults and 3rd commonest cause of death in children
- Developing countries – 95% of TB burden and 98% TB deaths
- SEA – highest burden of TB among all WHO regions (35%)
- Resurgence in developed countries with AIDS pandemic
- WHO declared TB as a global emergency in 1993
- **Sri Lanka**
 - Nearly 17,000 people are infected (89/100,000 pop)
 - >11,000 new cases each year

What's alarming?

- Emergence of resistance
 - MDR – multi-drug resistant TB
 - XDR – extreme drug resistant TB
 - Pan drug resistant
- Co-infection with HIV
 - High mortality
 - High drug resistance
 - Difficult to diagnose
 - Difficult to treat

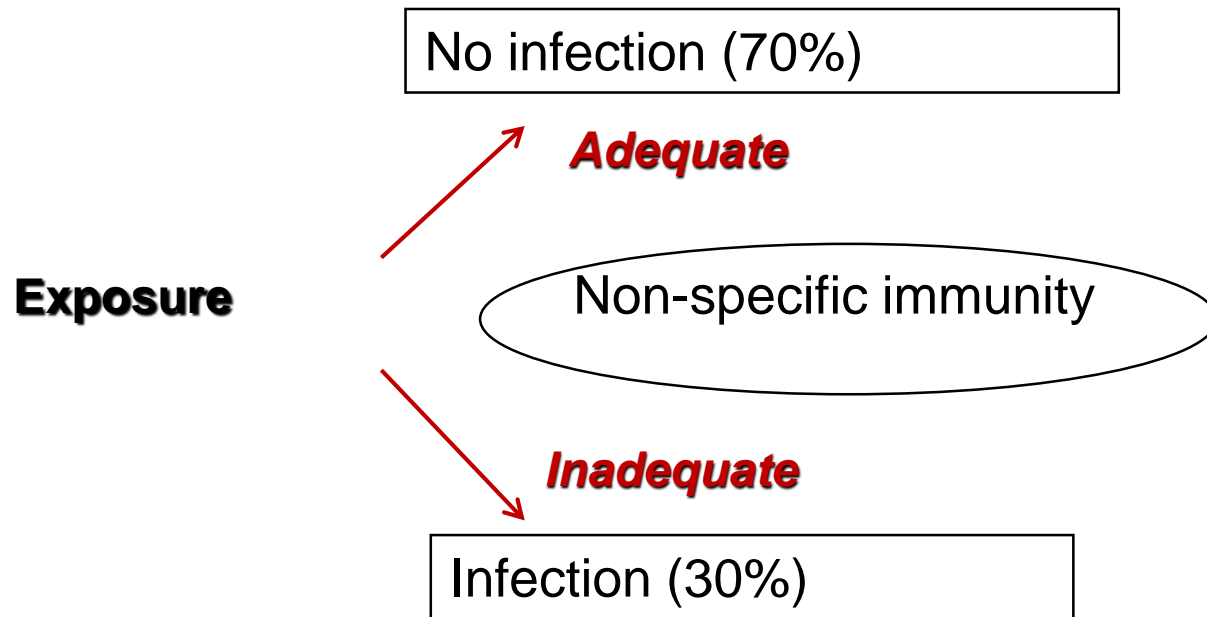
Transmission- Airborne



Left untreated, a person with active TB will infect 10-15 other people per year

- Coughing projects droplet nuclei into the air that contain tubercle bacilli
 - Large droplets settle to the ground quickly
 - Smaller droplets form “droplet nuclei” of 1–5 μ in diameter
 - Droplet nuclei can remain airborne

TB Transmission and Pathogenesis

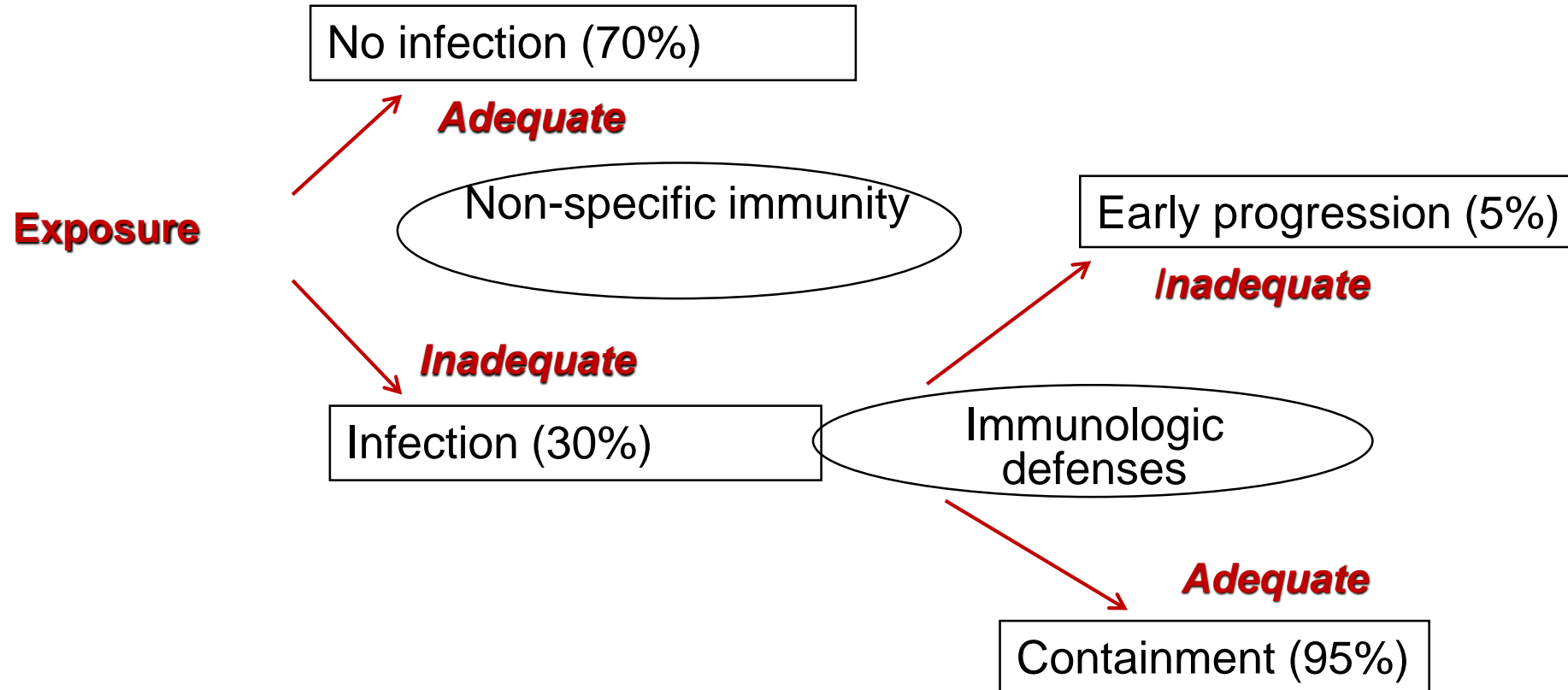


➡ Not everyone who is exposed to TB will become infected

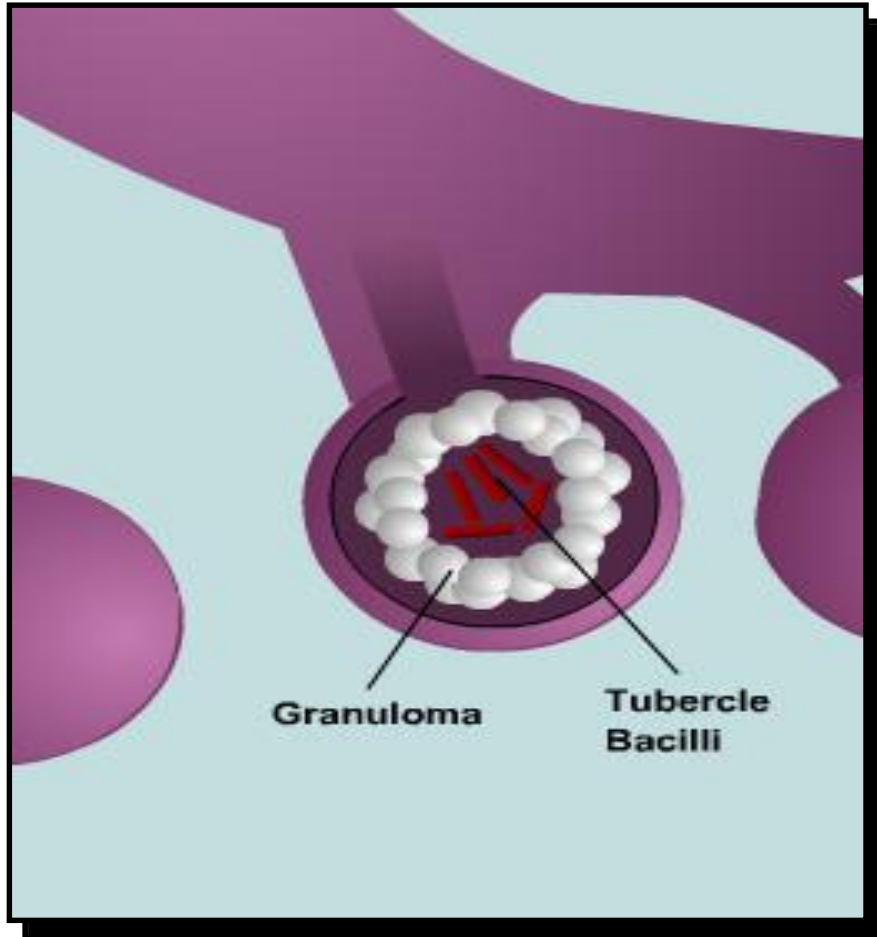
The Chance of Infection Increases...

- When the concentration of TB bacteria circulating in the air is greater
 - Coughing; smear +; cavitory disease
 - Exposure occurs indoors
 - Poor air circulation and ventilation; small, enclosed space
 - Poor or no access to sunlight (UV light)

TB Transmission and Pathogenesis (2)

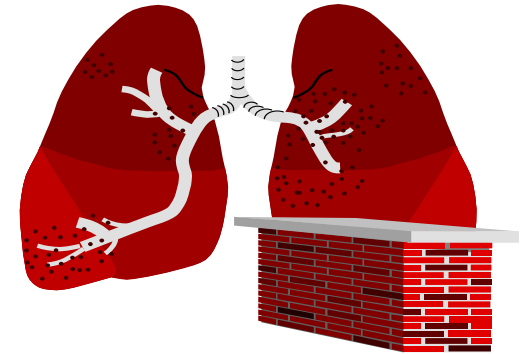


Latent TB Infection (LTBI)



Person:

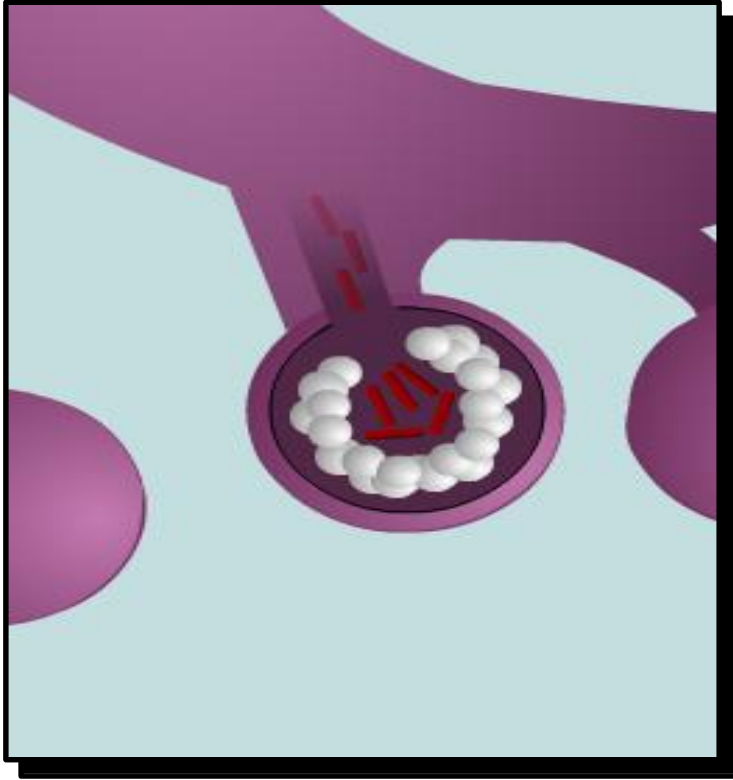
- Not ill
- Not contagious
- Normal chest x-ray
- Usually the tuberculin skin test is positive



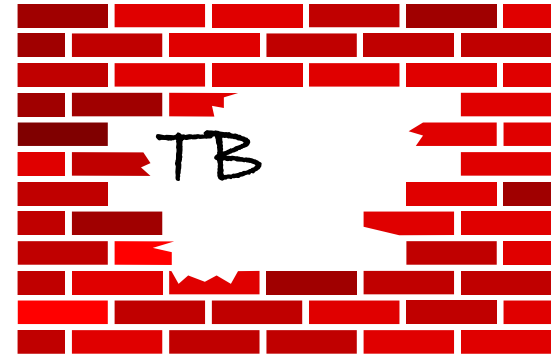
Germs:

- Sleeping but still alive, low amt
- Surrounded (walled off) by body's immune system
- Infected with TB, but no disease

Active TB Disease



Granuloma breaks down and tubercle escape and multiply



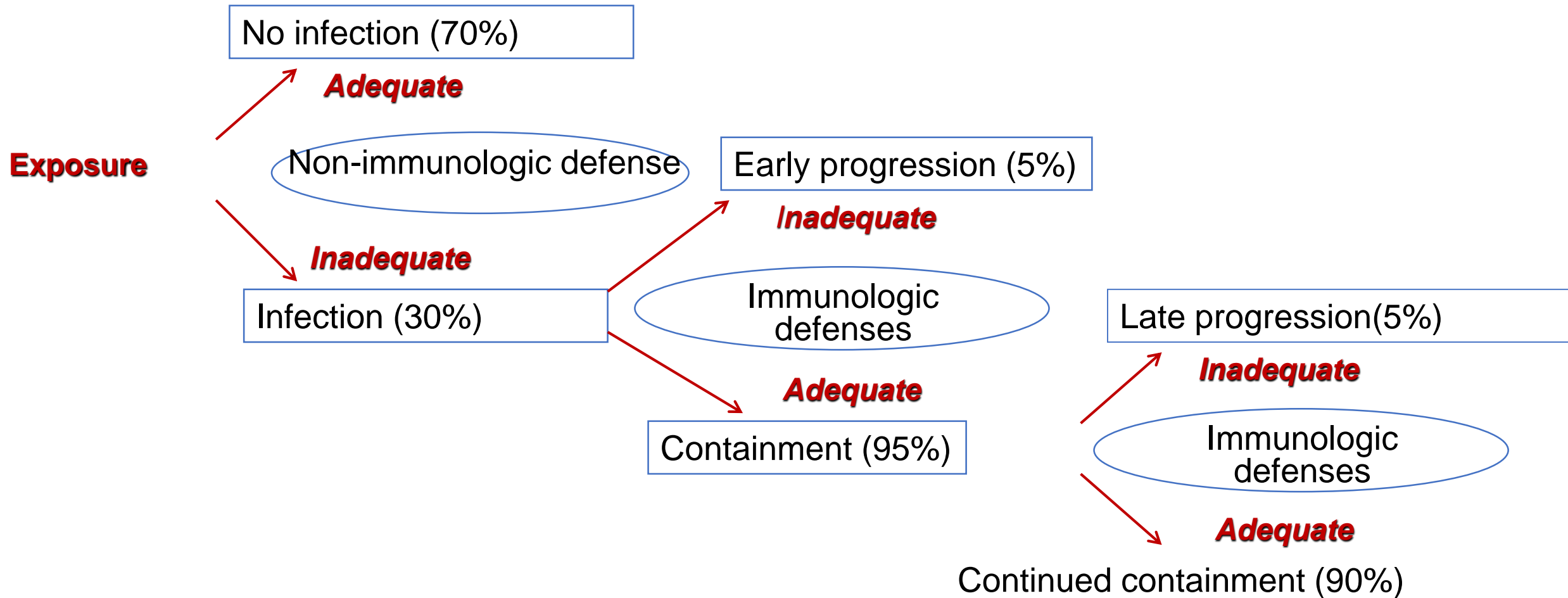
Germ:

- Awake and multiplying
- Cause damage to the lungs

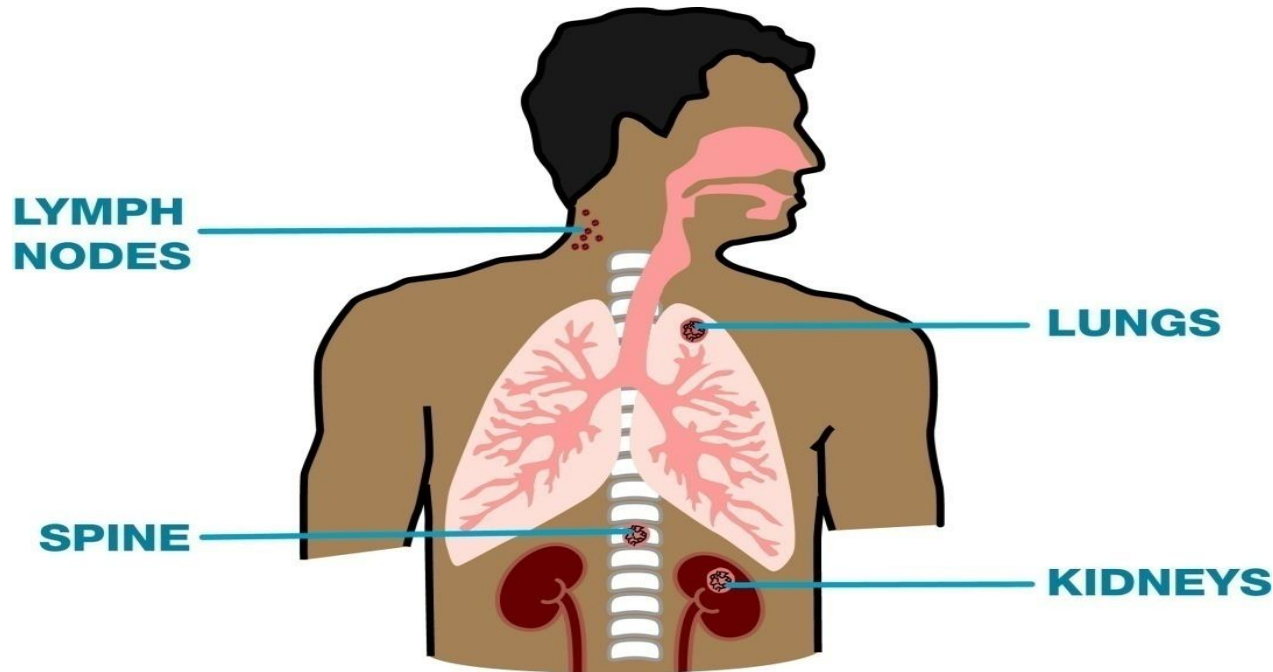
Person:

- Most often feels sick
- Contagious (before pills started)
- Usually have a positive tuberculin skin test
- Chest X-ray is often abnormal (with pulmonary TB)

TB Transmission and Pathogenesis (3)



Spread of TB to Other Parts of the Body



1. Lungs (85% all cases)
2. Pleura
3. Central nervous system
 - (e.g., brain, meninges)
4. Lymph nodes
5. Genitourinary system
6. Bones and joints
7. Disseminated
 - (e.g., miliary)

When to suspect?

- Unexplained weight loss
- Loss of appetite
- Night sweats
- Fever
- Fatigue
- Coughing for 3 weeks
- Hemoptysis
- Chest pain

Diagnosis

- History
 - Symptoms
 - Pulmonary
 - Extra-pulmonary
 - Contact history / past history
 - Risk factors
- Examination
- Laboratory evaluation

Types of Specimens

- Pulmonary
 - Sputum
 - Gastric lavage
 - Bronchoscopic specimens – bronchial washing/ brushing, BAL, PSB
 - ET secretions
 - Lung biopsy

Types of Specimens

- Extrapulmonary
 - CSF
 - Urine
 - Peritoneal fluid
 - Pleural fluid
 - LN aspirate/ biopsy
 - Blood
 - Joint aspirate
 - Skin biopsy/ wound tissue
 - Any other sterile fluid

Specimen collection

- For sputum microscopy
 - SPOT- EARLY MORNING- SPOT (for clinic/ OPD pts)
 - 3 x EARLY MORNING (for inward patients)
 - Collected into a clean cup
- For culture
 - SPUTUM - 3 x EARLY MORNING
 - URINE - 3 x EARLY MORNING
 - Collected into sterile containers
- Specimens for urine AFB microscopy are not accepted

Methods for diagnosis

1. Immunological - Detect sensitization for TB

1. Tuberculin test
2. Interferon gamma releasing assays

2. Direct detection

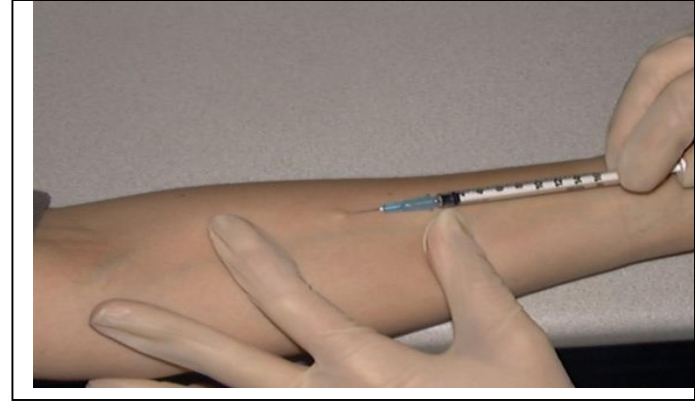
1. Microscopy - Z-N stain / Fluorescent stain
2. Molecular – amplification/ probe based

3. Growth methods

1. Conventional culture methods – on egg based/ agar based media
2. Rapid isolation methods - automated culture systems

4. Enzyme detection - ADA

Immunological



1. Tuberculin test - 5 tuberculin units of PPD → ID

→ Read after 48-72hrs → **induration** >10mm

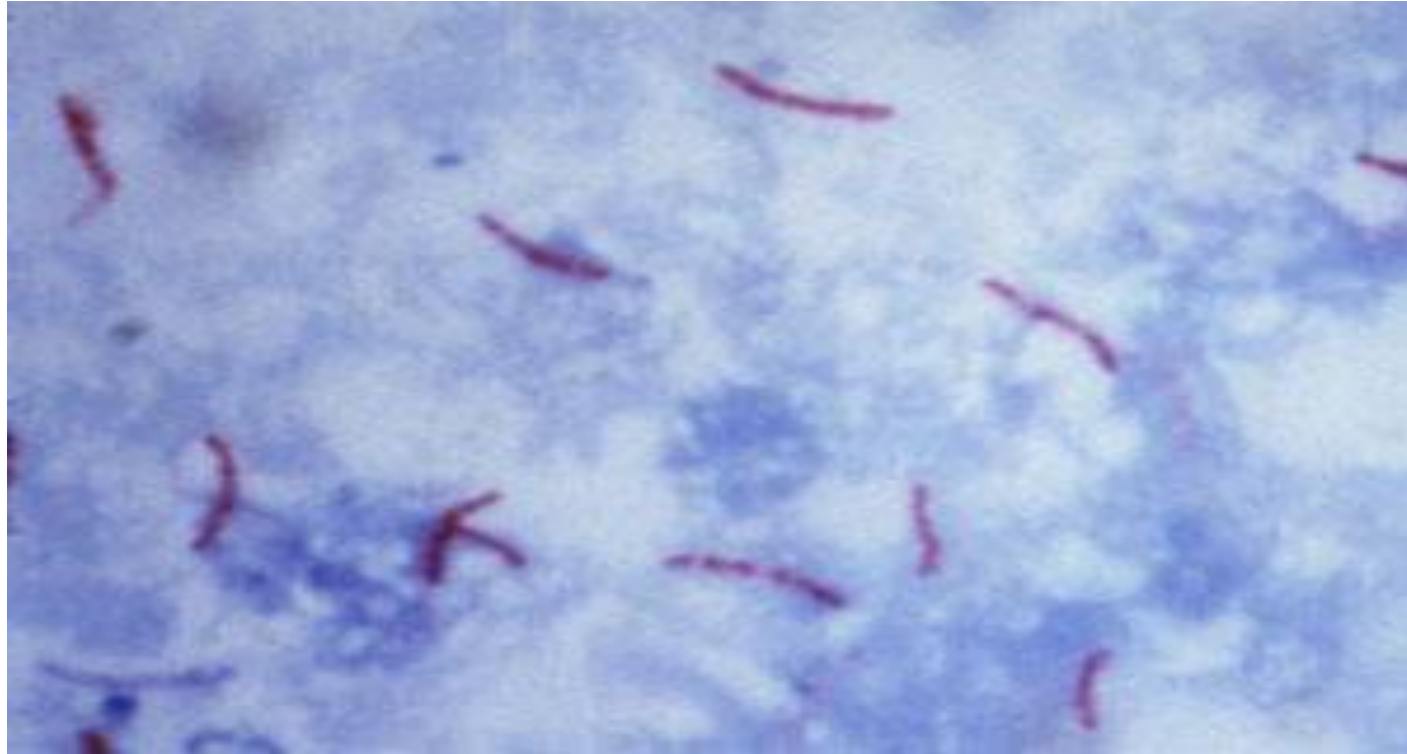
- Mantoux
 - Heaf
- } Represent DTH (delayed type hypersensitivity)

2. Interferon-Gamma Release Assays (IGRAs) - Blood Tests for TB Infection

- QuantiFERON®-TB Gold (ELISA)
 - T-SPOT®.TB test (T-Spot – ELISPOT)
 - A Blood test, not for diagnosis of TB, cannot differentiate infection with latent TB
- **Negative tuberculin or QFT-G results should not be used alone to exclude *M. tuberculosis* infection**

Sputum Microscopy

- Modified Z-N staining for AFB



Culture

- Gold standard for TB diagnosis
 - Use to confirm diagnosis of TB
 - Strict aerobe
 - Growth enhanced by 5-10% CO₂
-
- Samples – sterile container
 - Transport medium-1% cetyl pyridium chloride in 2% saline
 - Done at TB reference lab at Welisara

Culture

- Slow grower : doubling time 18 hours.
- Times needed:
 - Solid medium
 - 6-8 wks
 - Liquid medium
 - 2 wks

Culture

- Conventional methods
 - Egg based media - Lowenstein Jensen medium
 - Agar based – Middle Brook (7H9, 7H10 etc..)
- Identification of *M.tuberculosis* (Vs. Atypicals)
 - Slow growth rate.
 - Failure to grow at 25C.
 - Susceptibility to p-nitro-benzoic acid(PNB).
 - No pigment production.
 - Other tests - Nitrate reduction, niacin test



M. tuberculosis

Atypical mycobacteria



Culture

- Rapid methods
 - Automated –
 - BACTEC (radiometric) –positive within 9 days
 - MGIT (Mycobacterial growth indicator tubes) – fluorescent dye in fluid media – positive within 7.3 days



BACTEC



MGIT

Molecular

- Equal or less sensitive than conventional culture method
- If smear is negative → sensitivity is less
- False positive with cross contamination
- New techniques to detect bacilli in direct samples
 - Amplification based – PCR, 16s RNA ribotyping
 - Probe based

Treatment

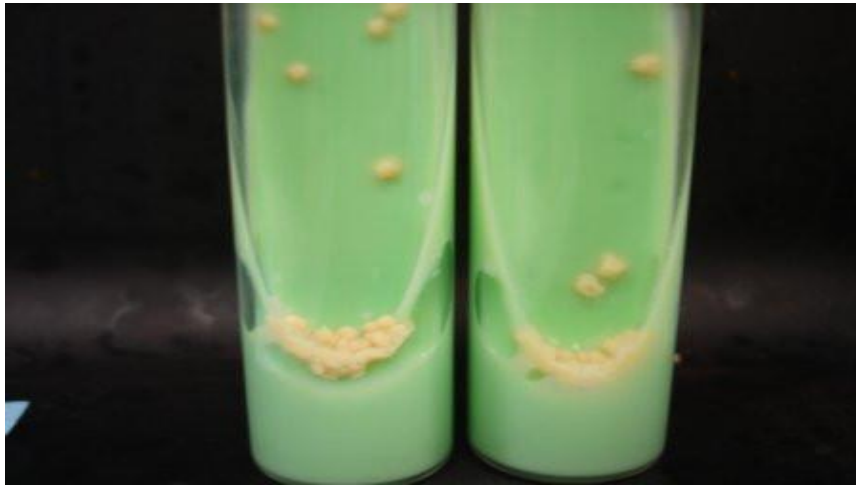
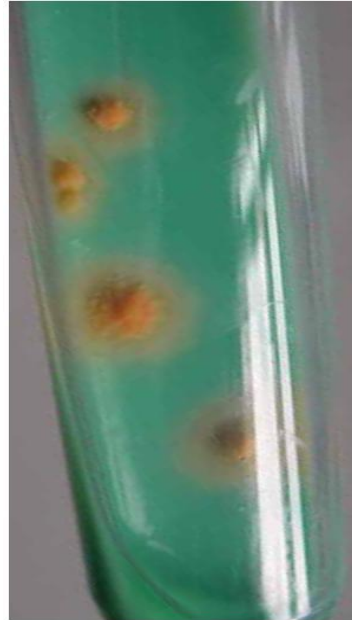
- Most TB is curable
 - There are currently 10 drugs used for active TB disease
 - The first lines drugs are isoniazid, rifampin, ethambutol, pyrazinamide
 - Four or more drugs required for the simplest regimen
 - 6-9 or more months of treatment required
- Person must be isolated until non-infectious
- Directly observed therapy to assure adherence/completion recommended (DOTS)
- Side effects and toxicity common

IMPORTANT INFORMATION ABOUT TB DRUGS!

Rifampin turns urine red, be sure to tell patient to expect it!

Isoniazid can cause peripheral neuropathy, be sure to pretreat with B6

Atypical Mycobacteria



Atypical Mycobacteria



- Frequently found in environmental habitats
 - Wet soil, rivers, marshlands, streams
- low virulence
- may infect with repeated exposure but very occasionally cause overt disease in humans
- Mainly cause disease in immunosuppressed host
- No man to man transmission
- Acquired from environment

Risk factors

- Immunosuppression (HIV, Medications)
- Aging
- specific mutations in the interferon (IFN- γ)/interleukin 12 (IL-12) synthesis and response pathways
- Predisposing lung diseases
 - COPD
 - Bronchiectasis (NTM often coexist)
 - CF
 - Pneumoconiosis
 - Prior TB
 - Esophageal motility disorders
- Body Morphotype (Lady Windermere syndrome)
 - Middle aged white females
 - Slender, tall
 - Scoliosis, Pectus excavatum
 - Mitral Valve prolapse
 - women who chronically suppress the normal cough reflex



NTM

Common clinical syndromes

1. Lymphadenitis
2. Chronic pulmonary disease
3. Skin and soft tissue infections (often associated with trauma or a foreign body) sometimes with extension to bone and joint
4. Disseminated disease

NTM Pulmonary disease

- Usually adults
- Insidious onset of cough, sputum production, dyspnea ,weight loss, malaise and diminished energy= TB
- Fever, chills, night sweats are not uncommon
- Recurrent “bronchitis,” or “walking pneumonia”

TABLE 2. SYMPTOMS AT TIME OF PRESENTATION

| Symptom | Patients (<i>n</i> = 63) |
|--|------------------------------|
| Cough, <i>n</i> (%) | 49 (78) |
| Phlegm | 42 (67) |
| Description of phlegm, <i>n</i> (<i>n</i> = 42) | |
| Thick | 37 |
| Green | 23 |
| Yellow | 11 |
| Clear | 4 |
| Hemoptysis, <i>n</i> (%) | 18 (29) |
| Fever, <i>n</i> (%) | 28 (44) |
| Fatigue, <i>n</i> (%) | 52 (83) |
| Shortness of breath, <i>n</i> (%) | 41 (65) |
| Night sweats, <i>n</i> (%) | 34 (54) |
| Mean weight loss attributed to PNTM infection or chemotherapy at time of enrollment \pm SD, kg | 3.7 \pm 5.2 |

Pulmonary disease

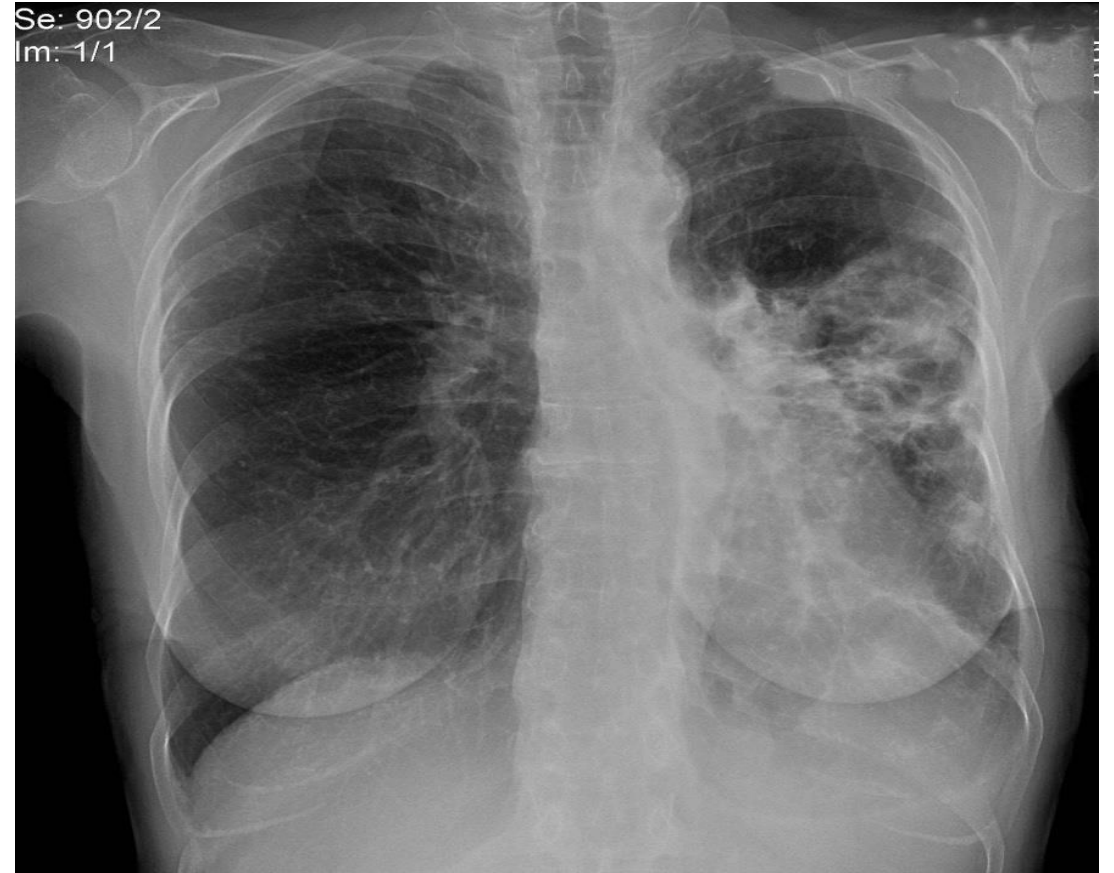
- Common causative agents
 - *M. avium*
 - *M. intracellulare*
 - *M. kansasii*
- M. avium* complex(MAC)

MAC

- Most common bacterial opportunistic infection in adults infected with HIV in the developed world.
- Also found in
 - Elderly men with COPD
 - Middle aged to elderly Non- smoking women (Lady Windermere syndrome)
 - CF patients
- Annual frequency in AIDS – 10-20%.
- Occurs late in HIV infection. Mean CD4+ Count ~ 10-30/ μ L. (TB occurs early.)
- Spreads via lymphatics then hematogenously to cause disseminated disease.
- Commonly affects right middle lobe or lingula

NTM Pulmonary disease

- Chest Radiography:
 - Chest X-rays typically reveal amorphous, lower zone shadowing
 - Upper lobe cavitory disease (like TB) is uncommon; however, cavities may be present in other zones



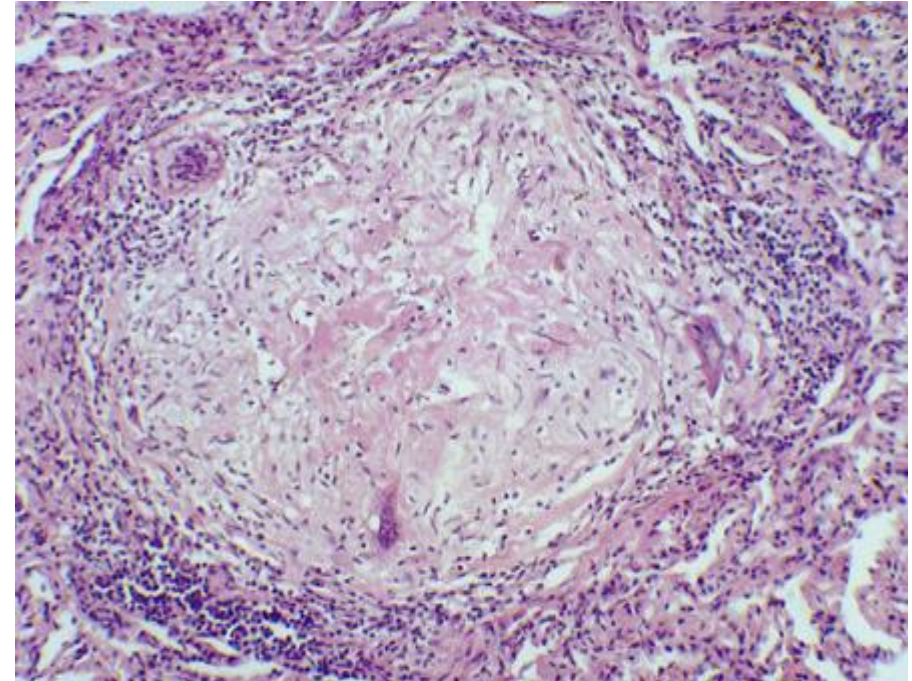
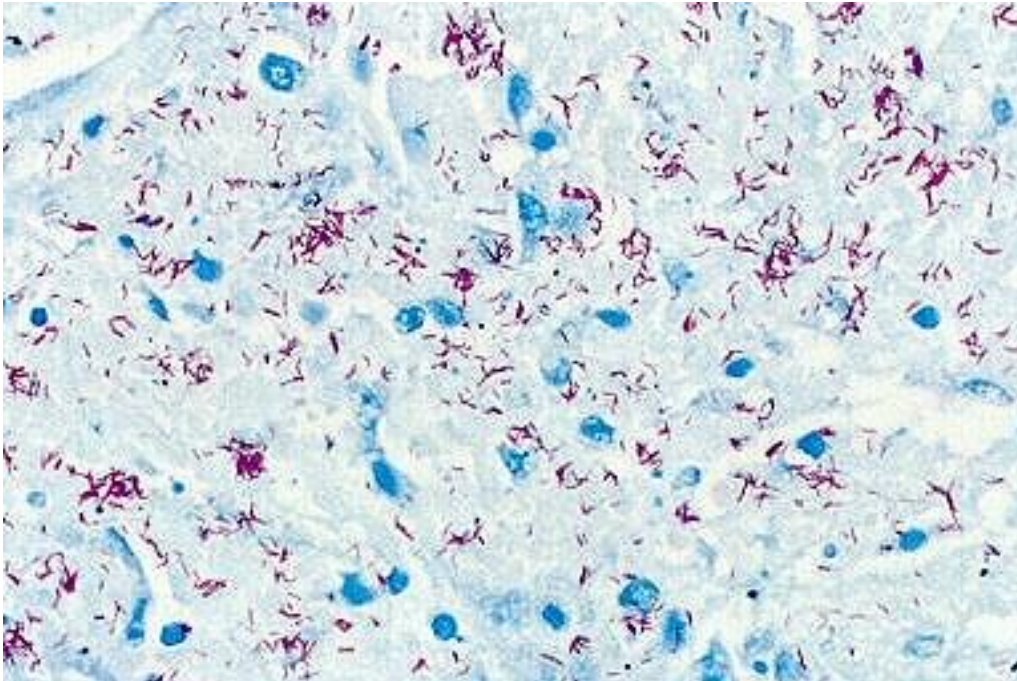
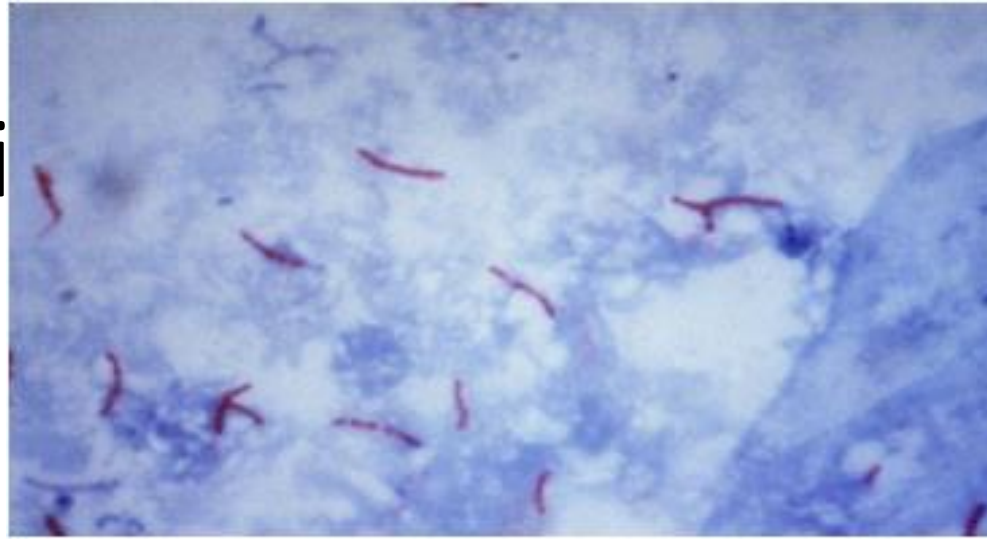
Diagnosis of NTM Pulmonary disease

- Clinical History
- Radiography
- Microbiology specimens
 - Spontaneous sputum sample (x 3 early morning/ Spot/ EM/ Spot)
 - Induced sample (hypertonic saline nebs)
 - Bronchoscopy (BAL/ Bronchial wash/ brush)
- General guidelines for NTMs
 - 2 or more (+) sputum cultures
 - or (+) sputum smear and (+) sputum culture
 - or (+) bronch wash culture

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AFB (Z-N Stai



Treatment of NTM pulmonary disease

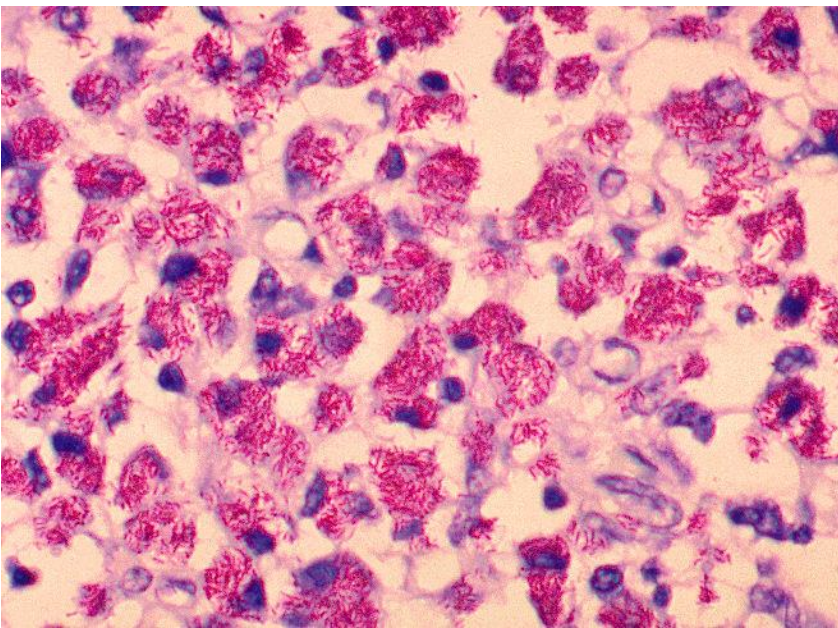
- Nodular/bronchiectatic MAC lung disease
 - 3 times weekly regimen
 - Clarithromycin
 - Rifampin
 - Ethambutol
- Severe disease
 - Daily Abx
 - 3 times weekly amikacin or streptomycin for 3 months
- Follow with sputum culture monthly
- Treatment to be continued until culture negative for 1 year

NTM Lymphadenitis

- Usually < 5 years of age
- Unilateral, submandibular site most common
- Onset of symptoms subacute
- Skin induration and sinus tract formation may occur
- Common species
 - MAC
 - *M. scrofulaceum*
- Dx Fine needle or excisional Bx (usually curative)
 - AFB stain
 - Culture



Unilateral, non-tender, cervical lymphadenopathy with violaceous discoloration.



NTM skin diseases

- Swimming pool granuloma/ fish tank granuloma/fish fancier's finger
 - Caused by *M. marinum*
 - found in salt and fresh water
 - Commonly seen in patients contact with
 - Aquariums (keeps fish tanks)
 - salt water
 - marine animals such as fish or turtles
 - Users of swimming pools- now rare
- Enter by scratches/ abrasions
- Chronic granulomatous warty skin lesions, purulent discharges
- Bx for diagnosis
 - AFB
 - Culture

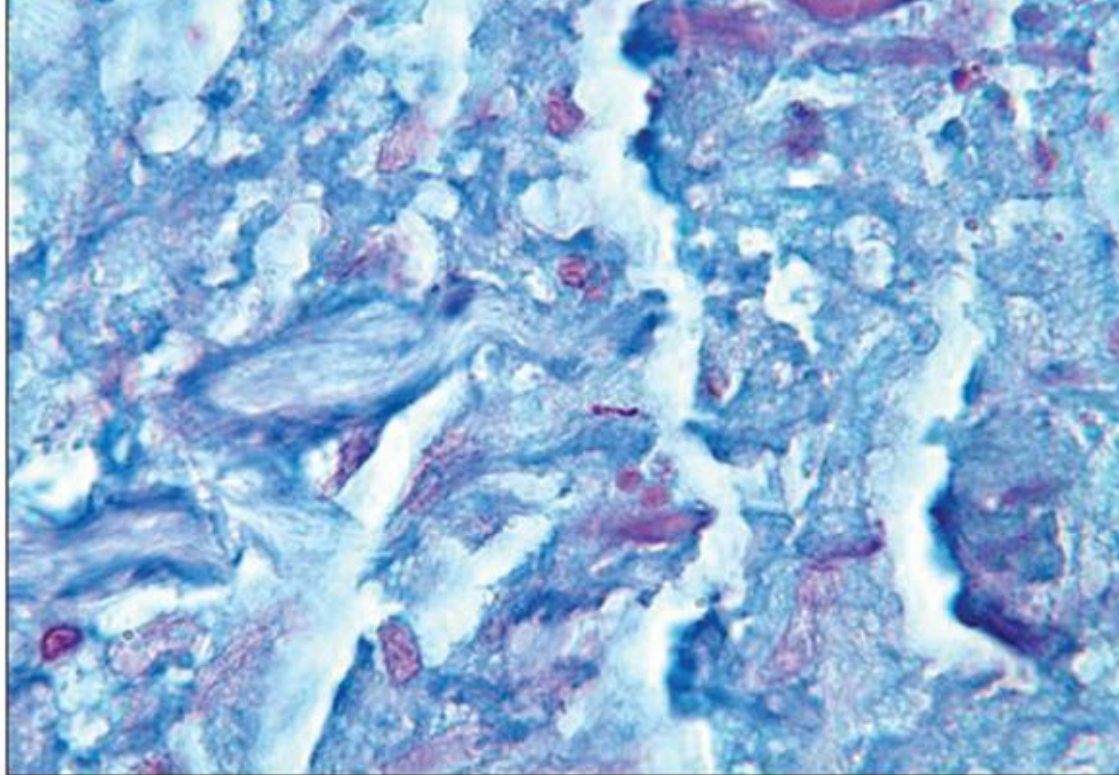


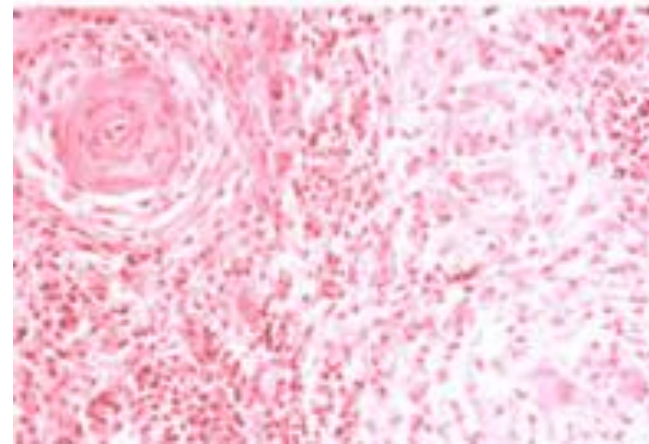
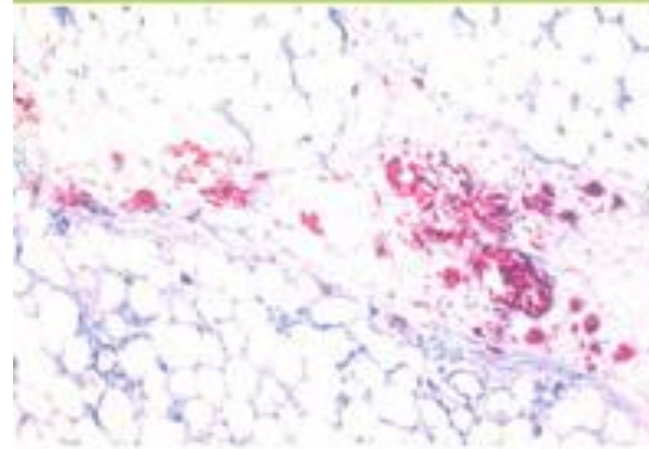
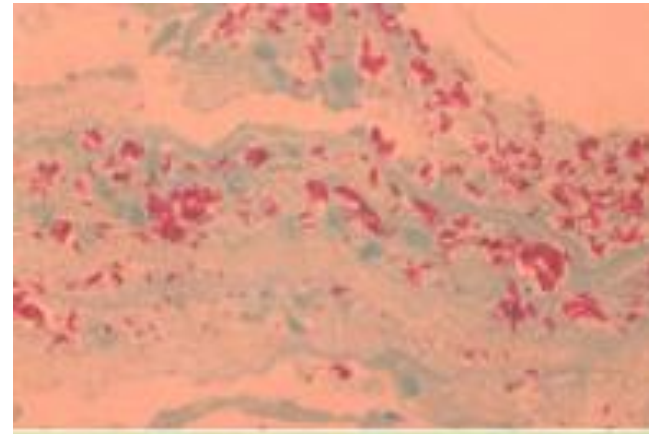
Figure 2 – Biopsy of the lesion revealed acid-fast bacilli within the granulomatous inflammation, consistent with *Mycobacterium marinum* granuloma.



NTM skin diseases

- Buruli ulcer
 - Caused by *M. ulcerans*
 - Tropical countries with marshy areas subject to periodic flooding
 - Uganda, Nigeria, Ghana, Mexico, Malaysia, Papua New Guinea
 - Entry through minor injuries by spiky grass
 - Hard nodule → itchy → necrosis with central softening → ulcerate → discharging ulcers with undermined edges → enlarge to involve large area
 - Diagnosis- Bx → granuloma, AFB
 - Treatment- surgical

Buruli ulcer



NTM skin diseases

- Post injection abscess
 - Caused by rapid growers
 - *M. chelonae*, *M. fortuitum*
 - Sporadic/ small outbreaks- when batches of injectable materials get contaminated
 - Painful abscesses develop within weeks or year/> after injection
 - Deeper abscesses following surgery, ocular injuries
 - Tx- by drainage

NTM disseminated disease

- HIV or other immunosuppressive disease
 - Commonest cause of death of HIV patients in USA
- Also seen in other immune deficient pts- transplant
- Symptoms: fever, weight loss, diarrhea
- Any site possible
 - AFB isolated in BM, blood, intestinal biopsies, stools
- Almost always due to MAC
- Needs aggressive Tx
 - Clarithromycin or azithro
 - Ethambutol
 - +/- Rifabutin
- HIV pts need prophylaxis when CD4 T-lymphocyte count is <50cells/mcL

Azithromycin weekly or clarithromycin daily

Leprosy



- A chronic granulomatous disease caused by the bacterium *Mycobacterium leprae*

Leprosy

Hansen's disease

- Old disease
 - Gerhard Henrik Armauer Hansen was a physician which first identified *Mycobacterium leprae* as the cause of leprosy in 1873
- Of all the diseases that continue to plague humanity, leprosy has the most notorious history as a cause of deformity, disability, loathing and fear.
- From ancient times until the recent past, the disease was considered both highly contagious and impossible to cure with associated social stigma

M. leprae

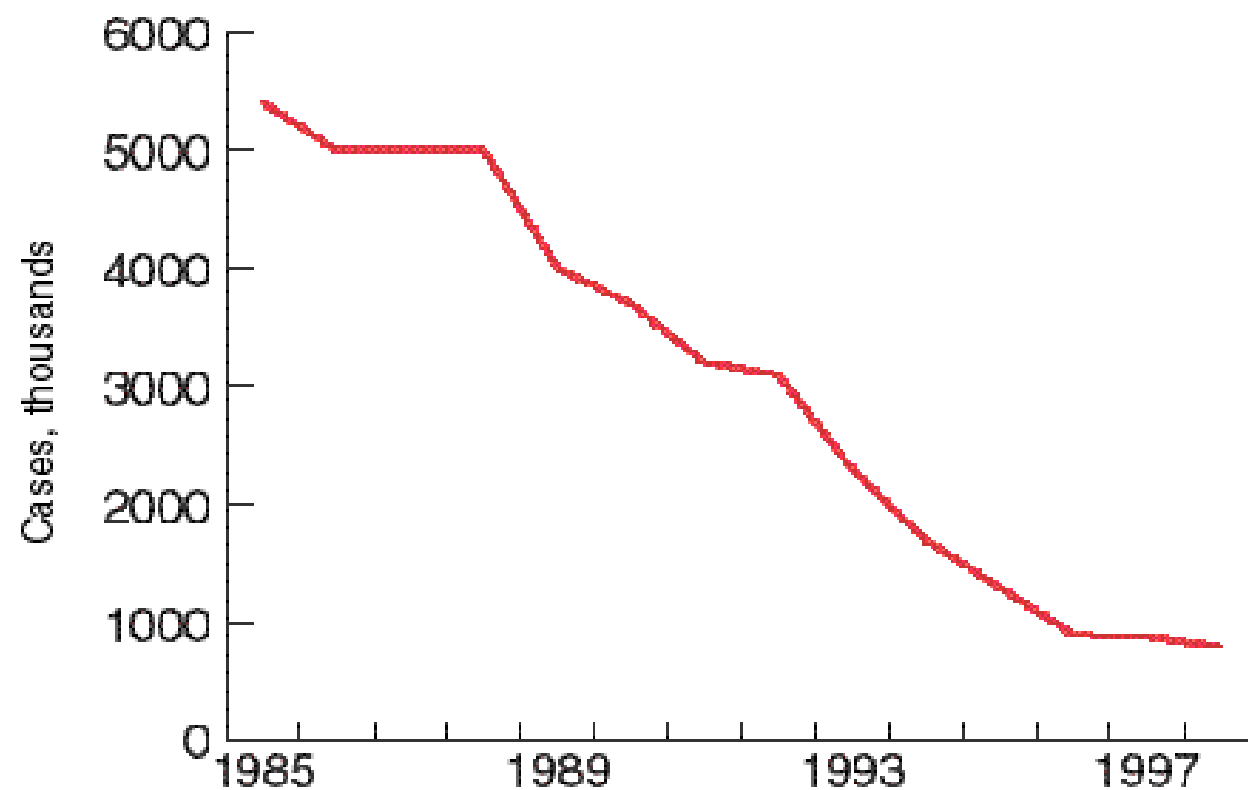
- Gram-positive, intracellular, aerobic bacilli with a waxy coating
- Unable to grow *in vitro*
- Therefore in vivo growth
 - armadillo
 - mouse footpad



- The transmission of leprosy is thought to occur through the respiratory track via droplets (from multibacillary patients)
- Infected individuals discharge bacilli through their nose
- The main reservoir is humans
- Risk group: children, people living in endemic areas, in low socio-economic conditions, compromised immune status (ie HIV)

Nearly eradicated or eliminated: Leprosy

Reported prevalence, worldwide



Source: WHO

Leprosy in Sri Lanka

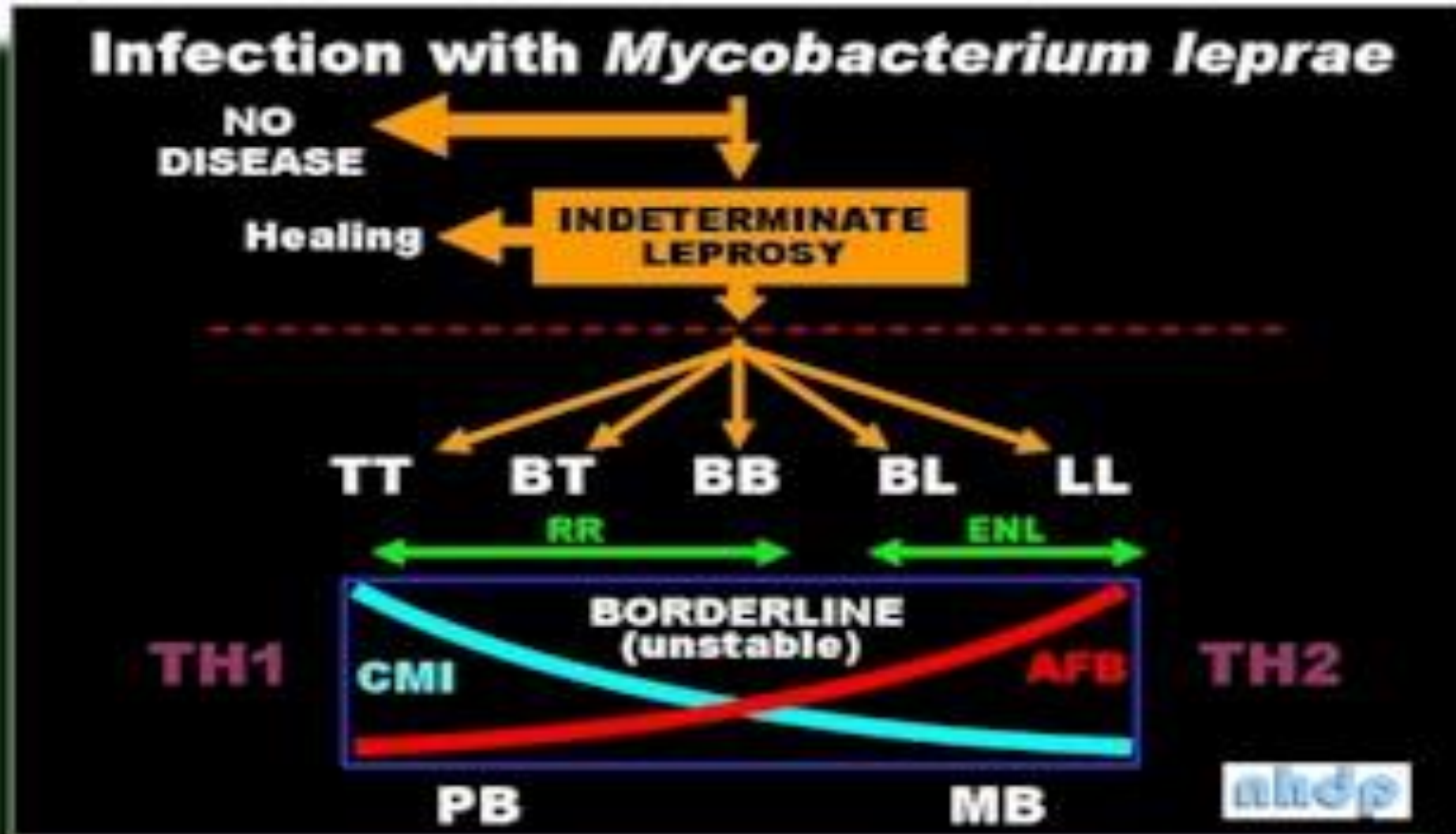
- The history of leprosy in Sri Lanka dates back to Dutch colonial times.
- The Dutch started the Leprosy Asylum at Hendala in 1701 to imprison the leprosy sufferers for life
- Leprosy is eliminated at national level as a public health problem.
- Achieved elimination target of prevalence less than 1 patient for 10,000 population.
- However, leprosy is not evenly distributed throughout the country.
- There still remain Western and Eastern provinces with prevalence more than the elimination target.

Clinical presentation

- Granulomatous disease affecting: peripheral nerves, mucosa of the upper-respiratory tract and skin
 - **Tuberculoid (Paucibacillary(PB) leprosy)**
 - few organisms
 - active cell-mediated immunity
 - have a Th1- type response
 - These strong cell-mediated responses clear antigens, but cause local tissue destruction
 - **Lepromatous (Multibacillary(MB) leprosy)**
 - many organisms → transmission
 - do not mount a normal cell mediated response
 - have specific T cell failure and macrophage dysfunction
 - But they do produce Th2-type cytokines

Ridley Jopling Classification of Leprosy

Basis - Immunological



| | Tuberculoid | Borderline Tuberculoid | Borderline | Borderline Lepromatous | Lepromatous |
|---------------------------------|---|---|--|---|---|
| Skin | | | | | |
| Infiltrated lesions | Defined plaques, irregular plaques, healing centers | Polymorphic, partially raised edges, satellites | Diffuse thickening | Papules, nodules, punched-out centers | Diffuse thickening Lionine face |
| Macular lesions | Single, small | Several, any size | Innumerable, small | Multiple, all sizes, bizarre | Innumerable, confluent |
| Peripheral Nerve lesions | Solitary, enlarged nerves | Irregular enlargement of several large nerves, asymmetrical | Late neural thickening, asymmetrical anaesthesia and paresis | Many nerves involved symmetrical patterns | Slow, symmetrical 'glove-and-stockings' anaesthesia |

Clinical diagnosis

- Painless skin patch, not itchy
- Typically anaesthetic and hypopigmented in tuberculoid leprosy (sensation preserved in LL)
- Thickened peripheral nerves
- Loss of sensation or paresthesias where the affected peripheral nerves are distributed
- Wasting and muscle weakness
- Foot drop or clawed hands
- Ulcerations on hands or feet
- Eye involvement
 - pain, sensitivity to light, decreased visual acuity, glaucoma, and blindness
- Erythema Nodosum Leprosum (ENL)
 - painful nodular skin lesions on the arms, legs and face
 - Type II hypersensitivity Immune complex

Paucibacillary(PB) leprosy:

< / = 5 skin lesions

No bacilli on smear

TT, BT

Multibacillary(MB) leprosy:

>5 skin lesions

possible visualization of bacilli on smear

LL, BL



Tuberculoid



Lepromatous



SDL

- Clinical and immunological differences b/w
 - TT
 - LL
 - Bordeline

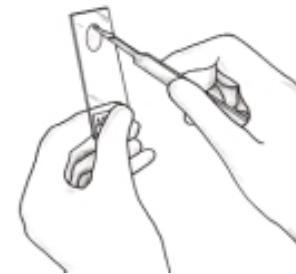
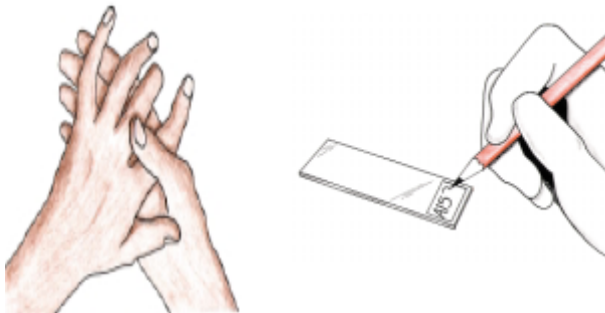
Diagnosis

- Usually clinical
 - More than 95% of cases can be diagnosed clinically even by paramedical workers
- Skin smears for *M.leprae* would assist in suspected infectious cases
- Detection of skin smear positive leprosy patients is more important as they infect others
- Biopsy/PCR may be needed rarely
- Lepromin test
 - A test utilizing an intradermal injection of a lepromin to classify the stage of leprosy
 - positive test in TT (PB leprosy) and negative test in LL (MB leprosy)

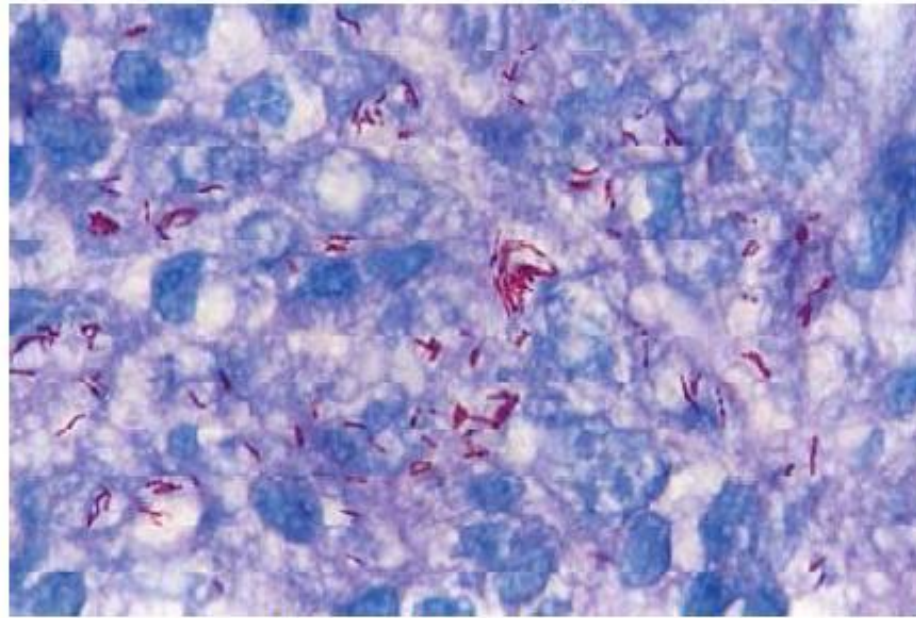
Microbiological diagnosis

– Demonstration of AFB

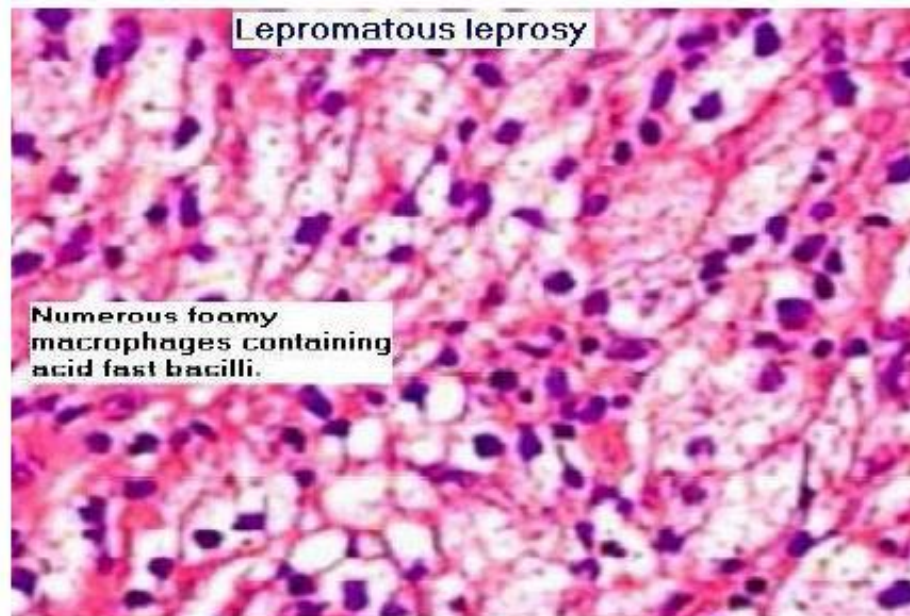
- Skin clippings / slit skin smears (from 2 sites)
 - One from ear lobe
 - One from active skin lesion



- Modified AFB stain (Z-N stain)



Modified Ziehl – Neelsen stain



Lepromatous leprosy

Numerous foamy
macrophages containing
acid fast bacilli.

H & E stain

Living (viable) leprosy bacilli

Solid-staining (S)



Dead leprosy bacilli

Fragmented (F)



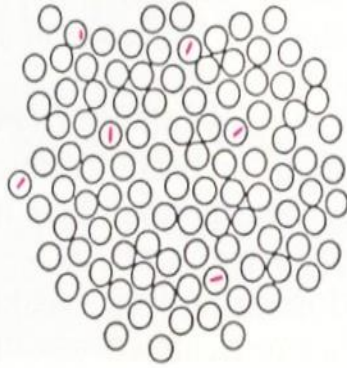
Granular (G)



Fig. 7 Solid-staining, fragmented and granular *M. leprae*

BI 1+

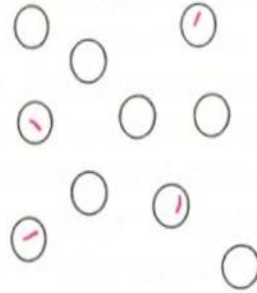
1–10 bacilli, on average, in 100 oil immersion fields



Examine 100 oil immersion fields

BI 2+

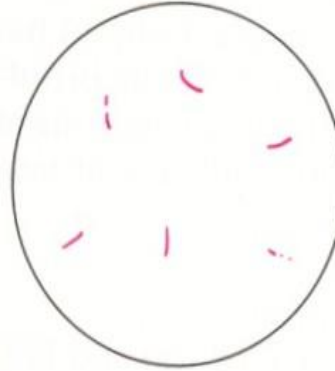
1–10 bacilli, on average, in 10 oil immersion fields



Examine 100 oil immersion fields

BI 3+

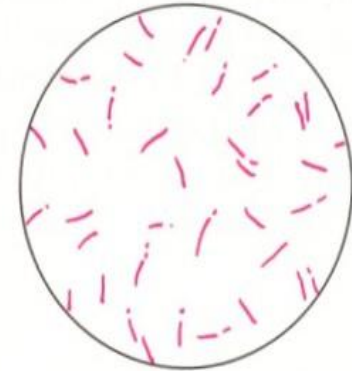
1–10 bacilli in an average oil immersion field



Examine 25 oil immersion fields

BI 4+

10–100 bacilli in an average oil immersion field



Examine 25 oil immersion fields

BI 5+

100–1000 bacilli in an average oil immersion field



Examine 25 oil immersion fields

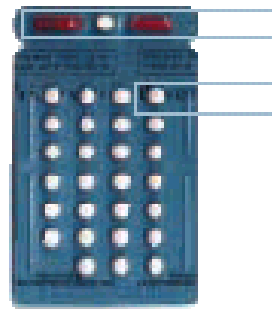
BI 6+

Over 1000 bacilli (many globi) in an average oil immersion field



Examine 25 oil immersion fields

Treatment



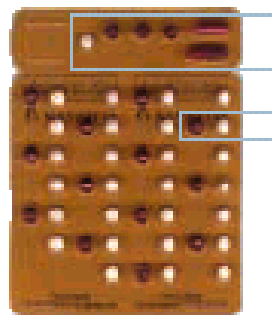
PB child blister pack

PB child treatment (10–14 years):

- Once a month: Day 1**
- 2 capsules of rifampicin (300 mg+150 mg)
 - 1 tablet of dapsone (50 mg)
- Once a day: Days 2–28**
- 1 tablet of dapsone (50 mg)

Full course: 6 blister packs

For children younger than 10, the dose must be adjusted according to body weight.



MB child blister pack

MB child treatment (10–14 years):

- Once a month: Day 1**
- 2 capsules of rifampicin (300 mg+150 mg)
 - 3 capsules of clofazimine (50 mg X 3)
 - 1 tablet of dapsone (50 mg)
- Once a day: Days 2–28**
- 1 capsule of clofazimine every other day (50 mg)
 - 1 tablet of dapsone (50 mg)

Full course: 12 blister packs

For children younger than 10, the dose must be adjusted according to body weight.

SDL

- Multi-drug treatment for leprosy
- Control of leprosy in Sri Lanka
- Information that should be given to patients