

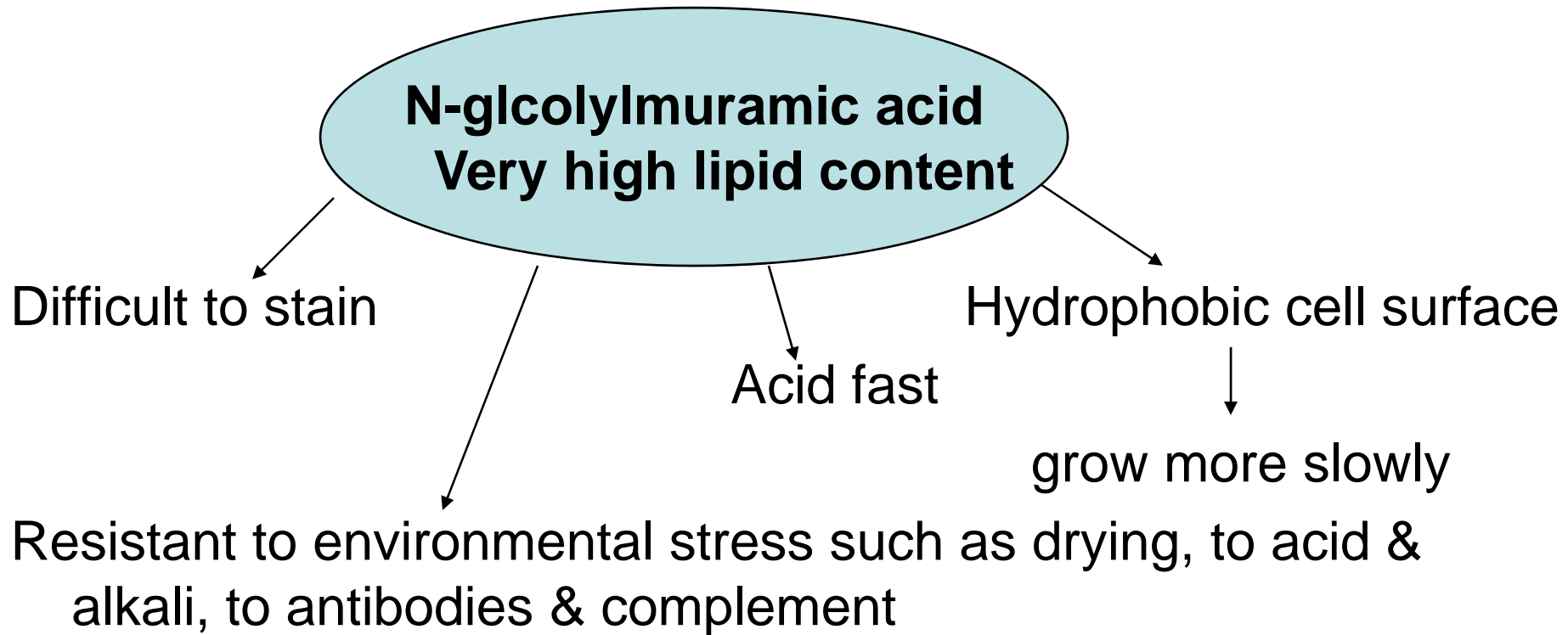
**MYCOBACTERIUM**

# Characteristics of Genus Mycobacterium

- Myco = Fungus
- Aerobic
- Nonmotile
- Nonsporing
- Slow growing
- Acid fast

- What is acid fastness?
- Why?

Unusual cell wall structure



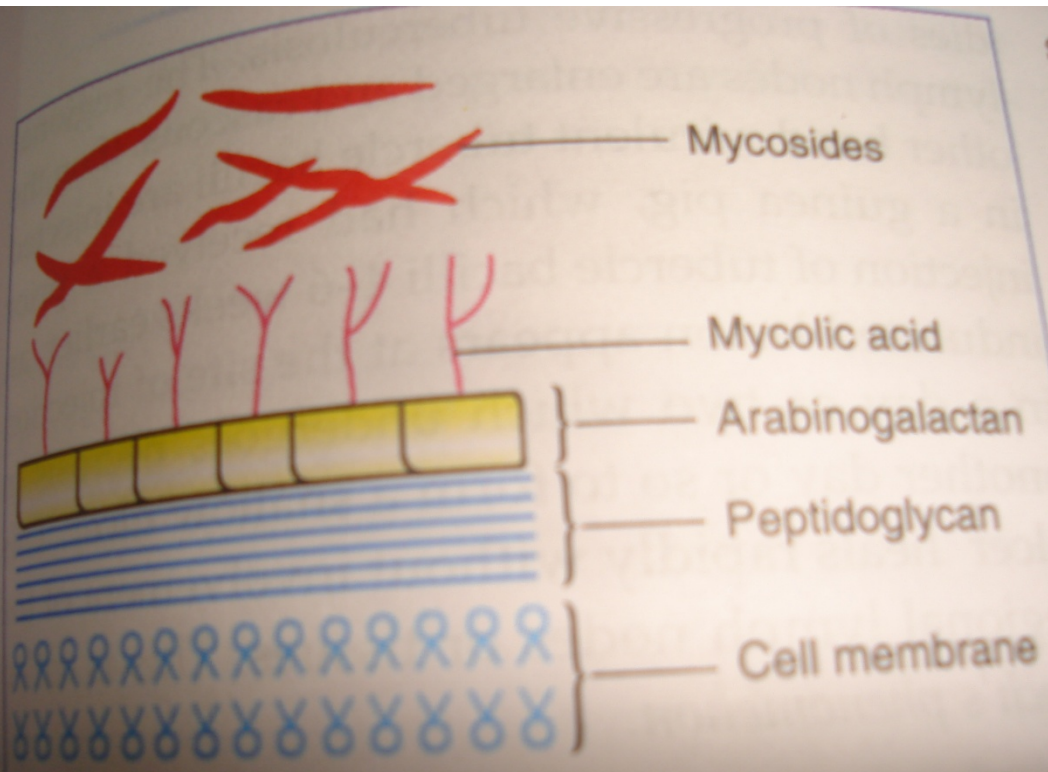


Fig. 42.5 Cell wall of Mycobacterium

# CLASSIFICATION OF MYCOBACTERIA

## **GROUP-I : Obligate pathogens**

### 1. *M. tuberculosis* complex

- *M. tuberculosis* (human tubercle bacillus)
- *M. bovis* (bovine tubercle bacillus)
- *M. africanum*

### 2. *M. leprae*

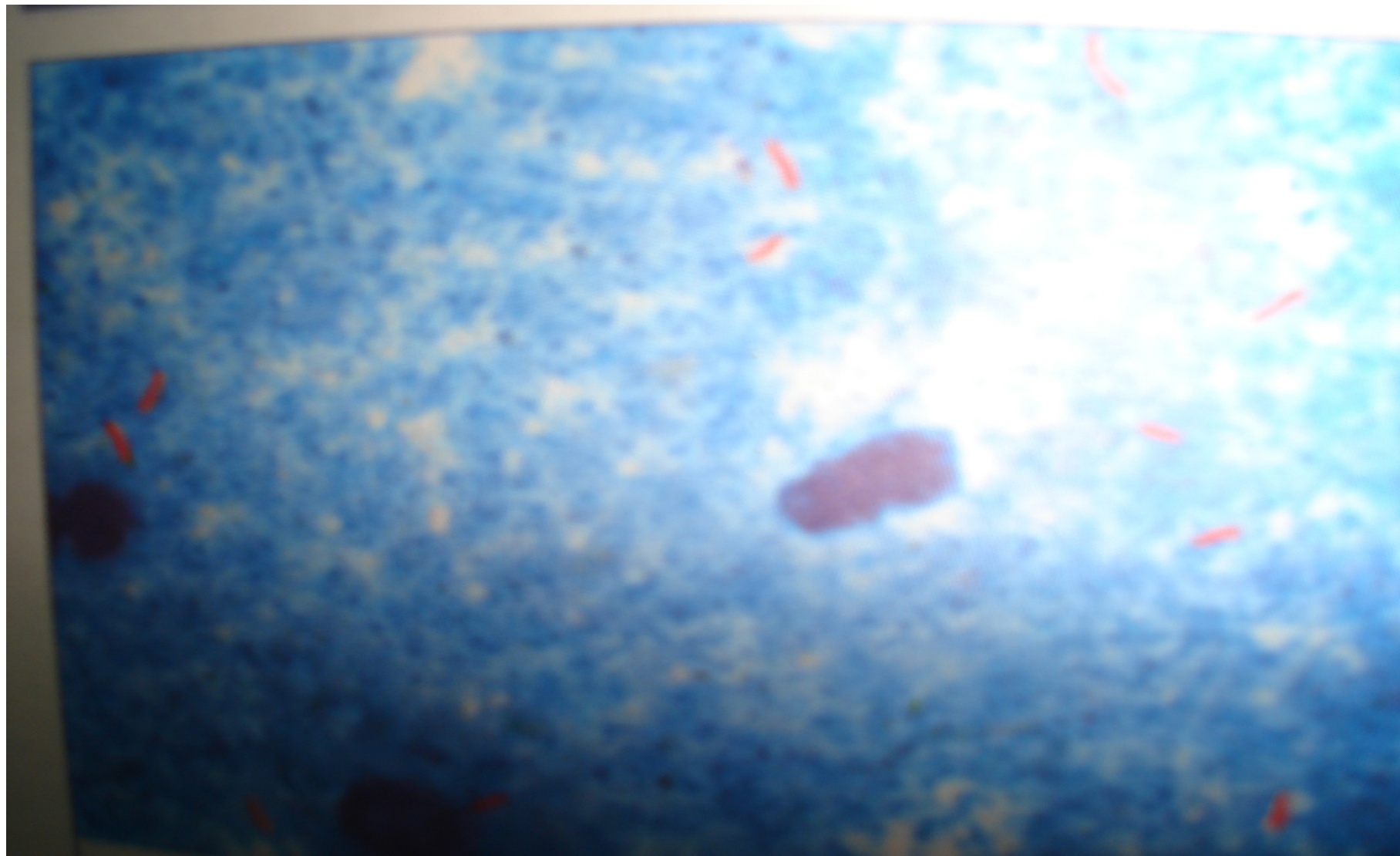
## **GROUP-II : Opportunistic pathogens (MOTT or NTM)**

## **GROUP-III : Saprophytic Mycobacteria**

- *M. smegmatis*
- *M. phlei*
- *M. stercoris*
- *M. butyricum*

# M. tuberculosis

- Acid fast rods –  $2-3\mu \times 0.3\mu$
- Alcohol fast
- Straight or slightly curved with rounded ends
- Arranged singly, in pair or clumps, sometimes branching & filamentous forms
- Gram positive
- Staining :1) Z.N. stain (bacilli appear red against blue or green background)  
2) Fluorescent stain



# Culture media

## 1. Solid media

– usually used for cultivation

Containing egg – Lowenstein-Jensen medium

Dorset egg medium

Petragnini medium

blood – Tarshis medium

potato – Pawlosky's medium

- ✓ Lowenstein-Jensen medium – IUAT L.J. (International Union Against Tuberculosis) is most commonly used laboratory media.



about two weeks  
up to 6-8 weeks.  
growth is 37°C (range  
5.4 to 7.0. Tubercle  
range of enriched  
stein-Jensen (L)  
sed. This medium  
paragine, mineral  
lycerol or sodium  
ing (inspissation).  
re solid without  
edium egg acts as  
reen inhibits the  
nycobacteria and  
n. The addition  
of human type  
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ium pyruvate



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## 2. Liquid media

– usually used for sensitivity testing, chemical analysis & preparation of antigens & vaccines

- Dubo's medium
- Middle brook's medium
- Proskaur's medium
- Beck's medium
- Sula's and Sauton's medium

# Growth characteristics

- Obligate aerobe
- Slow growing – takes 2-8 wks to grow
- Generation time - 14-15 hours
- Optimum temp. – 37<sup>0</sup> C (20<sup>0</sup>-40<sup>0</sup> C)
- Optimum pH 7.0 (6.4-7.0)

## Colony on solid media

- dry, rough, raised, wrinkled, irregular, creamy white (rough, buff & tough)
- luxuriant growth (eugonic growth)
- growth is improved with addition of 0.5% glycerol
- does not grow on medium with P-nitro benzoic acid

## Growth in liquid media

- growth begins at the bottom, creeps up the sides & forms a prominent surface pellicle which may extend along the sides above the medium

# Rapid culture methods

- BACTEC-460 System

- Rapid

- more sensitive than routine culture methods

- uses special broth (liquid media) with radiometric growth detection

- $^{14}\text{C}$  labeled substrate is used &  $^{14}\text{CO}_2$  evolved due to bacterial metabolism is measured

Disadvantages - cost of instrument

- inability to observe colony morph.

- need for disposal of radioactive sub

# Rapid culture methods

- BACTEC 9000MB and BACTEC MGIT use fluorescence quenching system .
- BacT/ALERT uses a colorimetric CO<sub>2</sub> sensor in each bottle to detect growth.

# Biochemical reactions

- ✓ Niacin test - Positive
- ✓ Nitrate reduction test - Positive
- Neutral red test - Positive
- Aryl sulphatase test - Negative
- Amidase test – Positive
- Peroxidase – Positive
- Catalase – weakly positive
- ✓ Susceptibility to pyrazinamide
- ✓ Resistant to TCH ( thiophen 2-carboxylic acid hydrazide) -

# Differences between M. tuberculosis & M. bovis

Characteristics	M. tuberculosis	M. bovis
Morphology	Straight or slightly curved	Straight, short & stout
AFB staining	Uniform or granular	Uniform
C/C	<ul style="list-style-type: none"><li>-Obligate aerobe</li><li>-luxuriant growth</li><li>-dry, rough, raised, creamy white</li><li>-not easily emulsifiable</li></ul>	<ul style="list-style-type: none"><li>-microaerophilic</li><li>-grows sparsely</li><li>-moist, smooth, flat, white</li><li>-easily emulsifiable</li></ul>



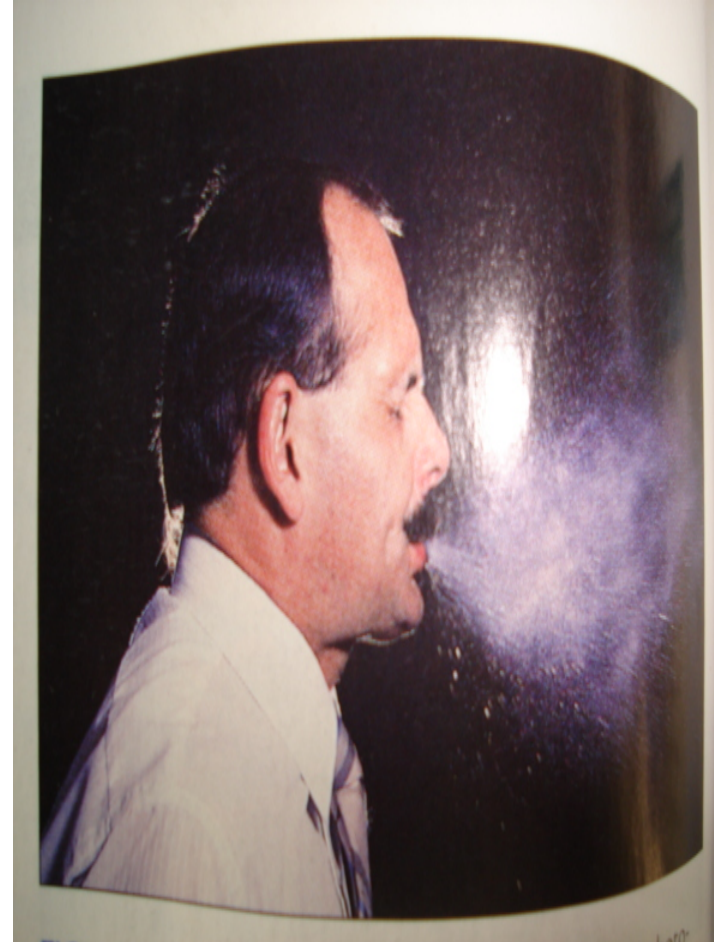
Characteristics	M. tuberculosis	M. bovis
Na pyruvate 0.5% glycerol	Helps in growth Improve growth	Helps in growth Impair growth
Biochemical reaction Niacin test NRT	Positive Positive	Negative Negative
Sensitivity to pyrazinamide	Sensitive	Resistant
Sensitivity to TCH	Resistant	Sensitive

# Sensitivity to physical & chemical agents

- Mycobacteria remain viable
  - In sputum for 20-30 hours
  - In droplet nuclei for 8-10 days
  - In cultures for 6-8 months at room temp. & for 2 years at  $-20^{\circ}\text{C}$
- Sensitive to formaldehyde, glutaraldehyde & ethanol
- Killed in 2 hours after exposure to direct sunlight & in 15-20 min. by heating at  $60^{\circ}\text{C}$

# Pathogenesis

- Source of infection – open case of pulmonary tuberculosis – it infects on an average some 25 contacts before death or cure in India.
- Mode of infection
  - 1) Inhalation of aerosolised bacilli contained in droplet nuclei of expectorated sputum
  - 2) Ingestion, through infected milk
  - 3) Inoculation (rare)



## Host factors

URT – ciliated epithelium

- mucus secretion
- cough reflex

LRT – alveolar macrophage

Genetic susceptibility

Age

Immunocompetence

Stress

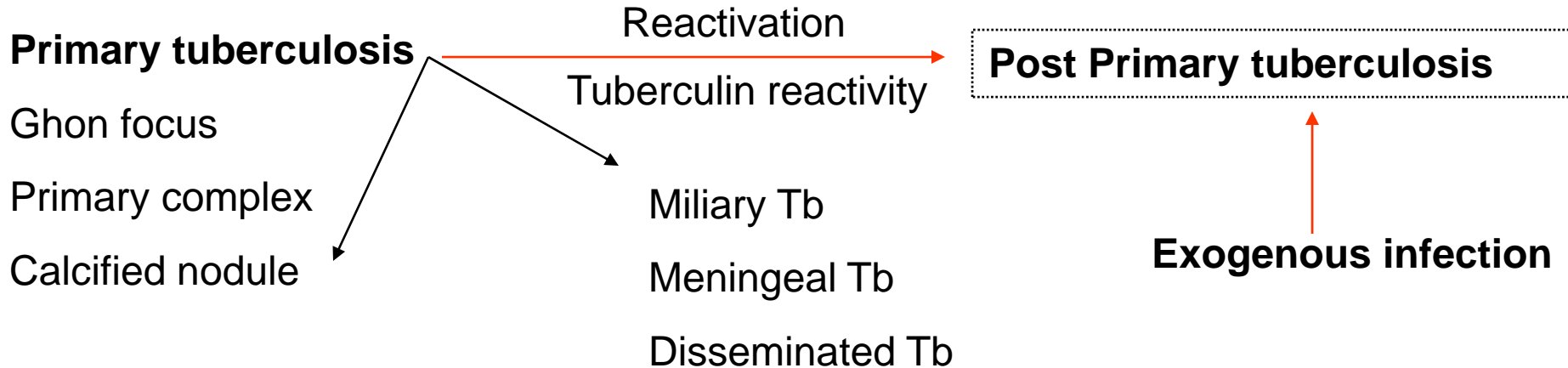
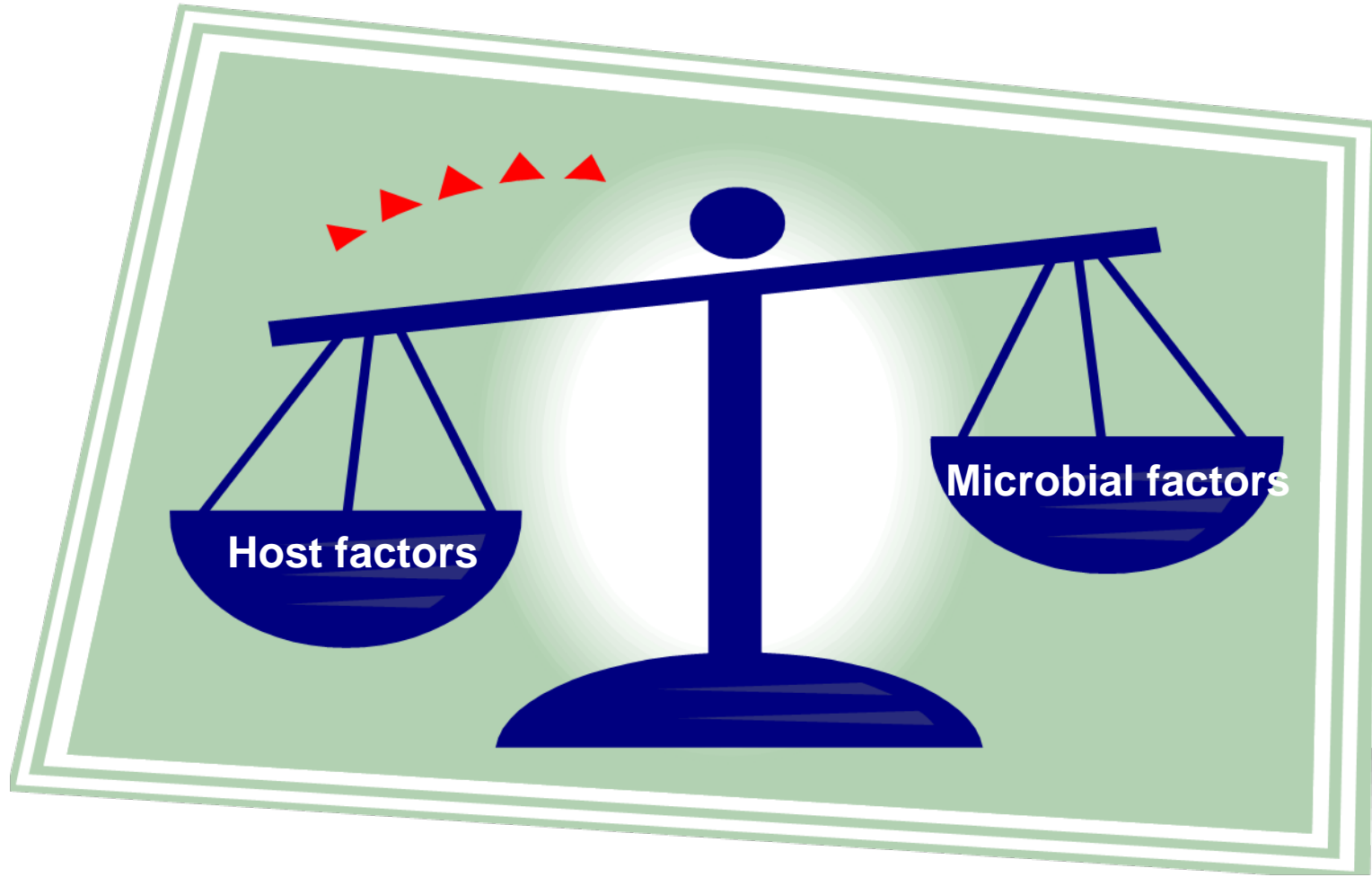
Nutrition

Coexisting illness

## Microbial factors

- Number of bacilli
- Virulence of bacilli
- Do not contain or secrete toxin
- Ability to survive & multiply in macrophages

- **Pathogenesis, allergy and immunity influence in the infection.**
- **Tenth of infected develop active TB.**
- **Cell-mediated immunity –tuberculosis**  
**Delayed type hypersensitivity &**  
**resistance to infection – identified by**  
**Koch – Koch phenomenon.**



# Laboratory diagnosis

## Specimens

### In Pulmonary tuberculosis

- sputum – Spot and early morning sample
- laryngeal swab
- bronchial washings
- gastric lavage

### In renal tuberculosis

- urine – 3 consecutive early morning

### In tuberculous meningitis

- c.s.f.

### Other specimen depending on site involvement

- ascitic fluid, pleural fluid, joint aspiration, pus aspirated from cold abscess, lymph node biopsy, tissue biopsy

1. Demonstration of AFB in specimens
2. Culture
3. Animal inoculation
4. Rapid methods
5. Serology
6. Skin test



# Demonstration of AFB in specimens

- Smear – staining – Z.N. method
  - Fluorescent staining

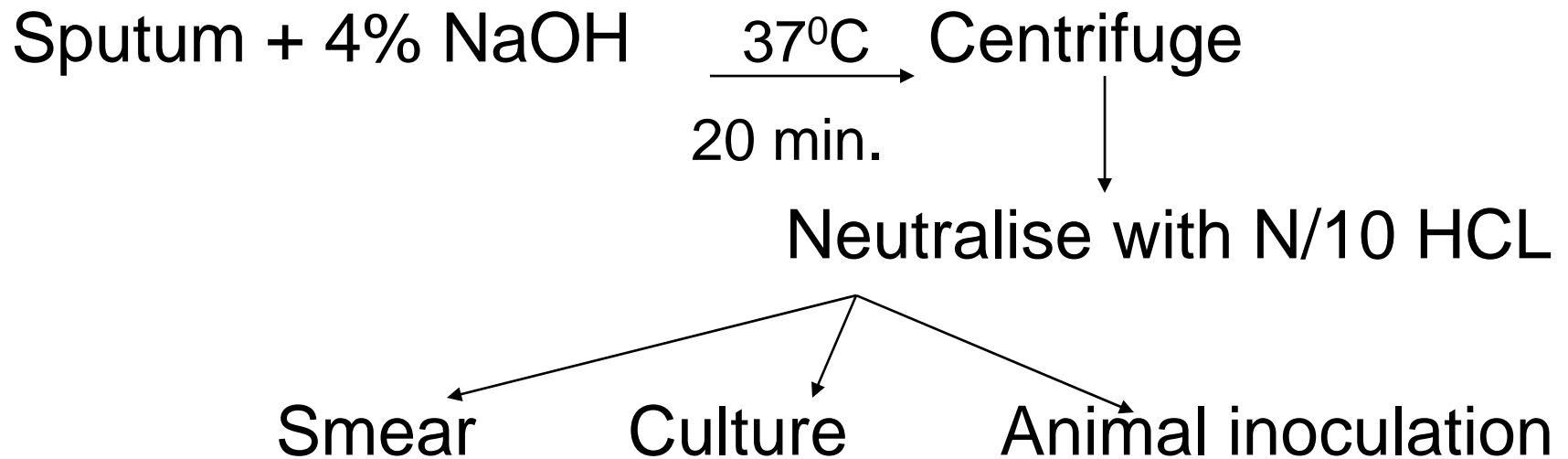
- Smear Reporting in case of sputum specimen

No. of AFB seen	in OIF	Interpretation
0	100	AFB not seen
1-9/100 field	100	scanty
10-99/100 field	100	1+
1-10/field	50	2+
$\geq 10$ / field	20 fields	3+

# Concentration methods

- To concentrate the AFB
- To kill contaminating or commensal organisms

Methods – Petroff's method



# Culture

- very sensitive (10-100 bacilli/ml)
- longer incubation required

Concentrated specimen



IUAT-L.J. ( International Union Against Tuberculosis)

37°C

see for growth twice weekly



Identify colony, Microscopy, Biochemical reaction  
(Slow growing, nonpigmented, niacin positive AFB)

- Negative report is given if no growth occurs after 8-12 weeks.
- Rapid culture methods – BACTEC-460

## Animal inoculation

- very sensitive
- loss of animal
- not used
- Guinea pig

## Nucleic acid technology – PCR & LCR , Typing methods.

## Serology – detection of Tb-IgM & IgG

- detection of Tb-Ag

## Other tests – X-ray

- E.S.R

# Extra pulmonary tuberculosis

C.S.F. – spider web clot on standing

- Lymphocytic leucocytosis

Pleural effusion & other exudates

- collected with citrate
- centrifuge – microscopy, culture & animal inoculation

# Skin test

Tuberculin test - delayed hypersensitivity  
- protein purified derivative

## Methods

- Mantoux method (Intradermal injection of PPD by tuberculin syringe)
- Heaf method (PPD is delivered through spring loaded gun which fires six prongs into the skin)

# Uses

- Epidemiologically to measure prevalence of tuberculous infection in community
- Diagnostically in young children with suspected clinical infection
- In immunisation campaigns in order to separate the positive and negative reactors and to assess the response to vaccination by B.C.G. vaccination

# Limitations

- Failure to distinguish active disease from quiescent infections and past BCG vaccination.
- Exposure to various mycobacteria in the environment may induce low level of tuberculin reactivity.



# Prophylaxis

- General measures

- adequate nutrition

- good housing

- health education

- early detection & treatment of cases

- chemoprophylaxis

- immunoprophylaxis – B.C.G. vaccine

(Bacille Calmette Guerin)

To whom B.C.G. is not given

- after the age of 2 years

- infants & children with active HIV disease

- babies born to mothers with AFB positive sputum

**THANK YOU**