Tuberculosis

Mycobacterium tuberculosis

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General Characteristics

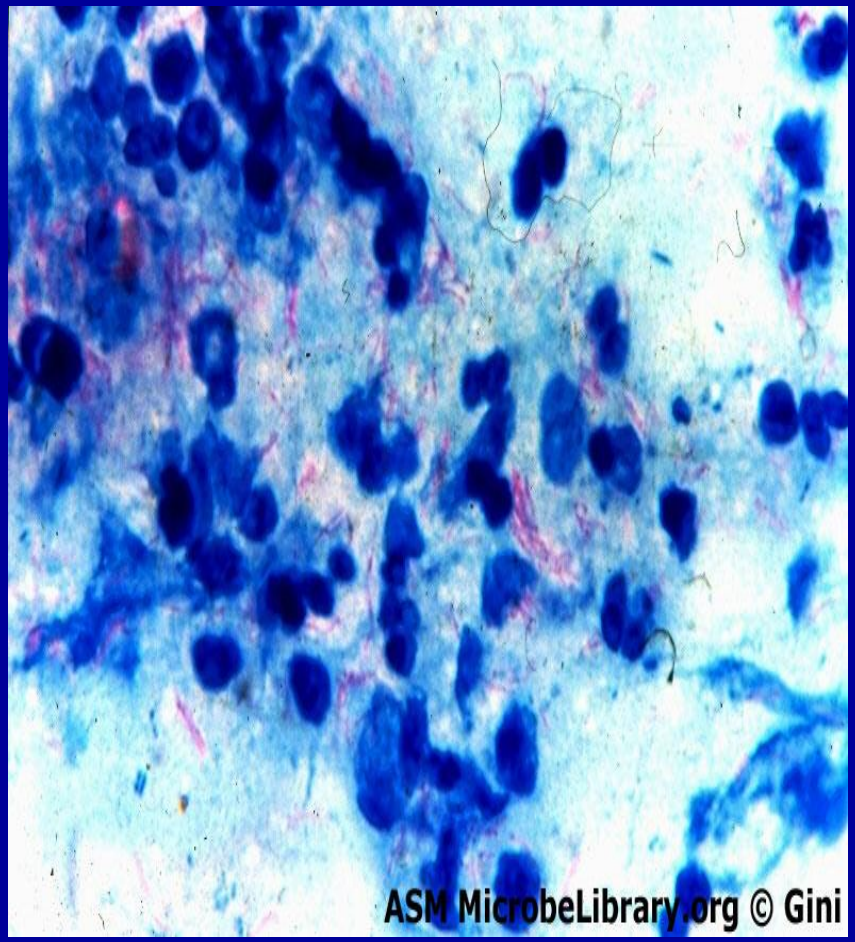
- Mycobacteria are strictly aerobic, non sporing intracellular bacilli. They are resistant to decolorization by mineral acid or alcohol after staining with carbol fuchsin, hence called ‘acid-fast bacilli’. The latter forms a strong complex with the mycolic acid content of the cell wall, therefore the organism is only decolorized with 20% sulfuric acid and alcohol. This is the base of the **Ziel- Neelson (ZN) stain**

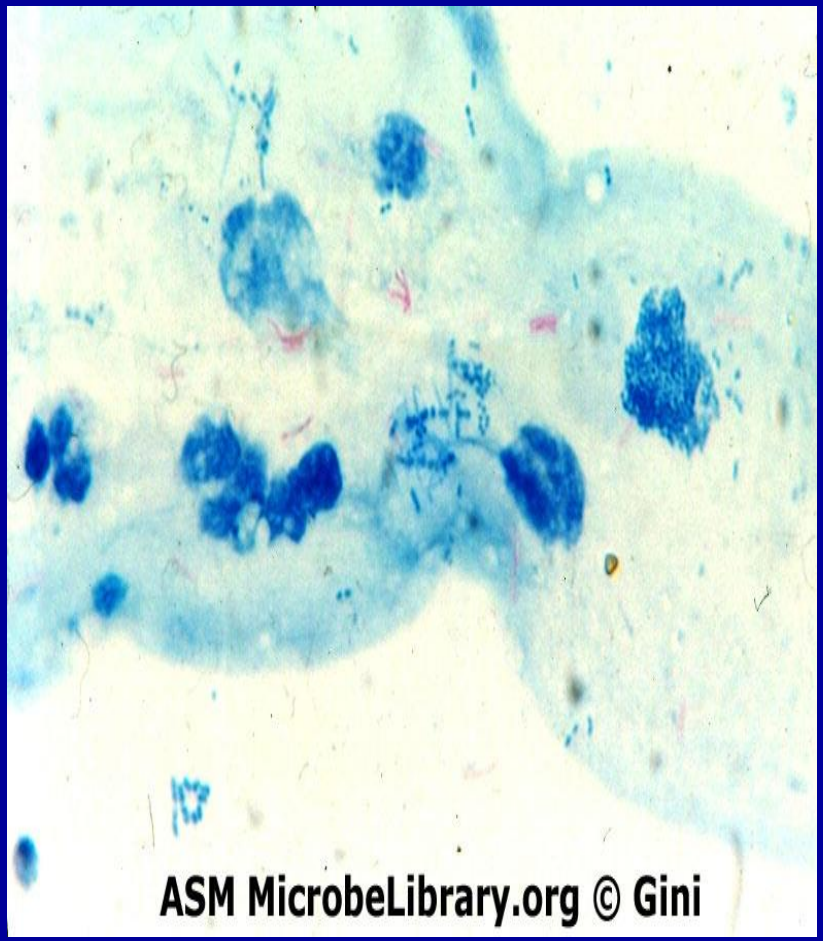
Acid fast stain method

- Dry smear preparation
- Strong Carbol-Fuchsine for 5 minutes with mild steaming
- Wash with water
- Decolorize with 20% H2SO4 for one minute
- Wash with water
- Flood slide with Methylen blue for 1 minute
- Wash with water , blot dry and examine under oil immersion lens.

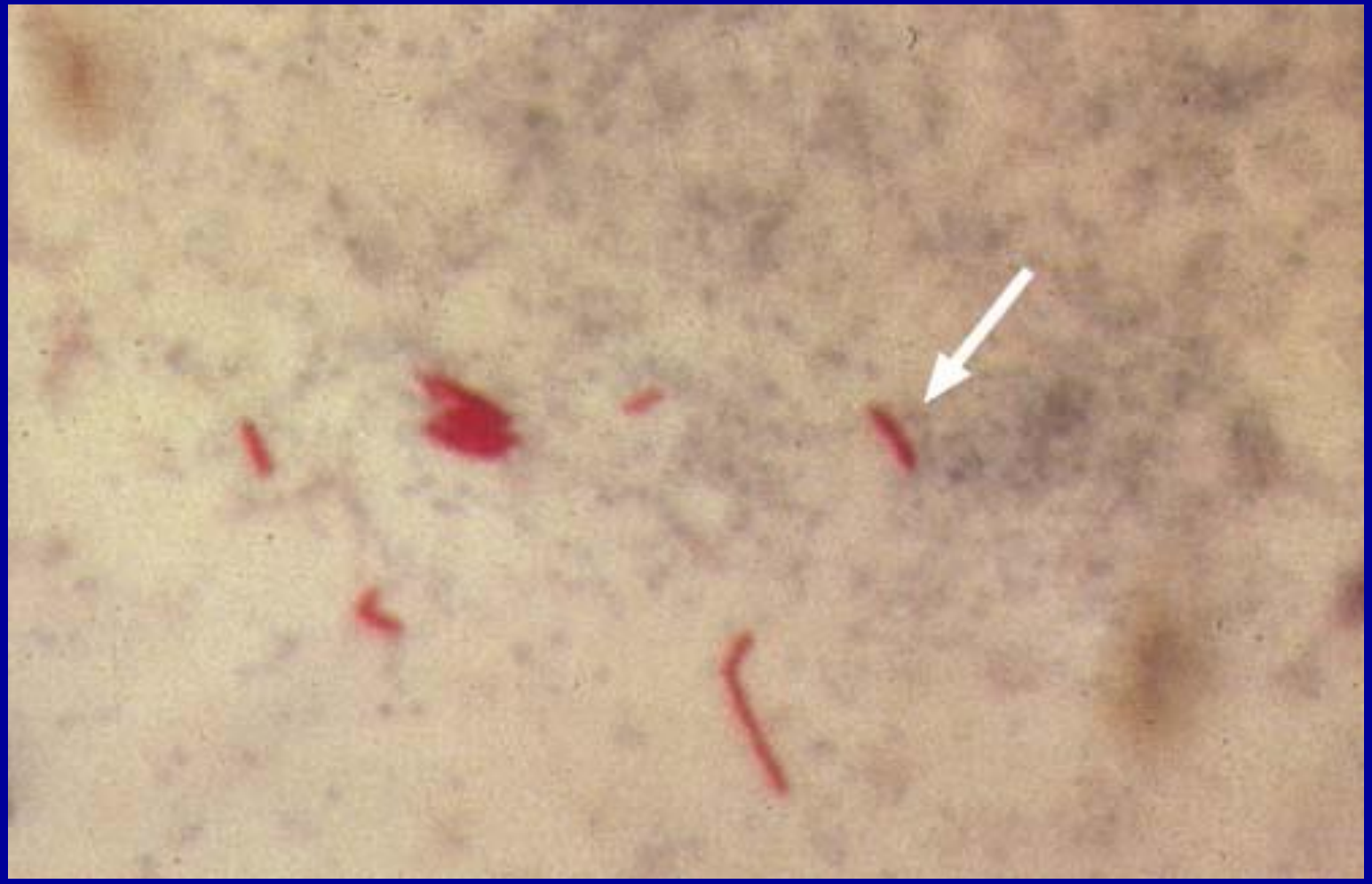
- Observations:
- Background of the slide seems blue while the acid fast bacilli take the red colr.

T.B (Scanty-versus-heavy infection)





These images show two different stages of tuberculosis disease. They come from sputum of two different Guatemalan patients with tuberculosis (TB) infections. Right figure shows scarce bacilli while left figure shows numerous bacilli representing a heavy TB infection



Mycobacterium tuberculosis. **Acid-fast stain.**

Smear result interpretation:

No. of AFB observed under 1000x	interpretation
≥ 9/field	4+(Strong +ve)
1-9 /field	3+ (Strong +ve)
1-9/10fields	2+ (Moderate +ve)
1-9/100fields	1+ (Moderate +ve)
1-2/300fields	+/- (Weak +ve)
No acid fast bacilli	Negative result

Cultural characters

- **There are different types of culture media suitable for *Mycobacterium tuberculosis:***

1- Lowenstien –Jensen medium , it is most widely used , containing egg, Malachite green , salt and Aspergin Glycerol .

2-Middle Brooks medium, it contains oleic acid , albumin, vitamines, salts, glycerol, glucose and malachite green

(It lok like LJ medium.)

3- Lofflers serum slopes

4- Dubos medium fluid medium containing Tween 80 as fat.

Mycobacteria do not grow on ordinary laboratory media but grow well on :
1-Lowenstein-Jensen (LJ) medium, containing egg, asparagine, glycerol and malachite green. The latter inhibits contaminants.

Colonial morphologies on LJ agar slopes are widely among Mycobacteria and are used in identification of

2- Dorset egg agar which is look like LJ medium.
3-Other media are Oleic acid medium and Middlebrook 7H10 and 7H11 **also enable Mycobacterium growth ,**
Fluid medium like Dubos medium consists of Tween 80 as fatty material .
Middlebrook 7H9 &7H12 are among fluid media also .

- Medically important mycobacterial species grow slowly. Their generation (doubling) time ranges from a few hours to >2 days, compared to 40–60 minutes for most other bacteria. Colonies may take 2–3 weeks to develop from the time of inoculation of culture media.

- Colonies on LJ medium are rough dry irregular they are white first become yellow or buff later. Mycobacterium tuberculosis colonies are well described as buff , tuff and rough take 2-3 weeks to develop at 37 C

Colonies of *Mycobacterium tuberculosis* on Lowenstein-Jensen medium.

Lab Diagnosis

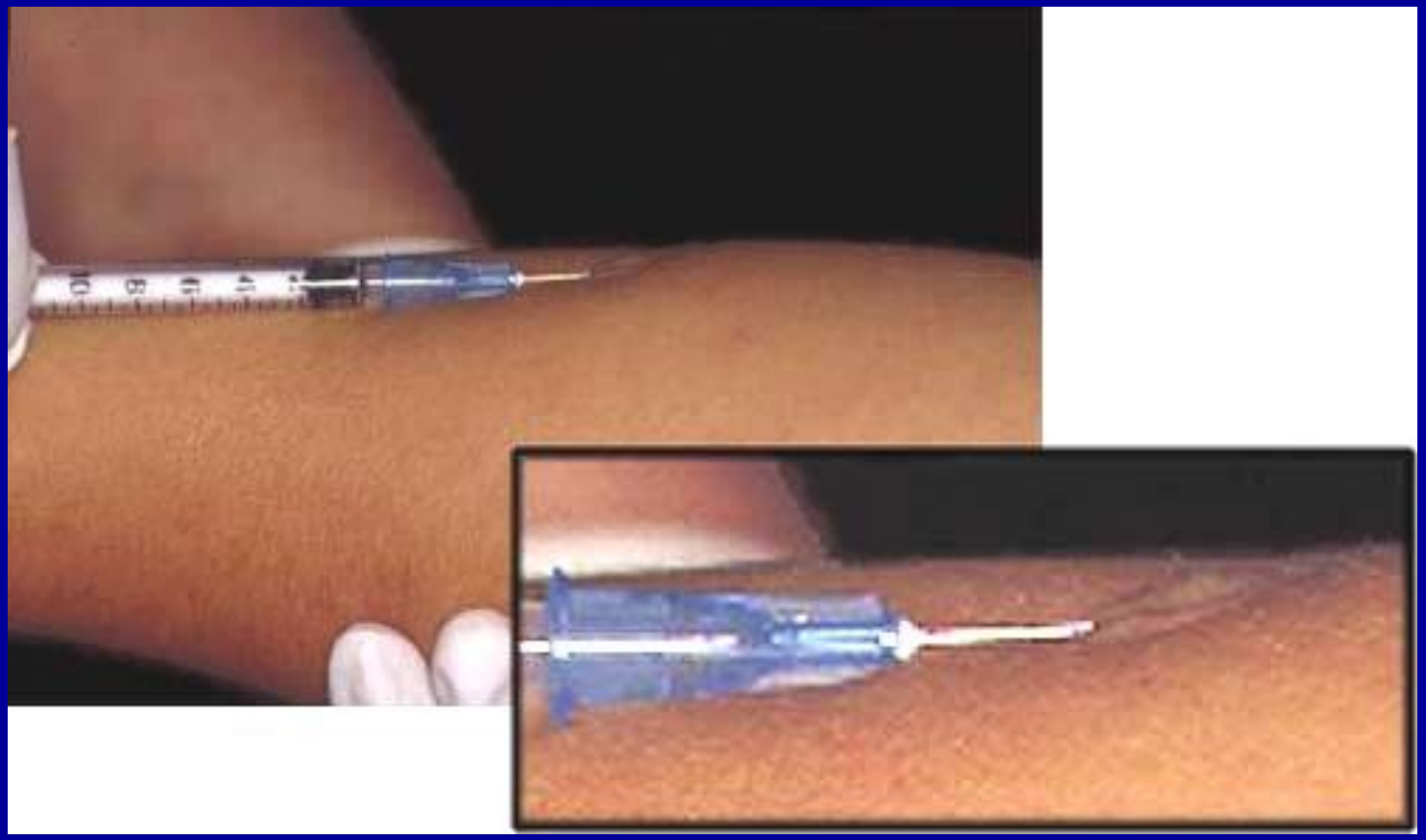
- Specimen depends on the site of infection :
- Sputum , fresh early morning sputum is recommended for the diagnosis of pulmonary TB.
- Gastric wash and laryngeal swab are indicated for pulmonary TB detection in children .
- Urine is most likely indicated in urinary TB.
- Cerebrospinal fluid CSF in case of neural TB.
- Pleural fluid and pleural biopsy in exudative and peracute TB.
- Blood for miliary TB.
- Synovial fluid for Joint TB.
- Peritoneal fluid and feces in digestive TB.

- The diganosis of tuberculosis requires detection of acid-fast bacilli in sputum via the Ziehl-Neelsen method. The organisms must then be cultured from sputum. First, the sputum sample is treated with NaOH. This kills other contaminating bacteria but does not kill the M.TB. present because M.TB. is resistant to alkaline compounds by virtue of its lipid layer.

- in a sputum sample, in excess In order to detect *Mycobacterium tuberculosis* of 10,000 organisms per ml of sputum are needed to visualize the bacilli with a 100X microscope objective. One acid-fast bacillus/slide is regarded as "suspicious" of an M.TB. infection.

Other diagnostic tests

- Tuberculin test:
- It is skin test depends on delayed hypersensitivity reaction in the host body to ***Mycobacterium tuberculosis antigens particularly protein antigen.***
- ***Mantux test it is widely used in doses of 50-250 tuberculin units , the volume is usually 0.1 ml of antigen is injected intradermal I/D on the fore arm . The result should be read after 72 hours.***



Administering the Mantoux test.

- result of skin test:
- Skin test Is considered positive if the reaction showed an increase in skin thickness with induration as shown below:

-ve	+ve	strong +ve
0-5mm	6-14mm	≥15

- Interpretation of Tuberculin test :

- 1- Actual positive test in real infection or vaccinated individual .
- False positive result due to reaction to other Mycobacteria like Mycobacterium tuberculosis.

- Actual negative result due to absence of infection
- False negative result in immunocopmromized individuals like HIV patients.

* Onsite TB IgG/IgM Cassette test

It is rapid screening test with about 85% sensitivity results.

Quantiferon-TBGold in tube test QFT-GIT)-

It is an ELISA test for Interferon Gamma released from special cells in TB infection.

*PCR test it is specific , sensitive rapid test for bacterial DNA in specimen.

* Fluorescent antibody test •

* The Gold test is culture because it is • diagnostic and supportive to identify anti TB resistant bacterial strains

Diagnosis of Skin Mycobacteria pathogens

- Mycobacterium leprae is weak acid fast , it requires 5% H_2SO_4 for decolorization step in Acid Fast staining technique.
- It is non cultivable organism ,it can not grow on cell free medium .
- Injection of the test material in the foot pad of mice is useful for their multiplication and antigen extraction

- Other diagnostic tests :
- Fluorescent antibody technique , her Mycobacteria will be revealed as fluorescent bacilli.

Updated diagnostic tests

- Polymerase Chain Reaction (PCR).
- Quantiferon test : it is a test for interferon Gama test in case of TB .
- Gene expert test , it is a test for *Mycobacterium tuberculosis* genes particularly ant TB. resistance genes .